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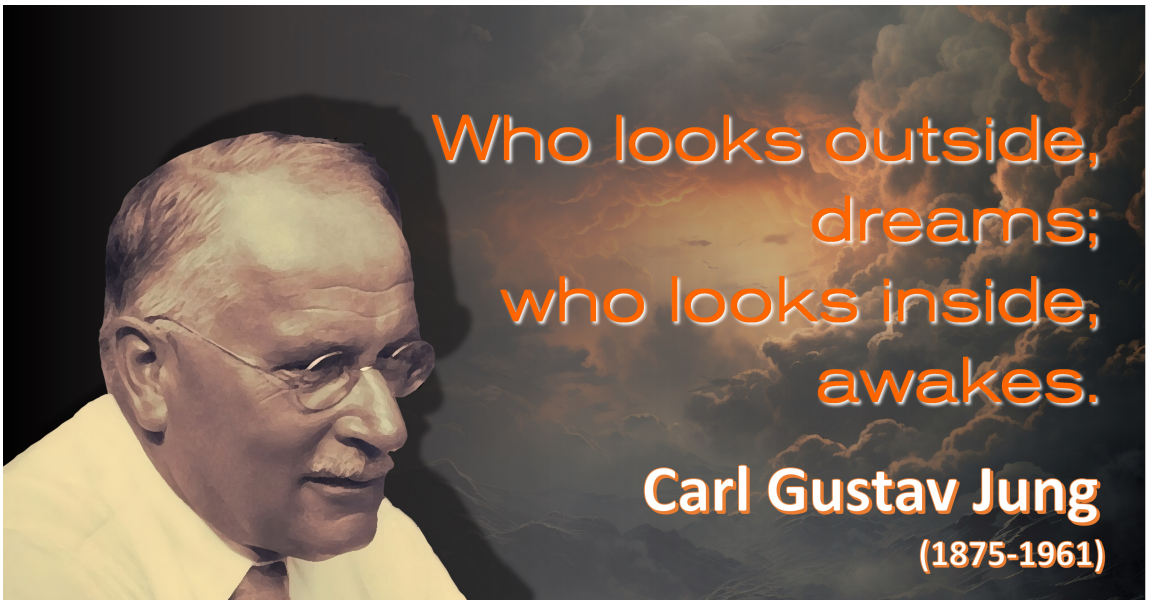

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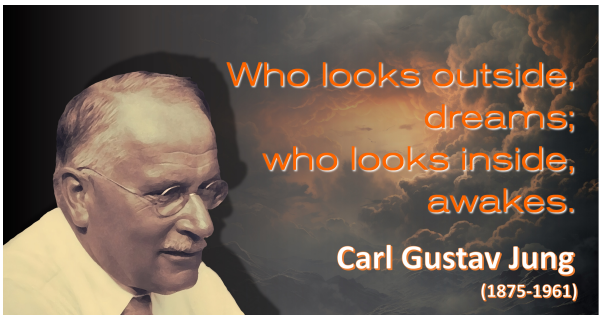

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Carl Gustav Jung (July 26, 1875–June 6, 1961) was a Swiss psychiatrist and psychologist, and one of the most influential thinkers in the history of studying the human psyche. He is most often referred to as the founder of "analytical psychology" and the creator of the concepts of the collective unconscious, archetypes, and the process of individuation. Jung's work remains an inexhaustible source of inspiration for everyone involved in the human psyche and those who wish to understand its essence.

Karl Gustav Jung (26. jul 1875—6. jun 1961) bio je švajcarski psihijatar i psiholog, jedan od najuticajnijih mislilaca u istoriji proučavanja ljudske psihe. O njemu najčešće govorimo kao o utemeljivaču „analitičke psihologije" i tvorcu koncepata kolektivnog nesvesnog, arhetipova i procesa individuacije. Jungov rad i danas predstavlja neiscrpan izvor inspiracije za sve koji se bave psihom čoveka i žele da razumeju njenu suštinu.

ORIGINAL ARTICLES

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Prevalence study on the use of antibiotics in a tertiary healthcare facility

Studija prevalencije o upotrebi antibiotika u ustanovi zdravstvene zaštite tercijarnog nivoa

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Abstract

Background/Aim. Prevalence studies, also known as cross-sectional studies, offer an effective approach for gathering high-quality data on particular health concerns. The aim of the study was to analyze the utilization of antimicrobial medications (AM) in a tertiary healthcare institution. **Methods.** A prevalence study on AM use was performed at the University Clinical Center Kragujevac, Serbia, in early November 2022. This research was conducted as part of the Fifth National Research Initiative and employed the standardized methodology established by the European Center for Disease Prevention and Control (ECDC). Data was collected on all patients admitted to the ward before 8 a.m. **Results.** Among the 567 patients included in the study, 246 received at least one AM, yielding a prevalence rate of 43.4% (95% confidence interval: 39.3–47.6). The most common indications for AM use were community-acquired infections (54.9%), followed by healthcare-associated infections (19.1%), surgical prophylaxis (20.7%), and medical prophylaxis (3.7%). Parenteral administration was the most common route for AM administration (89.6%). In 97.6% of cases, the rationale for the drug application was documented in the patient's medical records. Third-generation cephalosporins were the most frequently utilized antimicrobial group, comprising 17.9% of usage, followed by carbapenems at 12.7%, glycopeptides also at 12.7%, and fluoroquinolones at 11.8%. According to the Access-Watch-Reserve (AWaRe) classification, antibiotics under supervision were prescribed most frequently (66.4%), followed by first-choice antibiotics (28.1%), with reserve antibiotics comprising 5.5%. **Conclusion.** The implementation of measures to regulate the use of AM is required to retain the efficacy of these medications in the future.

Key words:
anti-bacterial agents; serbia; tertiary healthcare.

Apstrakt

Uvod/Cilj. Studije prevalencije, poznate i kao studije preseka, nude efikasan način prikupljanja visokokvalitetnih podataka o određenim zdravstvenim problemima. Cilj rada bio je da se analiza upotreba antimikrobnih lekova (AML) u ustanovi zdravstvene zaštite tercijarnog nivoa. **Metode.** Studija prevalencije o upotrebi AML sprovedena je u Univerzitetskom kliničkom centru Kragujevac, Srbija, početkom novembra 2022. godine. Ovo istraživanje sprovedeno je u okviru Pete nacionalne studije, i korišćena je standardizovana metodologija Evropskog centra za prevenciju i kontrolu bolesti (*European Center for Disease Prevention and Control* – ECDC). Prikupljeni su podaci o svim pacijentima primljenim na odeljenje pre 8 časova ujutru. **Rezultati**. Od 567 pacijenata uključenih u studiju, njih 246 primalo je najmanje jedan AML, što čini prevalenciju od 43,4% (95% interval poverenja 39,3–47,6). Najćešće idikacije za primenu AML bile su vanbolničke infekcije (54,9%), bolničke infekcije (19,1%), hirurška profilaksa (20,7%) i medicinska profilaksa (3,7%). Parenteralna primena predstavljala je najčešći put za aplikaciju AML (89,6%). U 97,6% slučajeva, razlozi za primenu leka dokumentovani su u medicinskom kartonu pacijenta. Cefalosporini treće generacije bili su najčešće korišćena antimikrobna grupa, sa 17,9% upotrebe, zatim karbapenemi sa 12,7%, glikopeptidi takođe sa 12,7% i

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fluorohinoloni sa 11,8% upotrebe. Prema *Access-Watch-Reserve* (AWaRe) klasifikaciji, najučestalije su propisivani antibiotici pod nadzorom (66,4%), potom antibiotici prvog izbora (28,1%), dok su rezervni antibiotici činili 5,5%. **Zaključak.** Neophodno je uvođenje mera za kontrolisanje upotrebe AML kako bi se očuvala efikasnost ovih lekova u budućnosti.

Ključne reči:
antibiotici; srbija; zdravstvena zaštita, tercijarna.

Introduction

Effective antimicrobial medication (AM) is essential in modern medicine for fighting bacterial infections. Nonetheless, data from nearly every part of the world show that microbes are becoming increasingly resistant to antibiotics, indicating that resistance is evolving into a serious health and socioeconomic problem [1]. If the warnings from the scientific community about the overuse and misuse of antibiotics are ignored, the overall burden of antimicrobial resistance is predicted to increase even further in the coming years. Despite a plethora of ongoing studies, no new AMs are anticipated any time soon; hence, greater efforts are required to maintain the sensitivity of existing medications [2].

Monitoring antibiotic use at the hospital, regional, and national levels is crucial, as it offers significant insights for the development of health policies and the implementation of control measures for antibiotic utilization. These studies serve as valuable and cost-effective instruments for monitoring antibiotic consumption, facilitating the collection of detailed and reliable information. In Europe, prevalence studies of healthcare-associated infections (HAIs) and antibiotic use are periodically conducted using standardized methodologies from the European Center for Disease Prevention and Control (ECDC), facilitating cross-country data comparisons. The ECDC study [3], conducted between 2011 and 2012 in European Union/European Economic Area (EU/EEA) countries, indicated that the average prevalence of patients receiving at least one prescribed AM was 35.0% (with country-specific rates ranging from 21.4% to 54.7%). The subsequent ECDC study, conducted from 2016 to 2017, reported a prevalence of 32.9% (with country-specific rates ranging from 16.0% to 55.6%) [4]. Despite being a non-EU member, the Republic of Serbia was invited to participate in this second study. It revealed a prevalence of patients with at least one prescribed AM at 41.3% [95% confidence interval (CI): 40.5–42.1]. The Republic of Serbia, in conjunction with Southern and Eastern European nations, has consistently ranked among the highest in Europe for antimicrobial consumption and bacterial resistance levels across all examined species. The Ministry of Health established a National Clinical Practice Guideline for Rational Antibiotic Use to tackle this issue [5]. This guideline offers healthcare professionals clear guidance on selecting appropriate therapeutic approaches in daily practice, optimizing antibiotic use, and reducing resistance rates.

This study aims to analyze the utilization of AMs in a tertiary healthcare institution and identify the risk factors influencing their use.

Methods

This prevalence study was conducted at the University Clinical Center Kragujevac, Serbia, during the first week of November 2022 as part of the Fifth National and Third European Point Prevalence Study on HAIs and antimicrobial use. The methodology of the ECDC [6] was implemented in the investigation. The research in Serbia was coordinated by a Working Group of the Ministry of Health and the Institute of Public Health of Serbia "Dr. Milan Jovanović Batut", which was specifically established. The Working Group operated through the appropriate institutions and public health centers. Hospitals participated voluntarily in the investigation. Our hospital, which is a tertiary healthcare institution with a total of 1,118 hospital beds, including 97 beds in intensive care units, was one of the participants.

The study adhered to the established methodology, encompassing all hospital departments and all patients admitted before 8:00 a.m. on the day of the study who remained hospitalized at the time of data collection. Emergency departments were excluded, except for those where patients are monitored for more than 24 hrs. Additionally, patients treated in day hospitals, those receiving one-day therapy or surgery on the study day, as well as individuals seen in outpatient clinics, emergency departments, or outpatient dialysis units, were also excluded. Data for each department and its hospitalized patients were required to be collected within a single day.

The research team, comprising physicians and medical technicians from the hospital and the pertinent public health institute, completed two days of theoretical and practical training before initiating the study. Surveyors gathered the requisite data by examining patient records and engaging in direct communication with physicians in the departments.

The collected data were inserted into two epidemiological questionnaires. The initial questionnaire gathered data regarding the department, which encompassed the department type, the bed count, and the number of patients admitted on the study date. In the second questionnaire, the patients were asked about sociodemographic information as well as specifics about their current hospitalization, such as the severity of their illness based on the McCabe score, the use of invasive procedures (such as surgery, central venous catheter, urinary catheter, or intubation), and their antimicrobial use. Antibiotic data were collected exclusively for patients receiving at least one antimicrobial during the study, except for surgical prophylaxis, where administration occurred up to 24 hrs prior to the study documentation.

Each antimicrobial drug was assigned to the fifth Anatomical-Therapeutic-Chemical (ATC) Classification level, along with details regarding the method of administration, indication [community-acquired infections (CAIs), acute HAIs, surgical or medical prophylaxes], diagnosis, documentation of the reason for drug use, initiation of therapy, and daily dosage. The duration of antibiotic use for surgical prophylaxis was also documented (one dose, one day, or multiple days).

The study included the following antimicrobials according to the fifth ATC level classification: J01 (antibiotics for systemic use), J02 (antimycotics for systemic use), A07AA (intestinal anti-infectives), P01AB (nitroimidazole derivatives – antiprotozoals), D01BA (antifungals for systemic use), and J04AB02 (rifampicin). Antiviral agents and antituberculous drugs were excluded unless utilized for the treatment of mycobacteriosis other than tuberculosis. All observed antibiotics were further categorized according to the Access-Watch-Reserve (AWaRe) classification [7] into first-choice antibiotics (Access), controlled antibiotics (Watch), and reserve antibiotics (Reserve).

To classify an infection as an active HAI, the established HAI criteria must be fulfilled, with signs and symptoms evident on the study date, or the patient must be undergoing treatment for the infection. Symptoms of infection must manifest after the third day of admission unless the patient was re-admitted to the hospital within 48 hrs. There were exceptions to these criteria for surgical site infections, infections caused by *Clostridioides* (*C.*) *difficile*, and infections associated with medical devices.

All collected data were systematically coded and stored in a database. SPSS 22.0 for Windows was used to analyze the data. A Chi-square test was used to determine the statistical significance of the variables. A separate test, univariate logistic regression, was also performed. The dependent variable was group membership (patients who received at least one prescribed antibiotic vs. patients who received no antibiotic), while all other factors were independent. All factors that showed statistically significant variations between groups were included in the multivariate logistic regression model. Before applying this multivariate technique, its assumptions were checked to see if they were satisfied (binary outcome, independence of observations, no multicollinearity, no extreme outliers, and a sufficient sample size). The quality of the regression models was checked by the Hosmer and Lemeshow test, Cox and Snell R-squared, and Nagelkerke R-squared. The results were considered statistically significant if the probability of the null hypothesis was 0.05 or below.

The prevalence of patients with AM was calculated, using patients receiving at least one antimicrobial at the time of the study as the unit of observation. The denominator comprised all patients treated at the hospital departments during the same period of antimicrobial drug usage. Patients receiving multiple antimicrobials were counted only once. Prevalences were calculated, accompanied by their 95% CI, which were determined using the Clopper-Pearson exact method for proportions and STATA version 18.0.

This study adhered to the ECDC methodology, which did not necessitate approval from the Institutional Ethics Committee. This ongoing practice, which has not been sufficiently regulated in the past, must undergo future changes to ensure the protection of patients and their data through the work of the Ethics Committees.

Results

The study sample comprised 567 patients, with 292 (51.5%) of them identified as male. The age of all patients was 51.4 ± 25.5 years (range: 0–93 years, median: 60.0), and the length of hospitalization was 10.1 ± 13.4 days (range: 0–116 days, median: 5.0). The majority of patients were admitted to surgical departments [212 (37.4%)] and internal medicine departments [154 (27.2%)]. This was followed by the intensive care unit [85 (15.0%)], gynecology and obstetrics [31 (5.5%)], pediatrics [21 (3.7%)], neonatology [9 (1.6%)], and other mixed departments [45 (7.9%)] (data not shown).

Out of the overall patient cohort, 246 received at least one AM, yielding a prevalence of 43.4% (95% CI: 39.3–47.6). The basic characteristics of the patients, categorized by whether they received AM, are presented in Table 1. A statistically significant difference was observed between the group receiving antibiotics and the group not receiving any in terms of patient age, length of hospital stay, the assessment of the patients' condition according to the McCrabe score, and the department in which they were hospitalized ($p < 0.05$). Patients who received AM more often had a fatal disease (24.0%) compared to those who did not receive AM (15.6%). The majority of patients with AM received treatment in surgical departments (39.4%), whereas most patients in the non-antibiotic group were treated in internal medicine departments (41.1%). Patients who received AM were significantly more likely to undergo medical procedures during their current hospitalization compared to those who did not. Specifically, they had higher rates of surgical intervention (32.6% vs. 15.6%), central venous catheter placement (13.4% vs. 3.7%), urinary catheterization (45.1% vs. 15.0%), and intubation (11.4% vs. 1.9%). Moreover, individuals who received at least one antimicrobial agent during the trial exhibited a statistically significantly higher incidence of HAIs (19.1%) compared to those who did not receive AM.

Approximately 74.0% of patients who received antimicrobial therapy did so for therapeutic purposes, with 54.9% treated for CAIs and 19.1% for HAIs. Surgical prophylaxis was the rationale for antibiotic use in 20.7% of patients, whereas 3.7% took it for medical prophylaxis (Table 2). Concerning the duration of surgical prophylaxis, 14 (27.5%) patients (prevalence: 2.5; 95% CI: 1.4–4.1) were administered a single dose of antibiotic, 10 (19.6%) patients (prevalence: 1.8; 95% CI: 0.8–3.2) underwent prophylaxis for one day, whereas over half of the patients [27 (52.9%)] (prevalence: 4.8; 95% CI: 3.2–6.9) received antibiotics for more than one day (data not shown).

Table 1

Distribution of patients based on the use of antimicrobial agents at the University Clinical Center Kragujevac in the first week of November 2022

Characteristics	Patients with AM (n = 246)	Patients without AM (n = 321)	χ^2 test	*p*
Male gender	132 (53.7)	160 (49.8)	0.811	0.368
Age groups (years)				
< 1	23 (9.3)	20 (6.2)		
1–14	15 (6.1)	16 (5.0)		
15–24	9 (3.7)	15 (4.7)	19.831	0.001
25–64	80 (32.5)	161 (50.2)		
≥ 65	119 (48.4)	109 (34.0)		
Length of hospital stay (days)				
1–3	76 (30.9)	140 (43.6)		
4–7	64 (26.0)	51 (15.9)		
8–14	62 (25.2)	54 (16.8)	20.656	< 0.001
15–29	29 (11.8)	44 (13.7)		
≥ 30	15 (6.1)	32 (10.0)		
McCabe score				
non-fatal disease	150 (61.0)	213 (66.4)		
fatal disease	59 (24.0)	50 (15.6)	9.727	0.021
rapidly fatal disease	28 (11.4)	52 (16.2)		
[1] unknown	9 (3.7)	6 (1.9)		
Department				
internal medicine	80 (32.5)	132 (41.1)		
surgery	97 (39.4)	79 (24.6)		
intensive care units	23 (9.3)	15 (4.7)	55.378	< 0.001
gynecology with obstetrics	13 (5.3)	28 (8.7)		
pediatrics	32 (13.0)	23 (7.2)		
other departments	1 (0.4)	44 (13.7)		
Surgical intervention				
none	166 (67.5)	271 (84.4)		
NHSH	55 (22.4)	33 (10.3)	22.730	< 0.001
non-NHSH	25 (10.2)	17 (5.3)		
Central venous catheter	33 (13.4)	12 (3.7)	17.847	< 0.001
Urinary catheter	111 (45.1)	48 (15.0)	62.817	< 0.001
Intubation	28 (11.4)	6 (1.9)	22.359	< 0.001
Healthcare-associated infection	47 (19.1)	1 (0.3)	63.43	< 0.001

AM – antimicrobial medication; NHSH – National Health Surveillance Network.
Values are given as numbers (percentages).
***Note*: [1] The questionnaire in the research trial used the option "unknown" for some data.**

Table 2

Prevalence of antimicrobial agent use by indication and route of administration at the University Clinical Center Kragujevac in the first week of November 2022

Variable	Patients	Prevalence (95% CI)
Total	246 (100)	43.4 (39.3–47.6)
Therapy		
community-acquired infections	135 (54.9)	24.2 (20.7–27.9)
healthcare-associated infection	47 (19.1)	8.5 (6.3–11.1)
total	182 (74.0)	32.5 (28.6–36.5)
Prophylaxis		
surgical	51 (20.7)	9.0 (6.8–11.7)
medical	9 (3.7)	1.6 (0.7–3.0)
[1] unknown	4 (1.6)	0.7 (0.2–1.8)
Route of administration		
parenteral	225 (89.6)	39.7 (35.6–43.8)
oral	25 (10.0)	4.4 (2.9–6.4)
[1] unknown	1 (0.4)	0.2 (0.0–1.0)
Reason for use noted		
yes	240 (97.6)	42.3 (38.2–46.5)

CI – confidence interval.
Values are given as numbers (percentages) or mean values (minimum–maximum).
***Note*: [1] The questionnaire in the research trial used the option "unknown" for some data.**

Parenteral administration constituted 89.6% of therapies for administering AM, whilst the oral route was utilized in 10.0% of therapies. The rationale for using AM was frequently recorded in the patient's medical documentation (97.6%) (Table 2).

Among the 246 individuals receiving AM, a total of 346 medications were administered, resulting in an average of 1.4 AM *per* patient. In the treatment of CAIs, categorized by anatomical localization, the most commonly prescribed antimicrobial agents were for respiratory tract infections (20.2%), urinary tract infections (15.3%), and clinical sepsis (14.6%). In the treatment of HAIs, the most commonly recommended medications were for respiratory tract infections (32.1%), surgical site infections (21.8%), and urinary tract infections (16.7%) (Table 3).

Antibacterial drugs for systemic use (ATC group J01) constituted the predominant category of AMs utilized (99.0%). The most commonly used AM group was third-generation cephalosporins (17.9%), followed by carbapenems (12.7%), glycopeptides (12.7%), and fluoroquinolones (11.8%). Among the antimicrobials prescribed for treating CAIs, the most frequently utilized were third-generation cephalosporins (22.2%), fluoroquinolones (14.6%), and carbapenems (12.6%). In contrast, for the

Table 3

Indications for the use of antimicrobial agents and prescribed classes of antibiotics, and antibiotics according to ATC 5th level classification

Variable	Therapy of infections n = 276 (79.8%)		Infection prophylaxis n = 66 (19.1%)		[1] Unknown n = 4 (1.1%)	Total n = 346 (100%)
	community-acquired infections n = 198 (71.7%)	healthcare-associated infection n = 78 (28.3%)	surgical prophylaxis n = 55 (83.3%)	medical prophylaxis n = 11 (16.7%)		
Indications						
surgical site infections	21 (10.6)	17 (21.8)	44 (80.0)	0 (0.0)	0 (0.0)	82 (23.7)
respiratory infections	49 (24.7)	25 (32.1)	0 (0.0)	0 (0.0)	0 (0.0)	74 (21.4)
urinary tract infections	40 (20.2)	13 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	53 (15.3)
clinical sepsis	29 (14.6)	10 (12.8)	0 (0.0)	0 (0.0)	0 (0.0)	39 (11.3)
cellulitis, wound, deep soft tissue infections	28 (14.1)	3 (3.8)	1 (1.8)	0 (0.0)	0 (0.0)	32 (9.2)
gastrointestinal infections	5 (2.5)	5 (6.4)	0 (0.0)	2 (18.2)	0 (0.0)	12 (3.5)
ear, nose, and throat infections	10 (5.1)	0 (0.0)	0 (0.0)	1 (9.1)	0 (0.0)	11(3.2)
gynecology and obstetrics	2 (1.0)	0 (0.0)	8 (14.5)	1 (9.1)	0 (0.0)	11(3.2)
laboratory-confirmed bacteremia	3 (1.5)	4 (5.1)	0 (0.0)	0 (0.0)	0 (0.0)	7 (2.0)
febrile neutropenia	4 (2.0)	0 (0.0)	0 (0.0)	1 (9.1)	0 (0.0)	5 (1.4)
intra-abdominal sepsis	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (1.2)
septic arthritis/osteomyelitis	0 (0.0)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
other/[1] unknown	3 (1.5)	0 (0.0)	2 (3.6)	6 (54.5)	4 (100.0)	15 (4.3)
Antibiotics						
penicillins with extended spectrum and combinations, including beta-lactamase inhibitors	9 (4.5)	0 (0.0)	0 (0.0)	1 (9.1)	0 (0.0)	10 (2.9)
beta-lactam sensitive penicillins	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.9)
first-generation cephalosporins	4 (2.0)	0 (0.0)	20 (36.4)	1 (9.1)	0 (0.0)	25 (7.2)
second-generation cephalosporins	3 (1.5)	0 (0.0)	8 (14.5)	0 (0.0)	1(25.0)	12 (3.5)
third-generation cephalosporins	44 (22.2)	8 (10.0)	7 (12.7)	3 (27.3)	0 (0.0)	62 (17.9)
fourth-generation cephalosporins	6 (3.0)	7 (9.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (3.8)
carbapenems	25 (12.6)	14 (17.9)	4 (7.3)	1 (9.1)	0 (0.0)	44 (12.7)
macrolides	9 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (2.6)
aminoglycosides	18 (9.1)	7 (9.0)	4 (7.3)	1 (9.1)	2 (50.0)	32 (9.2)
fluoroquinolones	20 (14.6)	5 (6.4)	5 (9.1)	1 (9.1)	1 (25.0)	41 (11.8)
glycopeptides	19 (9.6)	20 (25.6)	4 (7.3)	1 (9.1)	0 (0.0)	44 (12.7)
imidazole derivatives	13 (6.6)	7 (9.0)	3 (5.5)	0 (0.0)	0 (0.0)	23 (6.6)
colistin	4 (2.0)	6 (7.7)	0 (0.0)	1 (9.1)	0 (0.0)	11 (3.2)
antimycotics	1 (0.5)	2 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.9)
sulfamethoxazole and trimethoprim	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
clindamycin	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.6)
tetracyclines	2 (1.0)	2 (2.6)	0 (0.0)	1 (9.1)	0 (0.0)	5 (1.4)
others	6 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (1.7)

ATC – Anatomical Therapeutic Chemical.
Values are given as numbers (percentages).
***Note*: [1] The questionnaire in the research trial used the option "unknown" for some data.**

treatment of HAIs, the predominant agents were glycopeptides (25.6%) and carbapenems (17.9%). Over one-third of the drugs (36.4%) administered for surgical prophylaxis were first-generation cephalosporins (Table 3).

The predominant category of prescribed antimicrobials, as *per* the AWaRe categorization, was the Watch group, comprising 66.4%, followed by Access at 28.1%, and Reserve antibiotics at 5.5%. Furthermore, antibiotics were predominantly administered for both therapeutic and preventive objectives (Figure 1).

In multivariate logistic regression, the dependent variable was the use or absence of AM. All characteristics that were significant according to univariate logistic regression were included as independent variables. The results of multivariate logistic regression are shown in Table 4. Multivariate logistic regression identified the following independent risk factors for the use of AM: surgical intervention (OR = 1.501; 95% CI: 1.126–2.002; p = 0.006), placement of a urinary catheter (OR = 4.186; 95% CI: 2.562–6.841; p = 0.000), and presence of HAIs (OR = 60.239; 95% CI: 7.988–454.256; p = 0.000). The final binary logistic regression model demonstrated a satisfactory fit to the data: Hosmer and Lemeshow's test was 7.113 (p = 0.525), Cox and Snell's R-square was 0.251, and Nagelkerke's R-square was 0.370.

Discussion

Bacterial resistance to antibiotics poses a significant worldwide health challenge and endangers public health, as it affects treatment outcomes and escalates healthcare costs [8]. Since it is directly correlated with the use of AM, research of this type is a prerequisite for identifying targets and taking measures for their rational application.

The demographic structure of the patients included in this study shows that our results are consistent with the results of research at the national level [9]. Although men were more often represented in the group of patients who received AM, the difference was not statistically significant. Our results are similar to those from other studies [9, 10]. The findings indicate that the patient's age, length of hospital

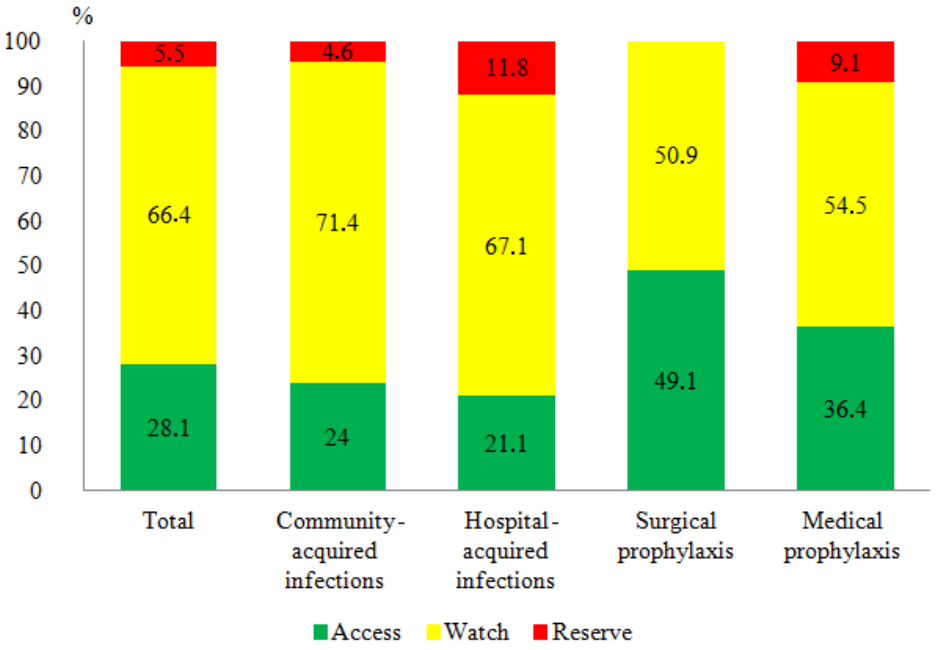

Fig. 1 – Antibiotic use according to the Access-Watch-Reserve classification.

Table 4

Multivariate logistic regression analysis for using antimicrobial agents

Variable	OR	95% CI		p
		lower	upper	
Age groups (years)	0.839	0.702	1.003	0.054
Length of hospital stay (days)	0.866	0.745	1.008	0.063
McCabe score	0.909	0.692	1.195	0.495
Department	0.998	0.893	1.116	0.979
Surgical intervention	1.501	1.126	2.002	0.006
Central venous catheter	0.922	0.378	2.253	0.859
Urinary catheter	4.186	2.562	6.841	0.000
Intubation	0.587	0.184	1.872	0.368
Healthcare-associated infection	60.239	7.988	454.256	0.000

OR – odds ratio; CI – confidence interval.

stay, and severity of illness (as measured by the McCabe score) were statistically significantly associated with more frequent use of AM. A longer hospital stay increases the likelihood that patients will acquire multiresistant nosocomial strains of various microorganisms. This can lead to the development of HAIs, which means that AM needs to be used. Additionally, various diagnostic and therapeutic methods, including surgery, vascular and urinary catheters, or intubation, are required. The processing of our study results indicated only the statistical association between the use of AM and these procedures.

In our study, 43.4% of patients received at least one antimicrobial agent (95% CI: 39.3–47.6), indicating that approximately one in two patients were treated with antibiotics during hospitalization. This prevalence exceeds that reported in the Third European study [11], which found that 35.5% of patients had received at least one antibiotic (with country-specific rates ranging from 20.8% to 56.5%). Following adjustments for the number of occupied hospital beds by country, the overall prevalence was estimated at 32.4% (95% CI: 29.7–35.1). This implies that 390,957 patients receive antibiotics daily in hospitals across the EU/EEA [11]. Studies conducted using the same methodology in various European countries reported comparable rates – for instance, Italy showed a prevalence of 42.1% (95% CI: 41.3–43.0) [12]. In contrast, Switzerland reported a notably lower prevalence of 27.6% (95% CI: 25.9–29.4) [13]. In certain regions of the world, there is a lower prevalence of antibiotic use. For instance, Li et al. [14] report a prevalence of 28.2% in Chinese hospitals, while Frenette et al. [15] indicate a prevalence of 34.0% in Canada.

The prevalence observed in our study is also slightly lower than that reported in the Fifth National Study [9], which found a rate of 44.8% (95% CI: 43.9–45.6). This means that 5,065 (95% CI: 4,765–5,369) patients receive at least one antibiotic every day in hospitals across the Republic of Serbia. The variability in prevalence rates among hospitals can be attributed to the distinct characteristics of individual institutions, such as the treatment of specific patient populations (e.g., surgical or pediatric patients), variations in medical practices, and differences in antimicrobial prescribing practices and the frequency of HAIs.

In this study, 74.0% of the patients received AM for medical reasons. Among these, 54.9% received antibiotics to address CAIs, whereas 19.5% utilized them for the treatment of HAIs. Other studies also indicate that infection treatment is the predominant reason for antibiotic administration in hospital settings [9, 14]. The elevated rate of antibiotic prescriptions for CAIs may be attributed to the demographic characteristics of these patients, who are typically older and often present with multiple comorbidities or compromised immune systems. They may have also received treatment for an infection prior to hospital admission. When all outpatient treatment options have been exhausted, patients are admitted to the hospital for continued care and antibiotic therapy requiring multiple daily parenteral administrations. Our study found that nearly 20% of patients were administered antibiotics for the treatment of HAIs. This indicates that healthcare workers must adhere more rigorously to infection prevention and control measures to reduce the reliance on antibiotics, which is essential in the strategy to prevent unnecessary antibiotic use.

The application of antimicrobial agents in surgical prophylaxis involves administering a single dose of an effective agent before surgery to minimize the risk of postoperative infections. In our study, 20.7% of patients received antimicrobial agents for surgical prophylaxis. However, it is significant that over half of the patients (52.9%) received these agents for more than one day without a clear indication. Nonetheless, this finding is relatively promising, given that a study conducted in Serbia five years earlier reported that over 70% of patients received surgical prophylaxis for more than one day [16]. Similar results have been reported in a related study [13]. Surgical prophylaxis must encompass the preoperative period, with a single dose of AM administered 60–120 min before the procedure, typically providing adequate protection against potential infection, barring instances of substantial blood loss or extended surgery. The rationale for extending surgical prophylaxis beyond one day is typically not substantiated [17]. The obtained result should be prioritized within our hospital to initiate measures that limit unnecessary prolongation of surgical prophylaxis, thereby enhancing the management of antimicrobial use in this patient population.

Contemporary antimicrobial administration practices favor oral routes over parenteral ones due to their reduced trauma to patients and lower treatment costs. Our study identified the parenteral route for antibiotic administration as the predominant choice (89.6%), aligning with findings from other researchers who report similar dominance in pediatric (93.3%) and adult patients (96.4%) [18]. Future efforts should prioritize transitioning to oral AM when the patient's condition allows.

A notable practice in our hospital is the thorough documentation of the reasons for prescribing AM in the patient's medical records (97.6%), a finding not observed in another similar study [18]. Documenting the rationale for initiating antibiotics enables all physicians involved in patient care to consistently review and assess the appropriateness of their use or duration.

Our study found that most of the AMs used to treat CAIs were prescribed for respiratory tract infections (24.7%), urinary tract infections (20.2%), and clinical sepsis (14.6%), which aligns with expectations. In the treatment of HAIs, the predominant indications for prescribing antibiotics were respiratory tract infections (32.1%), surgical site infections (21.8%), and urinary tract infections (16.7%). This distribution aligns with trends observed in other hospitals, although the ranking may differ.

In the management of CAIs, third-generation cephalosporins (22.2%), fluoroquinolones (14.6%), and carbapenems (12.6%) were the most commonly used antibiotics. In contrast, for HAIs, glycopeptides (25.6%) and carbapenems (17.9%) were the predominant antibiotics employed, indicating a reliance on broad-spectrum agents. This pattern of antibiotic use may be associated with the

emergence and dissemination of *C. difficile* and other multidrug-resistant bacteria linked with healthcare within our hospital. The prevalence of AM use in our hospital differs from that reported in hospitals across other European countries. Plachouras et al. [19], in their analysis of data from the Second study on AM use in European countries, reported that the most commonly prescribed antibiotics belonged to the penicillin combinations group, which includes beta-lactamase inhibitors (J01CR). Recent findings from the Third European Antibiotic Use Prevalence Study [11] indicate that other beta-lactam antibiotics (J01D) were the most frequently used, accounting for 35.6% of total antibiotic consumption. Among these, third-generation cephalosporins were the most prevalent (44.4%), followed by carbapenems (19.0%), second-generation cephalosporins (17.5%), first-generation cephalosporins (16.7%), and fourth-generation cephalosporins (1.4%). This study indicates that penicillins (J01C) represent the second most frequently utilized class of antibiotics, comprising 30.0%. Furthermore, combinations of penicillins, which include beta-lactamase inhibitors (J01CR), constitute 71.8% of total penicillin usage. A study on antimicrobial use in Greece [20] found that the most prescribed agents were penicillin combinations with beta-lactamase inhibitors (16.2%), followed by second-generation cephalosporins (10.9%), glycopeptides (9.4%), fluoroquinolones (9.2%), and carbapenems (8.3%). Sevin et al. [21] reported that in French hospitals, amoxicillin-clavulanic acid (27.6%) was the most frequently prescribed antibiotic for hospital-acquired pneumonia. For urinary tract infections, the most commonly prescribed antibiotics were ofloxacin (13.9%), amoxicillin (13.6%), and amoxicillin-clavulanic acid. In Italy, penicillin combinations, which include beta-lactamase inhibitors, were prescribed most frequently at a rate of 35.0% [22]. The utilization rates of these antibiotics in German hospitals were nearly identical, with 33.2% for the first category, followed by third-generation cephalosporins at 9.5% and second-generation cephalosporins at 9.1% [23]. The extensive use of broad-spectrum antibiotics necessitates a reevaluation of their indications and the implementation of more stringent management programs that address the risks of antimicrobial resistance and prioritize patient safety. A crucial component of an effective strategy to combat increasing resistance is the appropriate use and stringent regulation of broad-spectrum antimicrobials [24].

This study reveals that first-generation cephalosporins constituted 36.4% of the antimicrobial agents used in surgical prophylaxis, whereas second-generation cephalosporins represented 14.5%, and third-generation cephalosporins accounted for 12.7%. Cefazolin, a first-generation cephalosporin, is the preferred agent for providing sufficient coverage against the majority of pathogens responsible for postoperative infections. It rarely causes allergic reactions or side effects, achieves sufficient tissue concentrations, and is cost-effective, making it the optimal choice for prophylaxis in most surgical procedures [17].

According to the AWaRe classification, the majority of prescribed antibiotics belonged to the Watch group (66.4%), while the Access group accounted for less than one-third of all prescriptions (28.1%), and the Reserve group accounted for 5.5%. Comparable findings were reported in a recent study [12], where 71.8% of prescribed antibiotics were categorized as Watch, 19.6% as Access, and 8.6% as Reserve antibiotics. The AWaRe classification for measuring antibiotic consumption offers significant insights into the quality of antibiotic utilization in a specific context. The World Health Organization recommends that by 2023, at least 60% of all antibiotics nationally prescribed fall within the Access group. This group includes narrow-spectrum antibiotics that have a low potential for resistance and a favorable safety profile. In contrast, antibiotics in the Watch and Reserve groups are intended for the treatment of severe clinical manifestations or infections caused by pathogens resistant to other antibiotics, with the aim of mitigating the increasing issue of antibiotic resistance [25]. The data suggest that policymakers regarding antibiotic use in our hospital will encounter considerable challenges in the near future.

In the evaluation of risk factors associated with AM usage, univariate analysis indicated significance for various demographic characteristics and medical procedures during current hospitalizations. However, multivariate analysis identified three specific risk factors: surgical intervention, placement of a urinary catheter, and the presence of HAIs. These established risk factors are linked to the incidence of HAI, suggesting that these patients are likely to have increased exposure to various AM. Available studies indicate an association between secondary healthcare facilities and a potential risk factor for AM, attributed to insufficient diagnostic or interventional resources, along with antibiotic stewardship programs [20, 26].

A limitation of this study primarily arises from its design as a prevalence study, which involved data collection on a single day, potentially compromising the representativeness of the findings. Incidence studies yield more reliable data; however, they are time-consuming, resource-intensive, and consequently costlier. Additionally, our hospital, as a tertiary-level healthcare facility, manages more complex cases of patients, thereby enhancing the probability of AM utilization. Despite certain limitations, the data obtained provide valuable insights into AM use and can serve as a baseline indicator for managing their application.

Conclusion

This prevalence study provided valuable information on antimicrobial medication consumption in a tertiary-level healthcare facility. The high prevalence highlights the need for implementing antibiotic stewardship measures to maintain the long-term effectiveness of antimicrobial medication and ensure our ability to treat infections in the future. Healthcare workers must collaborate closely with policymakers at all levels of the healthcare system to promote the rational and responsible use of antibiotics and help combat antimicrobial resistance.

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Correlations of soluble TRAILR4, white blood cell count, and 25-hydroxyvitamin D with acute upper respiratory infection in children

Korelacije solubilnog TRAILR4, broja belih krvnih zrnaca i 25-hidroksivitamina D i akutne respiratorne infekcije gornjih disajnih puteva kod dece

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Abstract

Background/Aim. Rapid diagnosis and treatment guidance for acute upper respiratory infection (AURI) are challenging due to difficulties in specimen collection and the need for advanced laboratory conditions. The aim of this study was to investigate the correlations between soluble tumor necrosis factor-related apoptosis-inducing ligand receptor 4 (sTRAILR4), white blood cell (WBC) count, and 25-hydroxyvitamin D [25(OH)D] with AURI in children. **Methods.** A total of 80 children with AURI treated from January 2022 to January 2023 were enrolled in the study group. Another 80 healthy children undergoing physical examinations during the same period were enrolled in the control group. The levels of serum sTRAILR4 and 25(OH)D, and WBC count were measured. Receiver operating characteristic (ROC) curves were plotted to analyze the predictive values of serum sTRAILR4 and 25(OH)D levels and WBC count for AURI. Unconditional logistic stepwise regression analysis was employed for multivariate analysis. **Results.** Compared with the control group, the study group had a significantly elevated level of sTRAILR4 and WBC count and a reduced level of 25(OH)D ($p < 0.05$). The areas under the ROC curves of sTRAILR4 and 25(OH)D levels, and WBC count were 0.893, 0.765, and 0.937, respectively, suggesting that they were predictors of AURI. An elevated level of sTRAILR4 (≥ 88.751 pg/mL), elevated WBC count (≥ 10.223×10^9/L), along with a reduced level of 25(OH)D (≤ 48.943 nmol/L), were identified as significant influencing factors for AURI ($p < 0.05$). The level of sTRAILR4 and WBC count were positively correlated with AURI, while the 25(OH)D level had a negative correlation ($p < 0.05$). **Conclusion.** The children with AURI have a significantly higher level of sTRAILR4 and WBC count and a lower level of 25(OH)D than healthy children. In addition to WBC, sTRAILR4 and 25(OH)D may have a role in the assessment of pediatric upper respiratory infection.

Key words:
child; leukocytes; respiratory tract infections; tumor necrosis factor – alpha; vitamin d.

Apstrakt

Uvod/Cilj. Brza dijagnoza i smernice za lečenje akutne infekcije gornjih disajnih puteva (*acute upper respiratory infection* – AURI) predstavljaju izazov zbog teškoća u prikupljanju uzoraka i potrebe za visokospecijalizovanim laboratorijama. Cilj rada bio je da se ispitaju korelacije između solubilnog receptora 4 apoptoza-indukujućeg liganda povezanog sa faktorom nekroze tumora (*soluble tumor necrosis factor-related apoptosis-inducing ligand receptor 4* – sTRAILR4), broja belih krvnih zrnaca (*white blood cell* – WBC) i 25-hidroksivitamina D –[25(OH)D] i AURI kod obolele dece. **Metode.** Ukupno 80 dece obolele od AURI lečene od januara 2022. do januara 2023. godine, uključeno je u ispitivanu grupu. Još 80 zdrave dece koja su u istom periodu bila podvrgnuta fizičkim pregledima uključeno je u kontrolnu grupu. Izmereni su nivoi sTRAILR4 i 25(OH)D u serumu i broj WBC. Konstruisane su *receiver operating characteristic* – ROC krive kako bi se analizirale prediktivne vrednosti nivoa sTRAILR4 i 25(OH)D u serumu i broja WBC za AURI. Za multivarijantnu analizu korišćena je bezuslovna logistička regresija *stepwise* metodom. **Rezultati.** U poređenju sa kontrolnom grupom, ispitivana grupa je imala značajno povišen nivo sTRAILR4 i broj WBC i smanjen nivo 25(OH)D ($p < 0,05$). Površine ispod ROC kriva nivoa sTRAILR4 i 25(OH)D i broja WBC bile su 0,893, 0,765, 0,937, redom, što ukazuje na to da su prediktori AURI. Povišeni nivo sTRAILR4 (≥ 88,751 pg/mL), broj WBC (≥ $10,223 \times 10^9$/L), zajedno sa smanjenim nivoom 25(OH)D

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(≤ 48,943 nmol/L) identifikovani su kao značajni faktori koji utiču na AURI ($p < 0,05$). Nivo sTRAILR4 i broj WBC bili su u pozitivnoj korelacji sa AURI, dok je nivo 25(OH)D imao negativnu korelaciju ($p < 0,05$). **Zaključak.** Deca obolela od AURI imaju značajno viši nivo sTRAILR4 i broj WBC i niži nivo 25(OH)D, u odnosu na zdravu decu. Pored WBC, sTRAILR4 i 25(OH)D mogu imati ulogu u proceni infekcije gornjih disajnih puteva u pedijatrijskoj populaciji.

Ključne reči:
deca; leukociti; respiratorni trakt, infekcije; faktor nekroze tumora – alfa; vitamin d.

Introduction

Infectious diseases (IDs) of the upper respiratory tract include rhinitis, sinusitis, nasopharyngitis, the common cold, and laryngitis [1]. These conditions typically present as acute self-limited defensive responses occurring within 1–3 days after exposure to viruses, characterized by inflammatory symptoms such as coughing, sneezing, runny nose, nasal obstruction, and sore throat. IDs of the upper respiratory tract are the most frequent reason for hospitalization in children under 5 years of age, if not treated in time [2]. The diseases can be exacerbated through pathogen invasion into the sinuses, trachea, lungs, and other adjacent organs, thereby increasing the difficulty of treatment [3]. Acute upper respiratory infection (URI) – AURI is mainly diagnosed based on clinical findings; however, rapid diagnosis and treatment guidance are challenging due to difficulties in specimen collection and the high demands of laboratory testing [4]. Hence, it would be useful to have alternative approaches to confirm AURI.

White blood cell (WBC) count is a commonly used indicator in the diagnosis of IDs [5]. Although an elevated WBC count may signal an ongoing immune response, its low specificity restricts its ability to distinguish between different types of pathogens or the severity of the infection [6]. Soluble tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor 4 – sTRAILR4, an inflammatory factor receptor in the TNF family, participates in the progression of inflammation in IDs [7]. Although most studies of sTRAILR4 have focused on chronic inflammatory conditions [8], its potential role in the immune response during acute infections needs further investigation. An important indicator of vitamin D nutritional status, 25-hydroxyvitamin D [25(OH)D], has immune-modulating and immune response functions and plays a role in systemic inflammatory responses [9]. It has been demonstrated that sufficient levels of 25(OH)D, or supplementation in deficient individuals, are associated with lower risk and reduced severity of acute respiratory tract infections [10]. Until now, however, reports on WBC count and levels of sTRAILR4 and 25(OH)D in children with AURI, as well as their correlations with the disease, have been limited.

The aim of this study was to investigate the correlations between levels of sTRAILR4 and 25(OH)D and WBC count with AURI in children, in order to provide valuable evidence for future treatment.

Methods

A total of 80 children with AURI treated from January 2022 to January 2023 were enrolled in the study group. According to the diagnostic criteria for AURI [8], 22 cases were mild, 41 were moderate, and 17 were severe. Another 80 healthy children undergoing physical examinations during the same period were enrolled in the control group. The study was approved by the Ethics Committee of the Ningbo Yinzhou No. 2 Hospital, China (approval No. NYN2H202201003), and informed consent was obtained from all participating family members.

The inclusion criteria were as follows: children who met the diagnostic criteria for URI [11] and were in the acute stage, those with bacterial infections, and those aged ≤ 12 years.

The exclusion criteria involved children with the following: allergic diseases; malignancies; those with contraindications to pulmonary function tests; those with cardiovascular, central nervous system, connective tissue, metabolic, or endocrine diseases; those with infections at other sites; those receiving medications affecting the level of serum 25(OH)D; those who took hormones, immunotherapy, or vitamins two weeks before examination.

Fasting venous blood (6 mL) was drawn from each child in both groups from 8:00 am to 10:00 am and collected into Eppendorf Tubes® (Germany). Then, half of that dose, i.e., 3 mL of blood, was used for WBC detection using an automated hematology analyzer (Sysmex Shanghai Ltd., China). The remaining 3 mL of blood was centrifuged at 3,000 rpm for 15 min, and the serum was separated and stored at -80 °C for analysis.

Serum sTRAILR4 level was measured by UniCel DxI 800 immunoassay analyzer (Beckman Coulter, USA) using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Shanghai Xinyu Biotechnology Co., Ltd., USA). Serum 25(OH)D level was measured using the iBright electrochemiluminescence immunoassay system (Thermo Fisher Scientific, USA) with a commercial kit, following the manufacturer's instructions (DiaSorin Inc., USA). Quality control and calibration products were bought from Randox (UK). Quality control indicators such as intra-batch variability and inter-batch variability met the requirements.

Statistical analysis

All data were processed using SPSS 22.0 software. The measurement data were subjected to the Kolmogorov-Smirnov test, and the normally distributed data were expressed as mean ± standard deviation and compared by the independent samples *t*-test between the two groups. The count data were expressed as percentages and compared using the χ^2 test between groups. Receiver operating character-

istic (ROC) curves were plotted to analyze the predictive values of serum sTRAILR4 and 25(OH)D levels and WBC count for AURI. Multivariate analysis was conducted by unconditional logistic stepwise regression analysis. The correlations of sTRAILR4, WBC count, and 25(OH)D with AURI were detected through Spearman's rank analysis. The value of $p < 0.05$ suggested a statistically significant difference.

Results

A total of 80 children with AURI were selected as a study group, including 45 boys and 35 girls. Their age was 2–12 years (mean: 7.72 ± 2.86 years). The course of disease was 3–15 days (average: 6.24 ± 2.12 days). The body mass index (BMI) was 11.31–17.75 kg/m^2, with a mean of 14.25 ± 2.17 kg/m^2. Another 80 healthy children receiving physical examinations in the same period were enrolled as a control group, including 49 boys and 31 girls. They were aged 2–12 years (mean: 7.16 ± 3.13 years). BMI was 11.28–17.68 kg/m^2 (average: 14.16 ± 2.25 kg/m^2). The baseline data, such as gender, age, and BMI, showed no statistically significant differences between the two groups ($p > 0.05$).

Levels of sTRAILR4 and 25(OH)D and WBC count

The baseline data, such as gender, age, and BMI, showed no statistically significant differences between the two groups ($p > 0.05$). Compared with the control group, the study group had a significantly higher sTRAILR4 level and WBC count and a significantly lower 25(OH)D level ($p < 0.05$) (Table 1).

Results of ROC curve analysis of sTRAILR4 and 25(OH)D levels and WBC count for predicting AURI

The results of the ROC curve analysis revealed that the areas under the curves (AUCs) of sTRAILR4 and 25(OH)D levels and WBC count, were 0.893, 0.765 and 0.937 (Table 2), indicating that sTRAILR4 and 25(OH)D levels and WBC count can act as predictors of AURI. The ROC curves of sTRAILR4 and 25(OH)D levels and WBC count for predicting AURI are shown in Figure 1.

Table 1

Comparison of sTRAILR4 and 25(OH)D levels and WBC count between the two groups of patients

Parameters	sTRAILR4 (pg/mL)	WBC (×10^9/L)	25(OH)D (nmol/L)
Study group (n = 80)	114.28 ± 40.25	16.35 ± 5.82	41.58 ± 15.65
Control group (n = 80)	59.62 ± 18.94	7.24 ± 2.19	57.34 ± 16.37
t	10.974	13.103	6.224
p	< 0.001	< 0.001	< 0.001

sTRAILR4 – soluble tumor necrosis factor-related apoptosis-inducing ligand receptor 4; WBC – white blood cell; 25 (OH)D – 25-hydroxyvitamin D; n – number.
All values are given as mean ± standard deviation.

Table 2

Results of the ROC curve analysis of sTRAILR4 and 25(OH)D levels and WBC count for predicting acute upper respiratory infection

Variables	AUC	SE	*p*	95%CI	Cut-off	Sensitivity	Specificity
sTRAILR4	0.893	0.027	< 0.001	0.835~0.936	88.751 pg/mL	0.962	0.737
WBC	0.937	0.022	< 0.001	0.887~0.969	10.223 × 10^9/L	0.937	0.675
25(OH)D	0.765	0.038	< 0.001	0.691~0.828	48.943 nmol/L	0.787	0.712

ROC – receiver operating characteristic; AUC – area under the curve; SE – standard error; CI – confidence interval. For other abbreviations, see Table 1.

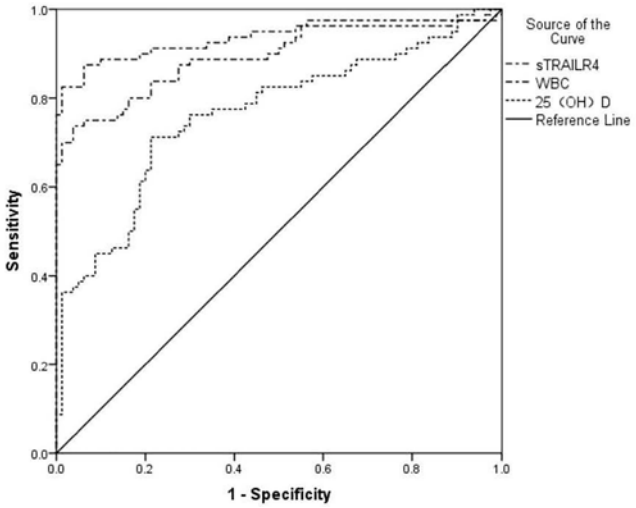

Fig. 1 – ROC curves of sTRAILR4 and 25(OH)D levels and WBC count for predicting acute upper respiratory infections. For abbreviations, see Tables 1 and 2.

Results of multivariate logistic regression analysis of AURI

The significantly different data from the univariate analysis were incorporated as the independent variables in the multivariate logistic regression analysis, with AURI as the dependent variable, followed by variable assignment for subsequent multivariate logistic regression analysis (Table 3). The results showed that sTRAILR4 (≥ 88.751 pg/mL), WBC count (≥ 10.223 × 10^9/L), and 25(OH)D (≤ 48.943 nmol/L) were influencing factors for AURI ($p < 0.05$) (Table 4 and Figure 2).

Correlations of sTRAILR4 and 25(OH)D levels and WBC count with AURI

The level of sTRAILR4 and WBC count were positively correlated with AURI, whereas the 25(OH)D level had a negative correlation with AURI ($p < 0.05$) (Table 5).

Table 3

Quantitative assignments for multivariate logistic regression analysis

Parameters	Assignment	
	1	0
sTRAILR4	≥ 88.751 pg/mL	< 88.751 pg/mL
WBC	≥ 10.223 × 10^9/L	< 10.223 × 10^9/L
25(OH)D	≤ 48.943 nmol/L	> 48.943 nmol/L

For abbreviations, see Table 1.

Table 4

Results of multivariate logistic regression analysis of acute upper respiratory infection

Factor	β	SE	Wald χ^2	*p*	OR	95%CI
sTRAILR4	1.211	0.303	15.918	< 0.001	3.358	1.852~6.088
WBC	1.301	0.442	8.630	0.003	3.674	1.542~8.753
25(OH)D	1.322	0.475	7.739	0.005	3.752	1.478~9.524

For abbreviations, see Tables 1 and 2, and Fig. 2.

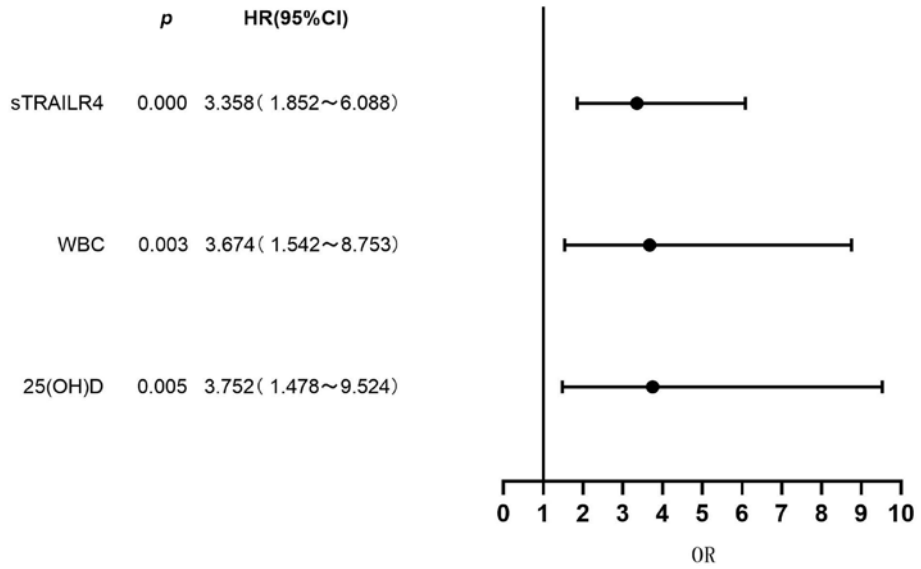

Fig. 2 – Forest plot of clinical characteristics based on multivariate logistic regression analysis. HR – hazard ratio; CI – confidence interval; OR – odds ratio. For other abbreviations, see Table 1.

Table 5

Correlations of sTRAILR4 and 25(OH)D levels and WBC count with acute upper respiratory infection

Parameters	Acute upper respiratory tract infection	
	r	*p*
sTRAILR4	0.643	< 0.05
WBC	0.582	< 0.05
25(OH)D	-0.517	< 0.05

For abbreviations, see Table 1.

Discussion

Pediatric AURI has a high incidence rate among children aged ≤ 10 years due to tonsillitis, laryngitis, and sinusitis. AURI caused by viruses has no routinely available diagnostic tests [12]. Bacterial culture of throat swabs is mainly employed for the diagnosis of acute bacterial URI, which, however, is time-consuming and not conducive to early diagnosis and treatment [13]. In addition, pediatric diseases often progress rapidly, making it difficult for pediatricians to assess the potential severity of an infectious disease based on initial symptoms alone. This greatly impacts the clinical decision-making process and can affect patient outcomes [14]. Therefore, it is necessary to explore the correlations of potential indicators with pediatric AURI in order to provide evidence for determining the onset and progression.

Blood indicators have been widely applied in the diagnosis of pediatric IDs in recent years, as laboratory equipment and testing techniques constantly improve [15]. The levels of sTRAILR4 and 25(OH)D and WBC count, in human peripheral blood, can be quickly and accurately detected. WBC count is one of the oldest laboratory markers of URI in use, as an important functional parameter of the immune system. It can reflect the inflammatory status and play a key role in the early diagnosis of IDs and medication guidance. However, WBC detection has low accuracy due to the influence of various external factors and is therefore often used in combination with other serum indicators for disease diagnosis in clinical practice [16]. In this study, WBC count showed a significant correlation with AURI, suggesting that elevated WBC values were associated with the onset and progression of the infection. In the case of bacterial URI, the WBC count is high, but its detection is not specific. For instance, it is also high in mycoplasma-induced URI, but there is no statistically significant difference between patients with viral URI and healthy populations. Thus, WBC count has high sensitivity but poor specificity in the assessment of IDs of the upper respiratory tract [6, 17].

As a receptor of TRAIL, sTRAILR4 is also an apoptosis-inducing ligand, which belongs to the TNF superfamily together with TNF-α. It is involved in the inflammatory processes of IDs, enhancing the immune response and anti-infective ability of the human body. Moreover, it can competitively suppress TRAIL-induced apoptosis through its extracellular structural domain and promote the activation of anti-apoptotic transcription factors [18]. In this study, the sTRAILR4 level was significantly higher in children with AURI than in healthy children. In a previous study [19], a higher expression of sTRAILR4 meant higher risk and faster progression of upper respiratory tract infection, similar to the results of the present study.

Through macrophages and T lymphocytes, 25(OH)D is capable of regulating the division and proliferation of WBC, thus potentially affecting the efficiency of an immune response [20]. Besides, 25(OH)D can also enhance the resistance of the human body to infection by promoting the expression of antimicrobial dopamine and Toll-like receptors and strengthening the innate defense of the immune system against viruses and bacteria [21]. Moreover, 25(OH)D has been closely related to autoimmune diseases, IDs, cardiovascular diseases, neuropsychiatric diseases, and even tumors, in addition to its traditional effects on the skeletal system [22]. In this study, the 25(OH)D level was significantly lower in the study group than in the control group. Additionally, there was a significant correlation between URI and 25(OH)D level, suggesting that the level was valuable for predicting URI. Jolliffe et al. [23] reported that 25(OH)D was of great significance to the diagnosis of respiratory tract infection, and the level rose significantly after treatment, which is in agreement with the results of the present study.

Nevertheless, this study had several limitations. First, the sample size was relatively small. In addition, subgroup analyses were not performed for specific diagnoses such as pharyngitis, rhinitis, and others. Moreover, the analysis focused exclusively on acute bacterial URI, without including cases caused by viral or mycoplasma infections. In future studies, the sample size will be expanded, and a comprehensive analysis will be conducted to examine the correlations between AURIs caused by various agents and the levels of sTRAILR4 and 25(OH)D and WBC count.

Conclusion

Children with acute upper respiratory infection tend to have relatively high levels of sTRAILR4 and white blood cell count and low 25(OH)D levels. Markers such as sTRAILR4, WBC, and 25(OH)D may be useful in assessing upper respiratory infection in children.

Conflict of interest

The authors declare no conflict of interest.

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Immunohistochemical analysis of IDH1, ATRX, p53, and Ki-67 in glioblastoma and diffuse infiltrative glioma: therapeutic and prognostic correlation

Imunohistohemijska analiza IDH1, ATRX, p53 i Ki-67 kod glioblastoma i difuznog infiltrativnog glioma: terapijska i prognostička korelacija

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Abstract

Background/Aim. The most common molecular alterations in high-grade astrocytoma include mutation of the isocitrate dehydrogenase (*IDH*) gene, loss of 1p19q, and *p53* mutation. The aim of the study was to determine the prevalence of high-grade astrocytoma and glioblastoma and to examine the immunohistochemical staining patterns of IDH1, alpha-thalassemia/mental retardation X-linked (ATRX), p53, and Ki-67, as well as neoplastic morphological findings, treatment response, and effects on prognosis. **Methods.** Patients with *IDH*-mutant or *IDH*-wild-type glial tumors diagnosed at our center between January 2016 and January 2022 were included in the study. Patients were divided into groups according to age as follows: 7–40, 41–55, 56–64, and ≥ 65 years. The impact of demographic and clinical features on survival was analyzed. The effects of IDH1, p53, ATRX, and Ki-67 parameters on treatment success and prognosis were investigated. The Chi-square test was used to compare independent categorical variables, while the McNemar test was used for dependent categorical variables between the groups. Kaplan-Meier method and Cox proportional regression model (forward model) were used to estimate the mean and median survival times, failure rates, and hazard ratios. **Results.** In the study, 115 (56.1%) patients were male and 90 (43.9%) were female. The patients ranged in age from 7 to 84 years. There was no significant relationship between gender and age groups on survival (p = 0.113). However, there was a significant association between the glioblastoma grade and survival (p = 0.024). There were 65 (31.7%) patients who died. The mean overall survival of all patients was 45.2 months (median: 24 months). While 45 (21.2%) patients were found to have *IDH1* mutation, the number of patients negative for the mutation was 160 (78.8%). Overall survival was significantly longer in *IDH1*-positive patients (mean: 65.8, median: 80) than in *IDH1*-negative patients (mean: 25.7, median: 22) (p = 0.019). **Conclusion**. It was found that mutations of *IDH1* and *ATRX* and overexpression of p53 alone significantly impacted the prognosis of glioblastoma patients. However, radiotherapy and chemotherapy had a positive effect on patient survival. Survival can be increased by adding additional treatments to patients with *ATRX* mutations.

Key words:
astrocytoma; glioblastoma; ımmunohistochemistry; mutation; neoplasm grading; survival.

Apstrakt

Uvod/Cilj. Najčešće molekularne promene kod astrocitoma visokog stepena uključuju mutaciju gena izocitrat dehidrogenaze (*IDH*), gubitak 1p19q i mutaciju *p53*. Cilj rada bio je da se utvrde prevalencija astrocitoma visokog stepena i glioblastoma, i da se ispitaju obrasci imunohistohemijskog bojenja IDH1, *alpha-thalassemia/mental retardation X-linked* (ATRX), p53 i Ki-67, kao i morfološki nalazi neoplazije, odgovor na lečenje i efekti na prognozu. **Metode.** Studijom su obuhvaćeni bolesnici sa *IDH* mutiranim ili *IDH-wild-type* glijalnim tumorima, dijagnostikovanim u našoj ustanovi od januara 2016. do januara 2022. godine. Bolesnici su bili podeljeni u grupe prema starosti: 7–40, 41–55, 56–64 i ≥ 65 godina. Analiziran je uticaj demografskih i kliničkih osobina bolesnika na njihovo preživljavanje. Ispitivani su efekti parametara IDH1, p53, ATRX i Ki-67 na uspešnost lečenja i prognozu. Za poređenje nezavisnih kategorijalnih varijabli korišćen je *Chi-square* test, dok je za zavisne kategorijalne

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varijable između grupa korišćen McNemar-ov test. Kaplan-Meier i Cox proporcionalni regresioni model (*forward* model) su korišćeni za procenu vremena preživljavanja [srednja vrednost (SV) i medijana (M)], stopa neuspeha i stepeni rizika (*hazard ratios*). **Rezultati**. U studiji je bilo 115 muškaraca (56,1%) i 90 žena (43,9%). Bolesnici su bili životnog doba od 7 do 84 godine. U pogledu preživljavanja, nije utvrđena značajna povezanost između grupa formiranih na osnovu pola i starosti bolesnika (p = 0,113). Međutim, postojala je značajna povezanost između stepena glioblastoma i preživljavanja (p = 0,024). Preminulo je 65 (31,7%) bolesnika. Srednje ukupno preživljavanje svih bolesnika iznosilo je 45,2 meseca (M: 24 meseca). Dok je 45 bolesnika (21,2%) imalo *IDH1* mutaciju, bez mutacije je bilo 160 bolesnika (78,8%). Ukupno preživljavanje bilo je značajno duže kod *IDH1* pozitivnih bolesnika (SV: 65,8 meseci; M: 80 meseci), nego kod *IDH1* negativnih bolesnika (SV: 25,7 meseci; M: 22 meseca) (p = 0,019). **Zaključak.** Otkriveno je da mutacije *IDH1* i *ATRX* i prekomerna ekspresija samog p53 značajno utiču na prognozu kod bolesnika sa glioblastomom. Međutim, radioterapija i hemoterapija imale su pozitivan efekat na preživljavanje bolesnika. Preživljavanje bolesnika sa *ATRX* mutacijama se može povećati dodavanjem dodatnih tretmana.

Ključne reči:
astrocitom; glioblastoma; imunohistohemija; mutacija; neoplazme, određivanje stadijuma; preživljavanje.

Introduction

Diffuse glial tumors account for 15–20% of all central nervous system (CNS) tumors and 80% of high-grade CNS tumors [1–3]. High-grade astrocytoma (HGA), particularly glioblastoma (GB), classified as World Health Organization (WHO) grade 4, is one of the most aggressive and poorly prognostic primary brain tumors. GB is the most common malignant glial tumor in adults [4–6]. Although morphology is important based on the new classification system, classification is based on molecular features in the case of incompatibility between histological and molecular features [7]. The most common molecular alterations in gliomas include mutations in the isocitrate dehydrogenase (IDH) gene, loss of 1p19q, and *p53* mutations. According to the 2021 WHO classification, all *IDH*-wild-type gliomas are classified as GB, while *IDH*-mutated high-grade gliomas are classified as grade 4 astrocytoma. With this classification, GB accounts for 15% of all brain tumors and 45% of malignant brain tumors [8].

Three different IDH enzymes convert isocitrate to alpha-ketoglutarate by oxidative decarboxylation. More than 90% of IDH mutations in gliomas affect the *IDH1* gene, with the remainder affecting the *IDH2* gene [9]. Glioma formation begins with an *IDH* mutation at an early stage and continues with a *p53* mutation in astrocytomas and loss of 1p19q in oligodendrogliomas [10, 11].

IDH1 and *IDH2* mutations have emerged as early mutations in diffuse astrocytic tumors, and determination of IDH status by immunohistochemical (IHC) staining or sequencing has become a standard in diagnosing these tumors [12]. Approximately 80% of adult grade 2/3 gliomas carry *IDH1* or *IDH2* mutations [13]. The presence of an *IDH* mutation is more important than histologic grade and other molecular features in determining prognosis in high-grade gliomas (grade 3/4) [14]. In studies, *IDH* mutation is observed in 54–100% of diffuse astrocytomas (WHO grade 2), 66.1% of anaplastic astrocytomas (WHO grade 3), and 64–93% of oligodendrogliomas. In addition, mutations are present in 50–88% of secondary GB and only 5% of primary GB [4, 11, 15].

IDH mutation is the strongest prognostic factor in gliomas, and studies show that tumors with *IDH* mutation have a better prognosis than those without *IDH* mutation (wild-type) [4, 11, 12, 16]. Moreover, another observation is that morphologically low-grade gliomas with *IDH*-wild-type behave like GB [11]. Indeed, studies have shown that an *IDH* mutation is a stronger predictor of average survival than a histologic grade [14, 15]. Based on these studies, *IDH*-negative tumors are classified as GB regardless of their histologic features. Positive tumors are classified as oligodendrogliomas or astrocytomas with *IDH* mutations (grades 2–4) according to the detection of 1p19q [8].

The tumor suppressor gene *p53* is located on the short arm of chromosome 17, and its mutations cause the continuous production of the p53 protein and accumulation in cells [17]. Although widespread and strong staining of gliomas with IHC p53 staining is indicative of a *p53* mutation, the sensitivity of this method varies among studies. In studies in which the threshold was set at 10% of tumor cells, the sensitivity of the p53 IHC method ranged from 77% to 91%, and the specificity ranged from 78% to 92% [18].

Alpha-thalassemia/mental retardation syndrome X-linked (ATRX) gene mutations were reported in 70–75% of grade 2 and 3 astrocytomas, 68% of oligoastrocytomas, and 57% of secondary GB, while it is rarely observed in primary GB [4, 11, 19]. *ATRX* mutations were first described in pediatric and adult GB in 2011 [11, 20]. While the *ATRX* mutation is observed in 90% of *IDH*-mutated astrocytomas, it is rare in *IDH*-wild-type GB [15, 21, 22].

A high Ki-67 labeling index in GB is an important indicator of malignancy. Levels of Ki-67 ≥ 20% are associated with more rapid tumor growth and poorer prognosis [23].

IHC staining is frequently used in neuropathology to indirectly demonstrate molecular changes because they are generally cheaper, more widely available, and provides faster results than molecular tests. IHC staining can reveal the absence of protein synthesis due to nonsense gene mutations or homozygous gene losses. Tumors with concurrent *IDH1* mutation and *ATRX* loss are typically associated with a more favorable prognosis and longer overall survival (OS) compared to GB patients with *IDH*-wild-type status.

The aim of this study was to determine the prevalence of HGA and GB diagnosed at our hospital, as well as to

analyze the IHC patterns of IDH1, ATRX, p53, and Ki-67, alongside neoplastic morphological findings, treatment response, and impact on prognosis.

Methods

This study was approved by the Baskent University Institutional Review Board (Project No. KA22/157, from March 29, 2022) and supported by the Baskent University Research Fund.

Parameters IDH1, p53, ATRX, and Ki-67 have been routinely used for the diagnosis of glial tumors in our center since 2016. Our study enrolled patients diagnosed with *IDH*-mutated or *IDH*-wild-type glial tumors between January 2016 and January 2022. Demographic and clinical features of the patients were analyzed. All variables were recorded at the time of enrollment in the study.

Sections from formalin-fixed paraffin-embedded tissue blocks were subjected to IHC analysis using a Leica/Bond-Max (Australia) automated immunostainer. Following our daily diagnostic and research practices, we used 4 μm-thick sections. IHC reactions were performed using the streptavidin-biotin-peroxidase complex method with antibodies listed in Table 1. Positive and negative IHC controls were used in all cases. Tumors exhibiting strong nuclear staining in more than 10% of cells were considered positive for p53 [18]. The Ki-67 proliferation index was determined by counting positively stained cells in 10 high-power fields (400× magnification) within the area of highest proliferation and expressed as a percentage. IHC evaluations were performed by two pathologists. According to the 2021 WHO classification, 160 cases were GB *IDH1*-wild-type, and 45 cases were HGA *IDH1* mutant.

All patients were divided into groups according to age as follows: 7–40 years, 41–55 years, 56–64 years, and ≥ 65 years.

Statistical analysis

The Chi-square test was used to compare independent categorical variables, while the McNemar test was applied for dependent categorical variables between the groups. Kappa and accuracy values were also calculated. OS was calculated from the time of diagnosis to the time of death. The Kaplan-Meier method and Cox proportional regression model (forward model) were used to estimate the mean and median survival times, failure rates, and hazard ratios (HR). Log-rank test was used to compare the survival distributions between groups. The prognostic ability of parameters was evaluated for OS in both univariate and multivariable Cox regression models. Categorical variables were expressed as numbers and percentages. The value of $p < 0.05$ was considered significant. The analyses were performed using the statistical package SPSS version 22.0.

Results

A total of 205 patients were evaluated in the study. The impact of IDH1, p53, ATRX, and Ki-67 parameters, along with demographic and clinical features, on patient survival was investigated (Tables 2 and 3).

Table 1

Technical specifications of antibodies used for immunohistochemical analyses

Primary antibody	Clone	Dilution	Origin
IDH1	H09	1/60	Dianova/Germany
ATRX	H-300	1/50	Santa Cruz-Biotechnology
p53	D0-7	1/200	Dako/Denmark
Ki-67	MIB-1	1/200	Dako/Denmark

IDH1 – isocitrate dehydrogenase 1; ARTX – alpha-thalassemia/mental retardation syndrome X-linked; Ki-67 – Kicl 67.

Table 2

Demographic and clinical features of the patients according to prognosis

Parameter	Alive (n = 140)	Dead (n = 65)	Total (n = 205)	*p*
Gender				
female	64 (71.1)	26 (28.9)	90 (43.9)	
male	76 (66.1)	39 (33.9)	115 (56.1)	0.443
Age groups				
7–40	21 (63.6)	12 (36.4)	33 (16.1)	
41–55	49 (80.3)	12 (19.7)	61 (29.8)	
56–64	35 (64.8)	19 (35.2)	54 (26.3)	
≥ 65	35 (61.4)	22 (38.6)	57 (27.8)	0.113
Diagnosis				
astrocytoma	22 (88.0)	3 (12.0)	25 (12.2)	
glioblastoma	118 (65.6)	62 (34.4)	180 (87.8)	0.024

Table 2 (continued)

Parameter	Alive (n = 140)	Dead (n = 65)	Total (n = 205)	*p*
Comorbidity				
no	135 (70.3)	57 (29.7)	192 (93.7)	
yes	5 (38.5)	8 (61.5)	13 (6.3)	0.017
Treatment				
other	67 (80.7)	16 (19.3)	83 (40.5)	
RT	6 (42.9)	8 (57.1)	14 (6.8)	
TMZ+RT	67 (62.0)	41 (38.0)	108 (52.7)	0.002
Recurrence				
no	133 (70.0)	57 (30.0)	190 (92.7)	
yes	7 (46.7)	8 (53.3)	15 (7.3)	0.062
Prognosis				
alive	133 (100.0)	0 (0.0)	133 (64.9)	
recurrence/alive	7 (100.0)	0 (0.0)	7 (3.4)	
dead	0 (0.0)	57 (100.0)	57 (27.8)	
recurrence/dead	0 (0.0)	8 (100.0)	8 (3.9)	0.001

RT – radiotherapy; TMZ – temozolamide; n – number.

Values are given as numbers (percentages). $p < 0.05$ was considered significant.

Table 3

Immunohistochemical characteristics of tumors according to patient prognosis

Parameter	Alive (n = 140)	Dead (n = 65)	Total (n = 205)	*p*
IDH1				
negative	111 (69.4)	49 (30.6)	160 (78.8)	
positive	29 (64.4)	16 (35.6)	45 (21.2)	0.530
p53				
negative	60 (66.7)	30 (33.3)	90 (43.9)	
positive	80 (69.6)	35 (30.4)	115 (56.1)	0.658
ATRX				
negative	13 (54.2)	11 (45.8)	24 (11.7)	
positive	127 (70.2)	54 (29.8)	181 (88.3)	0.113
Ki-67				
negative (< 20)	95 (72.5)	36 (27.5)	131 (63.9)	
positive (≥ 20)	45 (60.8)	29 (39.2)	74 (36.1)	0.084
IDH1 and p53				
no risk for both	21 (67.7)	10 (32.3)	31 (15.1)	
at least one risk	67 (68.4)	31 (31.6)	98 (47.8)	
risk for both	52 (68.4)	24 (31.6)	76 (37.1)	0.997
IDH1 and ATRX				
no risk for both	5 (55.6)	4 (44.4)	9 (4.4)	
at least one risk	32 (62.7)	19 (37.3)	51 (24.9)	
risk for both	103 (71.0)	42 (29.0)	145 (70.7)	0.386
IDH1 and Ki-67				
no risk for both	21 (77.8)	6 (22.2)	27 (13.2)	
at least one risk	82 (67.2)	40 (32.8)	122 (59.5)	
risk for both	37 (66.1)	19 (33.9)	56 (27.3)	0.518
ATRX and p53				
no risk for both	9 (56.3)	7 (43.8)	16 (7.8)	
at least one risk	75 (70.1)	32 (29.9)	107 (52.2)	
risk for both	56 (68.3)	26 (31.7)	82 (40.0)	0.540
ATRX and Ki-67				
no risk for both	9 (52.9)	8 (47.1)	17 (8.3)	
at least one risk	90 (74.4)	31 (25.6)	121 (59.0)	
risk for both	41 (61.2)	26 (38.8)	67 (32.7)	0.065
p53 and Ki-67				
no risk for both	51 (78.5)	14 (21.5)	65 (31.7)	
at least one risk	73 (62.9)	43 (37.1)	116 (56.6)	
risk for both	16 (66.7)	8 (33.3)	24 (11.7)	0.097

Values are given as numbers (percentages).

For abbreviations, see Table 1.

Among the patients, 115 (56.1%) were male and 90 (43.9%) were female, with ages spanning from 7 to 84 years. There was no significant association between gender and age groups (7–40, 41–55, 56–64, ≥ 65) in terms of survival ($p = 0.113$). However, there was a significant association between grade and survival ($p = 0.024$). A significant association was observed between GB and HGA concerning survival ($p = 0.024$). A total of 65 (31.7%) cases resulted in death. The mean OS in all cases was 45.2 months (median: 24 months).

While 45 (21.2%) cases were found to have the *IDH1* mutation, the number of cases without the mutation was 160 (78.8%). According to the 2021 WHO classification, 160 patients were diagnosed with GB. Among the *IDH1*-mutated cases, 14 were grade 2–3 gliomas, and 31 were classified as grade 4 astrocytomas. Of these 31 *IDH1*-mutated grade 4 astrocytomas, 19 (61.3%) were primary and 12 (38.7%) secondary.

There was a high concordance of 75% between *IDH1* and other markers, particularly between *ATRX* (positive/non-mutated) and *IDH1* (negative-wild-type) (Table 4). Loss of *ATRX* was observed in 21 (10.2%) cases, and 16 were GB patients.

OS was found to be longer in *ATRX* mutant negative ($p = 0.004$) (Figure 1A), *p53* positive ($p = 0.023$) (Figure 1B), and Ki-67 < 20% ($p = 0.060$) (Figure 1C) cases. In addition, OS was sifmeangnificantly longer in *IDH1* positive cases

Table 4

Compatibility of IDH1 with other markers (agreement)

Parameter	IDH1		
	no risk – positive (n)	risk – negative (n)	agreement (%)
p53			
positive	31	84	
negative	14	76	52
ATRX			
negative	9	15	
positive	36	145	75
Ki-67			
negative (< 20)	27	104	
positive (≥ 20)	18	56	40

n – number; % – percentage. For other abbreviations, see Table 1.

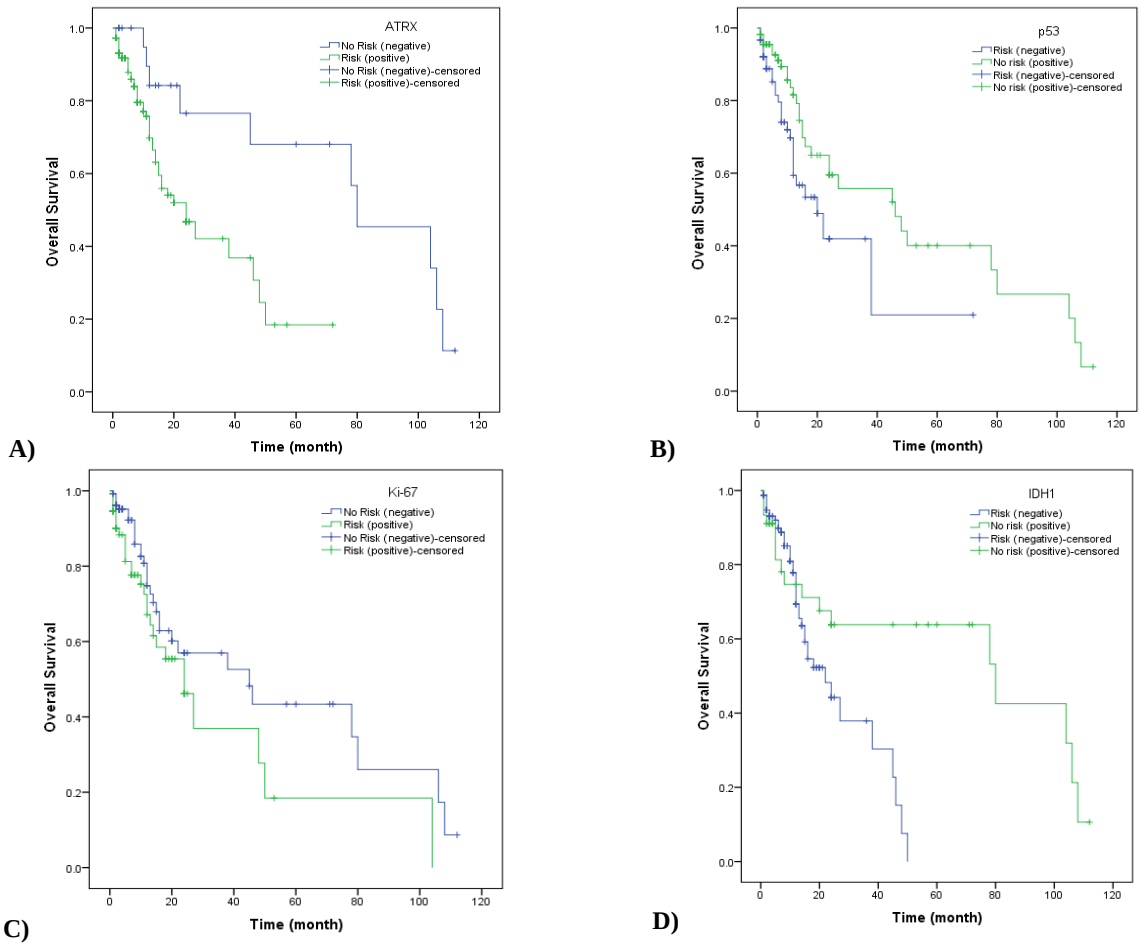

Fig. 1 – Overall survival curves following stratification by ATRX mutations (A), p53 mutations (B), Ki-67 < 20% positivity (C) and IDH1 mutations (D). IDH – isocitrate dehydrogenase; ATRX – alpha-thalassemia/mental retardion syndrome X-linked.

(mean OS: 65.8, median: 80) than in negative cases (mean OS: 25.7, median: 22) ($p = 0.019$) (Figure 1D).

When examining the prognostic ability of the combined markers (summarized in Table 5), patients with an *IDH1* mutation and *ATRX* mutation had the longest OS (mean: 98, median: 106 months; $p = 0.001$) (Figure 2A), while Ki-67 ≥ 20% and p53 negative patients had the shortest OS (mean: 13 months; $p = 0.003$) (Figure 2B).

Table 5

Mean and median overall survival according to related risk factors

Parameter	Overall survival				*p*
	total (n)	number of events	mean	median	
Gender					
female	90	24	31.3	38.0	
male	115	36	50.2	24.0	0.967
Age groups (years)					
7–40	33	10	72.1	80.0	
41–55	61	12	57.6	50.0	
56–64	54	19	14.6	15.0	
65+	57	19	13.8	13.0	< 0 .001
Grade					
2 + 3	25	2	95.3		
4	180	58	40.8	24.0	0.004
Diagnosis					
other	25	2	95.3		
glioblastoma	180	58	40.8	24.0	0.004
Comorbidity					
no	192	52	49.7	45.0	
yes	13	8	13.3	11.0	0.006
Treatment					
other	83	14	49.9	45.0	
RT	14	6	61.8	104.0	
TMZ+RT	108	40	46.1	38.0	0.328
IDH1					
negative	160	43	25.7	22.0	
positive	45	17	65.8	80.0	0.019
p53					
negative	90	28	29.3	20.0	
positive	115	32	52.8	46.0	0.023
ATRX					
negative	24	10	74.0	80.0	
positive	181	50	31.4	24.0	0.004
Ki-67					
negative (< 20)	131	32	52.9	45.0	
positive (≥ 20)	74	28	36.7	24.0	0.060
IDH1 and p53					
no risk for both	31	11	75.6	80.0	
at least one risk	98	27	28.3	20.0	
risk for both	76	22	22.5	22.0	0.004
IDH1 and ATRX					
no risk for both	9	5	98.0	106.0	
at least one risk	51	17	37.8	24.0	
risk for both	145	38	25.6	18.0	0.001
IDH1 and Ki-67					
no risk for both	27	7	82.2	80.0	
at least one risk	122	35	36.7	22.0	
risk for both	56	18	25.4	24.0	0.002
ATRX and p53					
no risk for both	16	7	85.8	104.0	
at least one risk	107	28	29.1	24.0	
risk for both	82	25	30.8	20.0	0.002
ATRX and Ki-67					
no risk for both	17	8	72.6	80.0	
at least one risk	121	26	49.1	38.0	
risk for both	67	26	25.2	24.0	0.010
p53 and Ki-67					
no risk for both	65	12	65.5	78.0	
at least one risk	116	40	37.6	24.0	
risk for both	24	8	13.7	-	0.003

For abbreviations, see Tables 1 and 2.

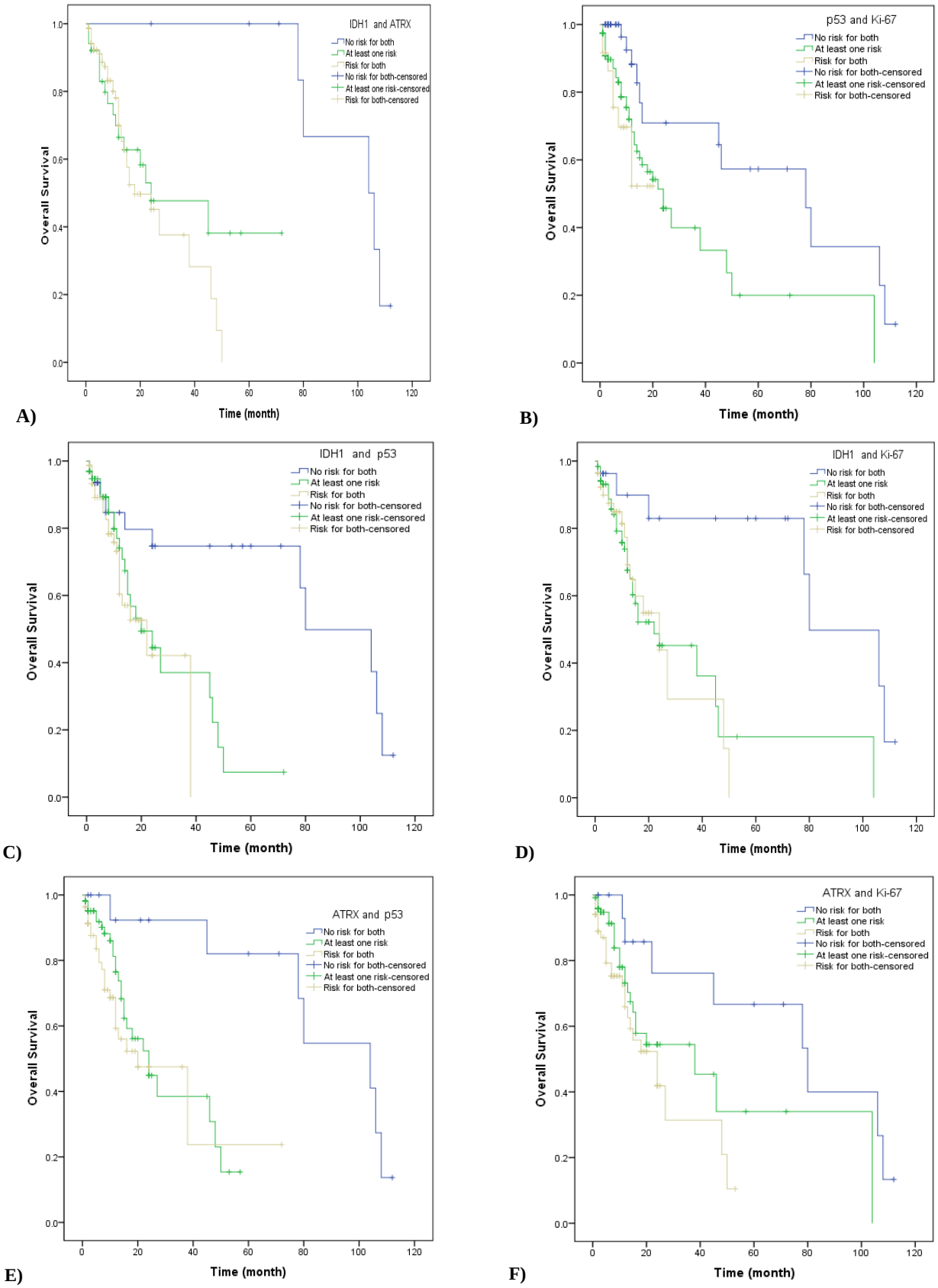

Fig. 2 – Overall survival curves following stratification according to IDH1, ATRX, p53 mutation status and Ki-67 positivity: A) IDH1 and ATRX; B) p53 and Ki-67; C) IDH1 and p53; D) IDH1 and Ki-67; E) ATRX and p53; F) ATRX and Ki-67. For abbreviations, see Figure 1.

Table 6

Results of Cox regression analyses

Parameter	β	SE	HR	95% CI for HR		Sig.
				lower	upper	
Age	0.034	0.011	1.0	1.0	1.1	0.002
Comorbidity	0.966	0.392	2.6	1.2	5.7	0.014
IDH1	0.731	0.372	2.1	1.0	4.3	0.049
ATRX	0.361	0.436	1.4	0.6	3.4	0.408
p53	0.470	0.287	1.6	0.9	2.8	0.101
Ki-67	0.388	0.256	1.5	0.9	2.4	0.130

For other abbreviations, see Table 1.

SE – standard error; HR – hazard ratio; CI – confidence interval.

Analysis of *IDH1* and *p53* expression patterns revealed that patients with *IDH1+*/*p53–* tumors had the most favorable outcomes, with the longest median OS of 75.6 months and progression-free survival (PFS) of 80 months. In contrast, the *IDH1–*/*p53+* group demonstrated the poorest prognosis, with the shortest median OS and PFS of 22.5 and 22 months, respectively. We identified that specific changes in protein profiles were associated with differences in patient survival times. Statistically significant differences in survival ($p < 0.05$) were observed across all combinations, including *IDH1*/*p53* ($p = 0.004$) (Figure 2C), *IDH1*/Ki-67 ($p = 0.002$) (Figure 2D), *ATRX*/*p53* ($p = 0.002$) (Figure 2E), *ATRX*/Ki-67 ($p = 0.010$) (Figure 2F), and *p53*/Ki-67. OS times according to a combination of indicators are illustrated in Figure 2 and summarized in Table 5.

After being standardized for age and comorbidities in the Cox regression model, only *IDH1* emerged as an independent and statistically significant predictor of OS (Table 6).

Radiotherapy (RT) and temozolomide (TMZ) were used in 108 (52.7%) cases, whereas RT only was used in 14 (6.8%) cases. The other 83 patients either did not start treatment at our hospital or were treated but could not receive regular treatment afterward.

Discussion

HGA and GB are the most common and aggressive primary brain tumors [1]. Although GB is frequently seen *de novo*, a few of them occur secondary to the transformation from low-grade astrocytoma (WHO grade 2) or anaplastic astrocytoma (WHO grade 3) [2]. In our series, 18 patients (5 stereotaxic, 13 resection biopsies) were previously diagnosed with low-grade glial tumors and transformed into grade 4 gliomas in the following years (1–11 years).

New molecular techniques and important biomarkers related to the prognosis and diagnosis of HGA/GB were discovered. These markers have emerged as targets for novel therapeutic strategies and offer important insights into the pathogenesis of gliomas [4]. While *IDH* gene mutations were observed with a frequency of 5% in primary GB, they were at a rate of 70–75% in grade 2–3 gliomas and secondary GB [24]. In our series, mutations were present in 11% of primary GB cases, 56% of grade 2–3 gliomas, and 67% of secondary GB. Mutations were more commonly observed in younger patients and were linked to a more favorable prognosis [25]. One of the most compelling clinical observations is the theory that *IDH* mutant gliomas follow a distinct biological course, with patients harboring these mutations demonstrating significantly longer survival compared to those with *IDH*-wild-type tumors [26].

The deoxyribonucleic acid (DNA) sequencing method and the IHC method were compared to detect *IDH1* mutations in 186 patients with glioma. Using antibodies specific for the *R132H* mutation, the sensitivity and specificity of the IHC method were determined to be 94% and 100%, respectively. The authors reported that IHC methods could be used as a standard procedure due to the difficulty of genetic analysis methods, such as DNA sequencing [27]. In subsequent studies, *IDH* mutation was between 10% and 14% in GB [4, 28]. Our study detected an *IDH1* mutation in 21.2 % of 205 patients with IHC methods.

IDH1 mutation is a good prognostic marker for OS and PFS in patients with GB.[13, 14]. Some meta-analyses have shown that *IDH1*/*IDH2* mutations are associated with longer OS and PFS in patients with GB [29]. However, some studies reported no statistically significant difference [4, 30]. Parsons et al. [31] reported that patients with *IDH*-mutated GB survived an average of 31 months, and patients with *IDH*-wild-type survived 15 months [31]. Similarly, Parsons et al. and other researchers found that the median OS of patients with stage III *IDH* mutant glioma was 20 months, whereas it was 65 months in patients with *IDH*-wild-type glioma [31, 32]. In our study, the mean and median OS were longer in patients with *IDH* mutations than in wild-type patients, and there was a statistically significant difference.

In GB, *IDH1* and *p53* mutations are frequently accompanied by *ATRX* mutations [4, 21]. Although *ATRX* mutations are more common in diffuse astrocytomas, they are rarely observed in oligoastrocytomas and GB [4]. Studies have reported *ATRX* loss of 15.3%, 18%, and 26% in GB [19, 33, 34]. In our study, loss of *ATRX* was observed in 21 (10.2%) cases, and 16 were GB patients. There were studies indicating that *ATRX* mutation was statistically significant in terms of survival in GB and that *ATRX* mutation was a good prognostic factor [33, 35]. Additionally, some studies have shown that there is no significant difference in survival in GB/HGA with *IDH* mutation, regardless of the *ATRX* mutation status. However, they associated the presence of the *ATRX* mutation in *IDH*-wild-type GB with better survival [3].

Our study found OS to be longer in patients with an *ATRX* mutation (negative), which was statistically significant. In addition, the longest OS was observed in patients with *IDH1*-mutant/*ATRX*-wild-type tumors (98 months), while the shortest OS was seen in those with *IDH1*-wild-type/*ATRX*-wild-type tumors (25.6 months).

Cells with impaired *p53* function can develop genetic abnormalities and lead to the development of malignancies. Alterations in the *p53* gene are more common in secondary GB than in primary GB, and *p53* may be mutated in more than 65% of cases [2]. There are conflicting reports on the impact of *p53* mutation on prognosis in patients with GB. While some studies have found no association between *p53* mutations and prognosis [35], some other studies have identified a significant correlation between *p53* positivity and clinical survival [36, 37]. Additionally, studies have observed shorter life expectancy with high p53 levels and reported that p53 is a poor prognostic factor [2]. In our study, *p53* was positive in 115 cases and negative in 90 cases. The OS was longer in *p53* positive cases (mean: 46 months) and shorter in negative cases (mean: 20 months), which proved to be statistically significant.

Surgery is the first step in treating all patients with intracranial gliomas, usually with tissue diagnosis and tumor resection [15]. Standard treatment for newly diagnosed patients with HGA/GB includes maximal resection of the tumor, 60 Gray RT for 6 weeks, concurrent administration of TMZ, and six cycles of adjuvant therapy [15, 38]. One study found that survival was 12.1 months in patients who received RT alone, compared with 14.6 months in GB patients who received RT after surgery, followed by adjuvant TMZ therapy [39]. In retrospective studies, the five-year survival probability for GB was reported to be approximately 5%, whereas the one-year survival rate was reported to be 36% [5]. Cancer progresses within one year despite standard treatment in 70% of cases. In our study, the number of patients receiving only RT was 14, and OS was 61.8 months, whereas 108 patients receiving TMZ+RT survived 46.1 months. Consistent with the literature, patients who received RT had longer life expectancy, but no statistically significant results were obtained. We can explain this by the small number of patients who received RT. Targeted therapies are available for *IDH1* mutant tumors, and research is ongoing for *ATRX* mutations. Losing *ATRX* leads to DNA repair errors and may increase sensitivity to poly-adenosine diphosphate ribose polymerase inhibitors. Agents that inhibit telomerase in cells with *ATRX* loss are being investigated. For instance, by demonstrating *ATRX* loss in the tumor, we can provide patients with access to new treatment options.

Recent studies have been conducted to determine molecular characteristics and specific genetic features of gliomas to improve prognosis [40]. There are studies on loss of 1p19q heterozygosity, *p53*, *EGFR* and *PTEN* mutation, *MDM2* amplification, *MGMT* promoter methylation, and *IDH1* mutation [11, 13, 41–43]. Given that more than 80% of low-grade gliomas carry an *IDH* mutation, this survival benefit strongly depends on the presence of an *IDH* mutation [15].

In summary, we found that *IDH1* and *ATRX* mutations significantly impacted prognosis in GB patients with *p53* overexpression alone. In addition, RT and chemotherapy had a positive effect on their survival.

Limitations of the study

Although our university hospital serves as a regional center and draws a diverse patient population from neighboring provinces, the relatively small sample size and single-center design may limit the generalizability of our findings. The identification of more cases may change with the availability of comprehensive clinical, morphological, and molecular profiles. There are inconsistent findings in the current literature regarding certain prognostic markers, which may complicate the interpretation of the results. Future studies employing molecular methods are needed to confirm the findings and deepen our understanding. In addition to IHC staining in our study, further prospective clinical and molecular studies are highly desirable.

Conclusion

IDH1 mutant patients have been shown to have a better prognosis, and now only those with the *IDH1*-wild-type are referred to as glioblastoma. Not only *IDH1* mutation status but also the combination of other protein expressions may subdivide glioblastoma from a diagnostic point of view in the future. These proteins may be used as prognostic markers. Our results should be supported by future studies in larger patient series using molecular methods.

Conflict of interest

The authors of this paper declare no conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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Rehabilitation for balance impairment in patients after stroke: a single-blind randomized controlled study

Rehabilitacija bolesnika sa poremećajem ravnoteže posle moždanog udara: jednostruko slepa randomizovana kontrolisana studija

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Abstract

Background/Aim. Balance problems in post-stroke patients should be recognized and treated with the most effective rehabilitation approaches. The aim of this study was to assess a group exercise program with chairs for improving balance and walking ability in post-stroke patients. **Methods.** This single-blind, randomized, controlled study included 86 post-stroke patients recruited from the only inpatient rehabilitation facility in the country. Patients were randomly assigned to one of the two groups: a group that had conventional rehabilitation only (control group) or a group that had a group exercise program with chairs, along with the conventional rehabilitation (treatment group). The methodology encompassed examination of demographics, history, clinical (balance was assessed using a Berg Balance Scale – BBS), and functional characteristics (walking ability and ability to use stairs). The assessments were made at baseline and after three weeks of rehabilitation. **Results.** All participants completed the exercise program. According to BBS, the balance improved in both examined groups: by 3.16 ± 2.16 (t = -4.989; p = 0.001) in the control group and by 8.33 ± 5.85 (t = -9.326; p = 0.001) in the treatment group. Significant improvement in balance and walking ability was registered in the treatment group compared to the control group. **Conclusion**. Group exercises with chairs appear to be effective in improving balance and stroke rehabilitation in post-stroke patients.

Key words:
core stability; physical and rehabilitation medicine; stroke; walking.

Apstrakt

Uvod/Cilj. Problemi povezani sa ravnotežom kod bolesnika nakon moždanog udara trebalo bi da se prepoznaju i leče najefikasnijim pristupima rehabilitacije. Cilj rada bio je da se proceni program grupnih vežbi sa stolicama za poboljšanje ravnoteže i sposobnosti hodanja bolesnika nakon moždanog udara. **Metode**. Ovom jednostrukom slepom randomizovanom kontrolisanom studijom obuhvaćeno je 86 bolesnika posle moždanog udara koji su regrutovani iz jedine ustanove za bolničku rehabilitaciju u zemlji. Bolesnici su nasumično raspoređeni u jednu od dve grupe: grupu koja je imala samo konvencionalnu rehabilitaciju (kontrolna grupa) ili grupu koja je uz konvencionalnu rehabilitaciju imala i program grupnih vežbi sa stolicama (testirana grupa). Metodologija je podrazumevala ispitivanje demografskih, anamnestičkih, kliničkih (procena ravnoteže primenom skale *Berg Balance Scale* – BBS) i funkcionalnih osobina (sposobnost hodanja i korišćenja stepenica). Procena je vršena na početku i nakon tronedeljne rehabilitacije. **Rezultati.** Svi učesnici su završili program vežbi. Prema BBS, ravnoteža je poboljšana u obe ispitivane grupe: za 3,16 ± 2,16 (t = -4,989; p = 0,001) u kontrolnoj grupi, i za 8,33 ± 5,85 (t = -9,326; p = 0,001) u testiranoj grupi. U testiranoj grupi je utvrđeno značajno poboljšanje ravnoteže i sposobnosti hodanja u odnosu na kontrolnu grupu. **Zaključak.** Grupne vežbe sa stolicama su korisne u poboljšanju ravnoteže i rehabilitacije bolesnika posle moždanog udara.

Ključne reči:
ravnoteža; medicina, fizikalna i rehabilitacija; moždani udar; hod.

Introduction

Stroke is a neurological disorder that affects roughly 13.7 million people annually [1]. Globally, stroke is the second leading cause of death, with significant increases in stroke incidence and stroke mortality [2]. The stroke incidence among younger adults, in contrast to the older population, is increasing globally [3].

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Hemiparesis is the most common consequence of a stroke, which affects about 65% of patients [4, 5]. In addition to the motor, there are numerous other disorders resulting from a stroke: loss of sensitivity, reduced attention, impaired vision, and spatial orientation, which contribute to balance problems and possible falls [6]. Balance is a complex system that includes motor, sensory, and cognitive components, as well as the interactions among these components and with the external environment. A deficit in any of these three systems after a stroke can lead to instability [7]. Good balance is a prerequisite for achieving independence in walking and performing activities of daily living (ADL) [8, 9]. For stroke patients and their families, establishing independent walking and achieving independence in ADL stand out as essential for functional recovery and quality of life (QoL) [10]. Therefore, it is important to give adequate importance to the problem of balance and its successful rehabilitation. Numerous rehabilitation programs have been implemented to improve balance in stroke patients, including maintaining different standing positions with external and internal perturbations [11], agility and stepping exercises [12], physioball exercises [13], virtual reality exercises [14], postural control exercises [15], robot-assisted therapy [16], balance platform exercises [17], etc. Although the effectiveness of different programs in the rehabilitation of balance after stroke has been proven, there are no general guidelines on the most effective therapeutic approach [9]. On the other hand, a large number of rehabilitation programs for improving balance use specific and sophisticated equipment (computerized balance platforms, virtual programs, robotics), which is quite expensive and not always available [18, 19]. It is important to adopt a unique rehabilitation approach with the primary goal of improving balance, gait, and functional independence after a stroke [20].

Starting from these grounds, we posed the question of proving the efficacy of a simple and achievable program of group exercises on improving balance in stroke patients. The recommendations based on the results of this and similar research could help physiotherapists make a better choice for improving balance, walking ability, and QoL after stroke.

In the present study, we aimed to determine whether group exercises with chairs, as an addition to conventional rehabilitation, can improve balance in post-stroke patients. The additional aim of the research was to establish the impact of the interventions on the patient's ability to walk and use stairs.

Methods

This single-blind, randomized, controlled study was conducted between November 2020 and July 2021 at the Institute for Physical Medicine, Rehabilitation, and Rheumatology "Dr. Simo Milošević", Igalo, Montenegro.

The study protocol was approved by the Ethics Committee of the University of Sarajevo, Faculty of Health Studies, Bosnia and Herzegovina (No. 04-7-99/20, from July 27, 2020). The study was conducted following the principles of the Declaration of Helsinki. Written informed consent was obtained from each patient.

A total of 86 stroke survivors who agreed to participate in the study and who met the inclusion criteria were recruited for research during rehabilitation. The patients were divided into two groups: the control group (n = 43), which consisted of patients who received standard rehabilitation, and the treatment group (n = 43), which consisted of patients who, in addition to standard rehabilitation, received a program of group exercises with chairs. All patients were examined by a physiatrist in the inpatient setting. Randomization occurred after the medical assessment, without the research physiotherapist having any prior knowledge of the patients' conditions. The patients were randomly and equally allocated into the control and treatment groups. Randomization (allocation ratio 1 : 1) was conducted with protocol numbers for each patient included in the study. Independent research assistants allocated patients to a control or treatment group. The assistants used numbered envelopes to conceal the patient allocation from the researcher. However, the research physiotherapist and patients were aware of the group allocation after the first evaluation, owing to the nature of the research process. Patients could not be blinded to their group allocation because there was no placebo intervention in this research.

Patients were excluded from the study if they could not walk independently for at least 10 m, could not stand independently or with support for at least 10 min, could not perform exercises in a standing position with or without holding onto the back of a chair, or if they were diagnosed with an unstable medical or psychiatric condition. The study flow chart is shown in Figure 1. All participants in each group completed the study.

Assessment and data collection

The medical history (diagnosis, type of stroke, number of strokes, date of the last stroke) and demographic information (age, gender, working status, family status) of all patients were collected from facility medical records and by self-report (with permission from the department and informed consent of the participants).

Balance ability was measured using the Berg Balance Scale (BBS), a measuring instrument designed in 1993 by Katherine Berg. The scale objectively assesses the patient's ability to safely maintain balance while performing a series of specific tasks. BBS measures several different aspects of balance, both static and dynamic, with relatively little equipment and space. This 14-item objective scale, with high inter- and intra-examiner reliability (98%) and specificity (96%), is the most frequently used instrument for balance assessment in neurological practice [8, 21].

To conduct the assessment, we needed the following equipment: a 25 cm long ruler, two standard chairs (one with armrests and one without), a footstool or step, and a stopwatch. The examination lasts for a total of about 20 min. The test consists of 14 different items—tasks evaluated on a five-point scale from 0 to 4, based on clearly established criteria. Grade 0 is for the lowest level of function, and grade 4 is for the highest level of function. The maximum possible score is 56, where a higher score means better stability.

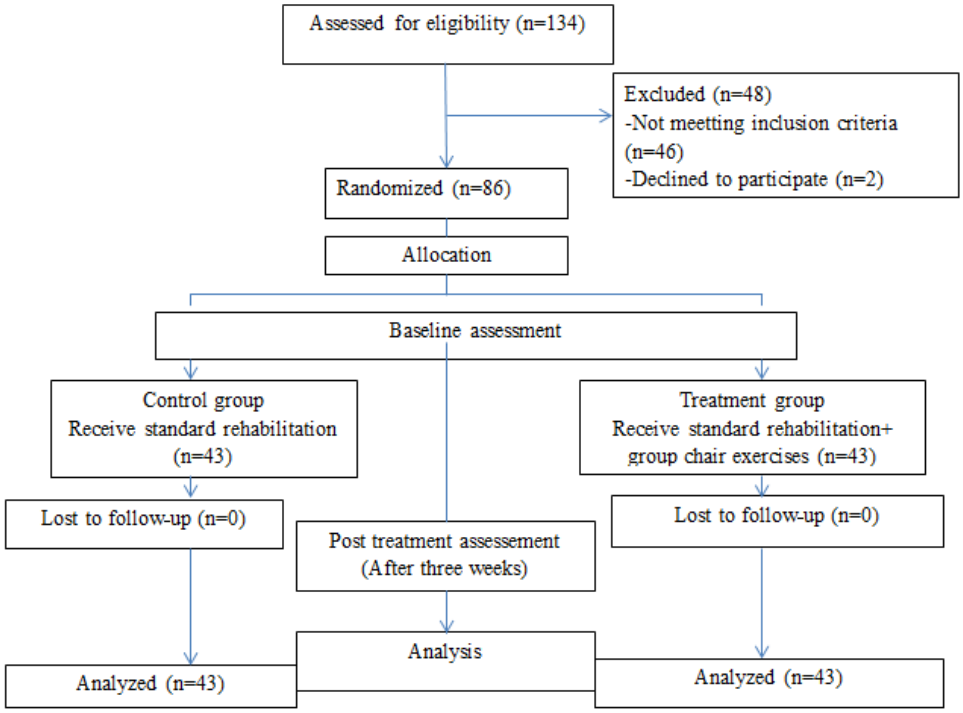

Fig. 1 – Study flow chart.

Walking ability and ability to use stairs were assessed based on careful observation of performance. After assessing the walking ability, all patients were classified into one of the three categories: walking indoors, walking outdoors, and walking as before the stroke. Regarding the ability to use stairs, all patients were classified into one of the five categories: independent, independent but holding handrail, supervision needed, help needed, and cannot use stairs. The testing was conducted on the baseline and after three weeks of rehabilitation. All tests, physiotherapy examinations, and chair exercises were carried out by a research physiotherapist with extensive experience in neurological rehabilitation.

Exercise program

A conventional inpatient stroke rehabilitation program, including a maximum of four therapeutic interventions (individual exercises, manual massage, occupational therapy, and physical modalities), was applied to both groups for three weeks, five days a week. The individual exercise program, lasting 45 min, consisted of strengthening and flexibility exercises for the affected half of the body, as well as walking exercises. Occupational therapy lasted a total of 30 min. The treatment group, in addition to conventional rehabilitation, received group exercises with chairs for three weeks, five days a week. There were at most six patients in the group. If there were more than six participants in a certain period, the exercises were organized in two sessions. The chair exercise program lasted a total of 30 min. During the first 20 min, the participants performed exercises in a sitting position, and the last 10 min in a standing position. During the last 10 min of the program, the patients stood between the chairs and the wall for additional safety during exercise. A combination of flexibility, body weight resistance strengthening, coordination, and static and dynamic balance exercises was applied. Each exercise was repeated up to 10 times. The same set of exercises was repeated daily, with progressive adjustments introduced based on individual tolerance. Progression in the exercises was achieved through increases in the number of repetitions, the time spent standing in tandem or single stance support, the distance reached forward, and the stride length, among other factors. The chairs in the exercise room were arranged in a semicircle to facilitate demonstration, monitoring, and correction of exercise performance.

These chair balance exercises introduced a group approach to exercise, unusual for stroke rehabilitation. The exercise program also promoted activities in upright, sitting, and standing positions, which are necessary for daily functioning. These exercises were designed according to available relevant programs, recommendations, and guidelines based on current best practices, research, and opinions of experts in the field [22–25].

Statistical analysis

Statistical analysis was performed using SPSS Statistics v.20.0. Descriptive statistics were used to express the data as the arithmetic mean ± standard deviation (SD) for both groups. Analysis of categorical variables was performed using Pearson's χ^2-test or Fisher's exact probability test. If the distribution of continuous variables was symmetrical, we used the arithmetic mean and SD to display the mean value and measures of dispersion; to compare these variables, we

used the analysis of variance (ANOVA) test. A student's *t*-test was used to compare the means of the two groups. The influence of non-changeable variables on changeable variables was analyzed by linear regression. We used Pearson's and Spearman's rank correlation coefficients to examine the linear relationship between ratio and ordinal characteristics. The threshold of statistical significance was set at the conventional level of $\alpha = 0.05$. The level of significance was established at $p < 0.05$.

Results

The general characteristics of the study subjects are summarized in Table 1. No statistically significant differences between the two groups were found in terms of patient demographics or clinical characteristics. The average age was 64.79 ± 9.21 years in the control and 66.97 ± 8.06 years in the treatment group. A chi-square test was used to analyze the gender distribution between the control and treatment groups, revealing no statistically significant difference ($\chi^2 = 0.047$; $p = 0.500$). Regarding employment status, most patients in this study were retired, with no statistically significant difference between groups ($\chi^2 = 0.544$; $p = 0.461$). In the control group, an average of 4.30 ± 2.13 months had passed since the stroke, compared to 4.67 ± 2.50 months in the treatment group. The chi-square test also showed no statistically significant difference in stroke frequency based on etiology ($\chi^2 = 0.806$; $p = 0.274$).

The average BBS value at admission for the control group was 46.51 ± 8.36, and for the study group, 44.77 ± 7.78. Using the ANOVA test, no statistically significant difference was found (F = 1.003; $p = 0.319$). In both study groups, there was an improvement after rehabilitation. The average BBS value at discharge for the control group was 49.67 ± 7.76, and for the treatment group, 53.09 ± 3.37. Using the ANOVA test, a statistically significant difference was found between the study groups (F = 7.028; $p = 0.010$) (Table 2).

In the control group, after rehabilitation, there was an improvement of 3.16 ± 2.16 compared to the BBS value at admission, which is a statistically significant difference ($t = -4.989$; $p = 0.001$). In the treatment group, after rehabilitation, there was an improvement of 8.33 ± 5.85 compared to the BBS values at admission, which is a statistically significant difference ($t = -9.326$; $p = 0.001$). By comparing BBS values before and after rehabilitation in relation to the examined

Table 1

The general characteristics of patients after stroke according to study groups

Parameters	Control group (n = 43)	Treatment group (n = 43)	Total
Gender			
female	20 (46.5)	21 (48.8)	41 (47.7)
male	23 (53.5)	22 (51.2)	45 (52.3)
Work status			
employed	7 (16.3)	5 (11.6)	12 (14)
retired	27 (62.8)	30 (69.8)	57 (66.3)
unemployed	9 (20.9)	8 (18.6)	17 (19.8)
Living status			
living alone	11 (25.6)	7 (16.3)	18 (20.9)
not living alone	32 (74.4)	36 (83.7)	68 (79.1)
Side of hemiparesis			
left	16 (37.2)	23 (53.5)	39 (45.3)
right	23 (53.5)	15 (34.9)	38 (44.2)
non-specific	4 (9.3)	5 (11.6)	9 (10.5)
Cause of stroke			
ischemia	38 (88.4)	35 (81.4)	73 (84.9)
hemorrhage	5 (11.6)	8 (18.6)	13 (15.1)

Values are given as numbers (percentages).

Table 2

Comparison of values of the BBS score before and after rehabilitation between groups (each consisting of 43 participants)

Parameters	Mean ± SD	SEM	95% CI		Min–Max	F	*p*
			lower	upper			
Before rehabilitation							
control group	46.51 ± 8.36	1.27	43.94	49.09	23.00–56.00	1.003	0.319
treatment group	44.77 ± 7.78	1.19	42.37	47.16	26.00–56.00		
After rehabilitation							
control group	49.67 ± 7.76	1.18	47.29	52.06	21.00–56.00	7.028	0.010
treatment group	53.09 ± 3.37	0.51	52.06	54.13	40.00–56.00		

BBS – Berg Balance Scale; SD – standard deviation; SEM – standard error of the mean; F – analysis of variance (ANOVA) test; CI – confidence interval; min – minimum; max – maximum; $p < 0.05$.

groups, a statistically greater improvement was found in the treatment group ($p = 0.006$) (Table 3).

The average value and comparison of individual BBS 14 items between the examined groups were determined at the time of discharge from rehabilitation (Table 4). The ANOVA test showed a statistically significant improvement in the treatment group for 6 of the 14 items, namely Standing unsupported ($p = 0.041$), Standing to sitting ($p = 0.049$), Transfers ($p = 0.007$), Standing unsupported with feet together ($p = 0.042$), Reaching forward with outstretched arms ($p = 0.002$), and Pick up (retrieving) object from the floor ($p = 0.004$).

The influence of the independent variables (gender, age, working status, living status, diagnosis, and cause of stroke)

Table 3

Comparison of values of the BBS score before and after rehabilitation within each particular group (each consisting of 43 participants)

Groups	Mean ± SD	SEM	95% CI		*t*	df	*p*	*P*
			lower	upper				
Control	3.16 ± 2.16	0.63	-4.44	-1.88	-4.989	42	0.001	0.006
Treatment	8.33 ± 5.85	0.89	-10.13	-6.52	-9.326	42	0.001	

***t* – paired *t*-test; For other abbreviations, see Table 2.**

Table 4

The average value of individual BBS items of the examined groups at discharge

Parameters	Mean ± SD	SEM	Min–Max
Sitting to standing			
control group	3.84 ± 0.37	0.06	3.00–4.00
treatment group	3.95 ± 0.21	0.03	3.00–4.00
Standing unsupported			
control group	3.91 ± 0.29	0.04	3.00–4.00
treatment group	4.00 ± 0.00	0.00	4.00–4.00
Sitting unsupported			
control group	4.00 ± 0.00	0.00	4.00–4.00
treatment group	4.00 ± 0.00	0.00	4.00–4.00
Standing to sitting			
control group	3.86 ± 0.35	0.05	3.00–4.00
treatment group	3.98 ± 0.15	0.02	3.00–4.00
Transfers			
control group	3.72 ± 0.59	0.09	2.00–4.00
treatment group	3.98 ± 0.15	0.02	3.00–4.00
Standing with eyes closed			
control group	3.63 ± 0.82	0.12	0.00–4.00
treatment group	3.88 ± 0.32	0.05	3.00–4.00
Standing unsupported with feet together			
control group	3.56 ± 0.96	0.15	0.00–4.00
treatment group	3.88 ± 0.39	0.06	2.00–4.00
Reaching forward with an outstretched arm			
control group	3.42 ± 0.73	0.11	2.00–4.00
treatment group	3.81 ± 0.39	0.06	3.00–4.00
Retrieving an object from the floor			
control group	3.67 ± 0.71	0.11	0.00–4.00
treatment group	4.00 ± 0.00	0.00	4.00–4.00
Turning to look behind			
control group	3.74 ± 0.66	0.10	1.00–4.00
treatment group	3.93 ± 0.34	0.05	2.00–4.00
Turning 360°			
control group	3.28 ± 1.03	0.16	0.00–4.00
treatment group	3.53 ± 0.93	0.14	0.00–4.00
Placing the alternate foot on a stool			
control group	3.47 ± 1.03	0.16	0.00–4.00
treatment group	3.79 ± 0.56	0.09	2.00–4.00
Tandem standing			
control group	2.95 ± 1.11	0.17	0.00–4.00
treatment group	3.33 ± 0.78	0.12	2.00–4.00
Standing on one foot			
control group	2.63 ± 1.33	0.20	0.00–4.00
treatment group	3.02 ± 1.03	0.16	0.00–4.00

For abbreviations, see Table 2.

on the dependent variable, the BBS score, at discharge, is shown in Table 5. It is obvious that the BBS score in the respondents of the control group was influenced by the cause of the disease, and in the treatment group by the number of strokes.

Walking ability and the ability to use stairs among the examined groups before and after rehabilitation are presented in Table 6. In the control group, no clinically significant improvement in walking ability was observed following rehabilitation. In contrast, the treatment group

Table 5

The influence of the independent research variables on the dependent variable BBS in the examined groups at discharge

Parameters	Unstandardized coeff.		Standardized coeff.	*t*	Sig.	95% CI for B	
	B	SE	ß				
Constant	51.622	10.685		4.831	0.000	29.952	73.291
Control group							
gender	-2.439	2.362	-0.159	-1.033	0.309	-7.230	2.351
age	0.176	0.128	0.209	1.372	0.179	-0.084	0.436
stroke	-0.705	4.694	-0.023	-0.150	0.882	-10.225	8.816
time	-0.518	0.551	-0.143	-0.940	0.354	-1.635	0.600
diagnosis	0.900	1.768	0.077	0.509	0.614	-2.686	4.486
cause	**-7.338**	**3.581**	**-0.307**	**-2.049**	**0.048**	**-14.601**	**-0.075**
Constant	59.279	6.651		8.912	0.000	45.790	72.769
Treatment group							
gender	0.702	1.013	0.105	0.693	0.493	-1.353	2.757
age	-0.50	0.071	-0.119	-0.697	0.490	-0.194	0.095
stroke	**-4.261**	**1.623**	**-0.411**	**-2.625**	**0.013**	**-7.553**	**-0.969**
time	0.030	0.215	0.022	0.140	0.889	-0.405	0.466
diagnosis	-0.081	0.823	-0.016	-0.098	0.922	-1.750	1.589
cause	0.722	1.482	0.084	0.487	0.629	-2.285	3.728

coeff. – coefficient; B (or b) – used for denoting the realization (value of) regression coefficient in the sample; SE – standard error; β – generally used for denoting population regression coefficient; n – number. For other abbreviations, see Tables 2 and 3.

***Note*: Influenced factors and their values are bolded (cause in the control group and the number of strokes in the treatment group).**

Table 6

Walking ability and the ability to use stairs among the examined groups before and after rehabilitation

Parameters	Control group (n = 43)	Treatment group (n = 43)
Walking ability		
indoors		
before	11	13
after	11	5
outdoors		
before	25	29
after	25	36
walking as before the stroke		
before	7	1
after	7	2
Ability to use stairs		
independent		
before	11	8
after	12	15
independent, but holding a handrail		
before	16	22
after	20	25
supervision needed		
before	4	1
after	5	1
help needed		
before	9	11
after	4	2
cannot use the stairs		
before	3	1
after	2	0

showed a statistically significant improvement (Z = -2.887; $p = 0.004$). The majority of patients in both groups were able to walk independently before and after rehabilitation, which is consistent with the study's inclusion criteria.

Regarding the ability to use stairs, a statistically significant improvement was established in the treatment group compared to the control group. In the treatment group, 8 patients were able to use the stairs independently, without holding the handrail. After rehabilitation, the number increased to 15, which is a statistically significant improvement compared to the control group ($\chi^2 = 4.764$; $p = 0.029$).

Discussion

The results showed that a statistically significant balance improvement was achieved in both examined groups. By comparing the BBS values before and after rehabilitation, it was found that a statistically significantly greater improvement was achieved in the treatment group. Analysis of the influence of independent variables on the BBS score at discharge showed that, in the control group, the BBS score was associated with the cause of the disease, while in the treatment group, it was influenced by the number of strokes. A higher BBS score indicates better postural stability. A significant negative correlation between the BBS score and the number of registered strokes in the treatment group is logical and expected, as individuals with fewer strokes tend to exhibit greater stability. In various studies, authors have analyzed the correlation between independent variables and the BBS score; however, the variables are different from ours, which makes comparing the correlation results difficult [26–28].

The improvement in balance most likely occurred as a result of rehabilitation programs. The results indicate that conventional rehabilitation improves balance; however, the combination of conventional rehabilitation and group chair exercises is significantly more efficient. The positive change in balance in the treatment group was probably due to the content of the chair exercise program. The chair exercises included a combination of flexibility, body weight resistance strengthening, coordination, and static and dynamic balance exercises. A statistically significantly greater improvement in 6 out of the 14 BBS items in the treatment group speaks in favor of the program's effectiveness.

Currently, post-stroke rehabilitation is most often carried out in institutions with the aim of establishing functional independence after discharge [29], following the realization of our research. Our program targeted stroke survivors who were admitted for inpatient rehabilitation. We were guided by the presumption that improving balance function would enable stroke survivors to effectively participate in routine daily activities. We assumed that, along with the improvement of balance, there would also be a recovery of the ability to walk, which is crucial for the functional independence of stroke patients. Many studies and review studies show that rehabilitation and various rehabilitation approaches are effective in improving walking ability after stroke [12, 30–32].

In our research, walking ability was significantly improved in the treatment group after rehabilitation. Our results on walking ability improvement are in agreement with those of a study by Mohd Nordin et al. [33]. They reported walking improvement with group-supervised exercises, which were organized in stations once a week for 90 min *per* session over 12 weeks. The very composition of the exercise program can explain the impact of the group chair exercises on walking ability. Body weight-bearing by the lower limbs, body weight-shifting, and multi-directional step exercises are essential for functional mobility and independence in ADL [34]. The chair exercises included weight-bearing exercises on the affected leg in sitting and standing positions, which could increase proprioceptive awareness and sensorimotor integration, necessary for adaptive and anticipatory aspects of postural control. Another explanation of the chair exercise's influence on walking ability is about their implementation in a sitting and standing position, which contributes to faster recovery and restoration of function after a stroke, as was also reported by Logan et al. [35]. It should be noted that all patients in our study, according to the inclusion criteria, were able to walk for at least 10 m without assistance before rehabilitation.

Previous studies showed that different therapy approaches are beneficial for balance after a stroke [9–15]. Madhuranga et al. [36] concluded that wobble board exercises, in combination with standard physiotherapy, can restore balance function in stroke patients. Various exercises were applied as individual therapy, such as intensive trunk training exercises in multiple planes, as implemented in the study by Ahmed et al. [37].

However, in our research, we introduced exercises organized in small groups, which is not the usual approach in stroke rehabilitation. Unlike individual exercises, group exercises involve a smaller number of physiotherapists and are therefore more economical. Group exercises using chairs require no additional investment and can be easily integrated into the existing workflow of a rehabilitation department, making this approach particularly advantageous. Furthermore, it is important to underline the socialization achieved through group exercises. The participants provided mutual support and motivation, which is the obvious advantage of exercising in a group. The adherence rate was optimal, probably due to the group nature of the exercises.

The presence of a physiotherapist at each session played a crucial role in achieving the observed results and must be emphasized as essential in the exercise organization. Demonstration, active participation, and explanation of each exercise by the physiotherapist were essential for the process of motor learning, proper performance, adherence, safety, and motivation. Social interaction and mutual support are unique to group exercise [38], and are another benefit of these exercises.

Our research has demonstrated that chair exercises are safe and effective in the rehabilitation of patients with mild and moderate stroke. These group exercises can be easily applied and organized in various institutions and offices dealing with post-stroke rehabilitation. This method promotes recovery with minimal use of resources and can effectively enhance standard physical therapy; therefore, it can be potentially used in addition to conventional rehabilitation or as an extension following inpatient rehabilitation. The chair exercise program promotes balance recovery and functional recovery with minimal resources and can effectively improve standard stroke rehabilitation.

As an advantage of our research, we highlight the randomization in the distribution of subjects and the homogeneity, and thus the comparability of the examined groups. However, the limitation of our research is that no long-term follow-up was conducted to determine the stability of the achieved outcomes, which is recommended for future research. The research physiotherapist conducted daily group exercises and performed the final assessment, knowing the distribution of the subjects by group; therefore, they were not blinded to the participants' group assignments. This limitation, although unavoidable, must be highlighted as a weakness and bias in the research.

Conclusion

The results of our research suggest that post-stroke rehabilitation effectively improves balance, but also that it is significantly more effective with the addition of daily 30-min group chair exercises. We sincerely hope that the results of this study will be used to develop recommendations or guidelines for best practices in post-stroke balance rehabilitation.

Conflict of interest

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Prevalence of the most common external manifestations and comorbidities in men with decompensated alcoholic liver cirrhosis

Prevalencija najčešćih spoljašnjih manifestacija i komorbiditeta kod muškaraca obolelih od dekompenzovane alkoholne ciroze jetre

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Abstract

Background/Aim. External manifestations and comorbidities represent important clinical aspects of decompensated alcoholic liver cirrhosis (ALC), providing insight into disease severity and systemic involvement. The aim of the study was to examine the prevalence of external signs and comorbidities in male patients with decompensated ALC. **Methods.** A prospective, comparative, descriptive, and analytical study was conducted at the Clinic for Internal Medicine, University Clinical Center of the Republic of Srpska, in Banja Luka, Bosnia and Herzegovina. The study included 123 male patients diagnosed with decompensated ALC. All necessary diagnostic evaluations, including laboratory, microbiological, serological, radiological, and endoscopic assessments, were performed during their first hospitalization. **Results.** The mean age of the patients was 59.09 ± 9.32 years. The most common external manifestations were jaundice (79.67%), spider nevi (54.47%), palmar erythema (36.58%), and gynecomastia (18.69%). The most frequent comorbidities were diabetes mellitus (19.51%), congestive heart failure (17.88%), and chronic kidney disease (11.38%). A significant correlation was found between disease severity and the presence of external signs, with jaundice being the most prevalent. **Conclusion.** External manifestations and comorbidities are frequent in male patients with ALC, reflecting the systemic impact of the disease. Recognizing these clinical markers can aid in early diagnosis, risk stratification, and tailored therapeutic strategies.

Key words:
comorbidity; early diagnosis; liver cirrhosis, alcoholic; men.

Apstrakt

Uvod/Cilj. Spoljašnje manifestacije i komorbiditeti predstavljaju važne kliničke aspekte dekompenzovane alkoholne ciroze jetre (ACJ), pružajući uvid u težinu bolesti i sistemsko zahvatanje. Cilj rada bio je da se ispita učestalost spoljašnjih znakova i komorbiditeta kod bolesnika muškog pola obolelih od dekompenzovane ACJ. **Metode.** Sprovedena je prospektivna, komparativna, opisna i analitička studija na Klinici za internu medicinu, Univerzitetskog kliničkog centra Republike Srpske u Banja Luci, Bosna i Hercegovina. Istraživanje je obuhvatilo 123 bolesnika muškog pola kojima je postavljena dijagnoza dekompenzovane ACJ. Sve neophodne dijagnostičke procene, uključujući laboratorijske, mikrobiološke, serološke, radiološke i endoskopske preglede, obavljene su tokom njihove prve hospitalizacije. **Rezultati.** Prosečna starost bolesnika iznosila je 59,09 ± 9,32 godina. Najčešće spoljašnje manifestacije bile su žutica (79,67%), paučinaste vene (54,47%), palmarni eritem (36,58%) i ginekomastija (18,69%). Najčešći komorbiditeti bili su dijabetes melitus (19,51%), kongestivna srčana insuficijencija (17,88%) i hronična bubrežna bolest (11,38%). Ustanovljena je značajna korelacija između težine bolesti i prisustva spoljašnjih znakova, pri čemu je žutica bila najzastupljenija. **Zaključak.** Spoljašnje manifestacije i komorbiditeti su česti kod bolesnika muškog pola obolelih od ACJ, što odražava sistemski uticaj bolesti. Prepoznavanje ovih kliničkih pokazatelja može doprineti ranijoj dijagnozi, stratifikaciji rizika i prilagođenim terapijskim strategijama.

Ključne reči:
komorbiditet; dijagnoza, rana; jetra, bolesti izazvane alkoholom: muškarci.

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Introduction

Alcoholic liver cirrhosis (ALC) is a significant global health issue, which represents the final stage of chronic liver disease resulting from excessive alcohol consumption. According to the World Health Organization, variations in alcohol consumption range from as low as 0.01 L *per* year in Sudan and Luxembourg to as high as 16.8 L *per* year in Russia, highlighting stark regional differences in alcohol use [1]. Globally, ALC is responsible for approximately 1.32 million deaths annually, underscoring the severity of this health crisis. The rising trend in alcohol consumption, with over 2.6 million alcohol-related deaths in 2019, presents an urgent challenge for legislative bodies and healthcare systems [2].

In recent years, the increasing prevalence of alcohol use and its association with liver-related complications have drawn significant attention to the need for better understanding and management of this condition. ALC leads to progressive liver dysfunction, which manifests not only through liver-specific biochemical markers but also through a variety of external clinical signs and comorbidities that can serve as indicators of disease severity and systemic involvement. This disease is particularly prevalent in developed countries in Europe and the United States, where it is considered the fourth leading cause of premature death among adults [3, 4].

Between 2022 and 2040, the total economic burden of alcohol-associated liver disease is expected to reach approximately $880 billion. This figure encompasses about $355 billion in direct healthcare costs and an estimated $525 billion in losses due to reduced workforce productivity and economic consumption. The annual cost of alcohol-associated liver disease is projected to rise significantly, from $31 billion in 2022 to $66 billion by 2040, marking an increase of 118% [5].

External manifestations of cirrhosis, such as jaundice (icterus), spider nevi, palmar erythema, and gynecomastia, are common and have been widely recognized as indicative of liver dysfunction. These signs are often related to the severity of liver damage and may reflect underlying pathological processes, such as hormonal imbalances, altered bilirubin metabolism, and vascular changes [6–10]. A study by Tapper and Parikh [11] highlights the relationship between these external signs and the stage of liver disease, indicating that their presence can offer valuable clues for early diagnosis and monitoring the progression of cirrhosis.

In addition to external manifestations, patients with ALC often present with a range of comorbidities, which complicate their clinical course and affect their overall prognosis. Diabetes mellitus (DM), cardiovascular disease, and chronic kidney disease (CKD) are frequently observed in this patient population, suggesting that liver cirrhosis has widespread systemic effects [12]. The interplay between liver dysfunction and these comorbid conditions, as noted by Dugum and McCullough [13], underscores the complexity of managing patients with decompensated cirrhosis. Understanding the prevalence and relationships between these comorbidities is crucial for developing effective management strategies.

By examining the distribution of these clinical markers and their associations with disease severity, this study provides valuable insights into the systemic impact of ALC. The findings underscore the need for comprehensive care strategies that address both liver-specific and extrahepatic complications. Moreover, these insights could contribute to faster and earlier diagnosis of alcohol-related diseases, primarily decompensated ALC, which would consequently enable earlier and more comprehensive treatment.

The aim of this study was to examine the prevalence of external manifestations and comorbidities in male patients with decompensated ALC, with an emphasis on how these factors vary with age.

Methods

This prospective, comparative, descriptive, and analytical study was conducted at the Internal Medicine Clinic, University Clinical Center of the Republic of Srpska, in Banja Luka, Bosnia and Herzegovina, within the Department of Gastroenterology and Hepatology. The study was conducted from June 2021 to January 2025, and a total of 123 male patients over the age of 18 years hospitalized for decompensated ALC were included. The mean age of patients was 59.09 ± 9.32 years. The diagnosis of cirrhosis was based on clinical, laboratory, radiological, and endoscopic criteria. The study was conducted in accordance with the Helsinki Declaration and was approved by the Ethics Committee of the University Clinical Center of the Republic of Srpska, Banja Luka, Republic of Srpska, Bosnia and Herzegovina (No. 01-19-462-2/24, from November 28, 2024). All participants provided informed consent for participation in the study.

Exclusion criteria included female patients, patients under the age of 18 years, patients with cirrhosis of other etiologies (viral, autoimmune, metabolic, or biliary), those with concurrent malignancies except hepatocellular carcinoma (HCC), patients with active infections, and those with incomplete medical records.

All patients underwent a comprehensive diagnostic evaluation, which included: clinical examination – focused on identifying external signs of chronic liver damage, such as jaundice, spider nevi, palmar erythema, gynecomastia, and other manifestations; laboratory tests – complete blood count, biochemical parameters for liver and kidney function, coagulation profile, and serological tests for viral hepatitis; radiological methods – abdominal ultrasound to assess liver structure, spleen size, and the presence of ascites and endoscopic examinations – esophagogastroduodenoscopy to evaluate esophageal and gastric varices.

Associated comorbidities were identified based on medical history, clinical records, and previous diagnostic findings. Patients were categorized into age groups to analyze the prevalence of external signs and comorbidities across different age categories.

Statistical analysis

The data were analyzed using descriptive and inferential statistical methods with version 29 SPSS. Categorical variables were presented as absolute and relative frequencies (num-

bers and percentages). The Chi-square test was applied to assess statistical significance, with a significance level set at $p < 0.05$. A detailed description of the statistical tools and methods used for the analysis is provided below. For comparative purposes, patients were divided into the following age groups: under 50 years, 51–60 years, 61–70 years, and over 71 years. The Chi-square test was applied to compare the distribution of categorical variables (such as the presence of external signs of the disease and comorbidities) across the different age groups. This allowed for the assessment of potential associations between age and the frequency of these variables.

Results

The majority of patients belonged to the 61–70 age group (39.80%), followed by the 51–60 age group (36.60%). Patients under 50 years accounted for 14.60%, while those over 71 years made up 8.90% of the study population.

External signs of liver cirrhosis varied among patients, with jaundice being the most common manifestation, present in 98 (79.67%) patients. Spider nevi were observed in 67 (54.47%) patients, followed by palmar erythema in 45 (36.58%) patients. Gynecomastia was diagnosed in 23 (18.69%) patients, while parotid gland enlargement was recorded in 10 (8.13%) cases. Dupuytren's contracture and lacquer tongue were the least frequent, occurring in 1.62% of patients each (Table 1).

The distribution of external signs across different age groups showed significant differences. Jaundice was most prevalent in patients over 61 years ($p = 0.002$), while spider nevi and palmar erythema were more common in younger patients ($p = 0.014$ and $p = 0.027$, respectively). Gynecomastia was significantly associated with the 51–60 age group ($p = 0.032$), whereas parotid gland enlargement was more frequent in patients over 71 years ($p = 0.041$) (Table 2).

Comorbid conditions were frequently observed in the study population. The most common comorbidity was DM, present in 24 (19.51%) patients, followed by congestive heart failure (CHF) in 22 (17.88%) patients. CKD was identified in 14 (11.38%) cases, while peptic ulcer disease (PUD) was found in 7 (5.69%) patients. Chronic obstructive pulmonary disease (COPD) was present in 6 (4.87%) cases, and HCC was diagnosed in 5 (4.06%) patients. Cerebrovascular and peripheral vascular diseases were equally distributed, each occurring in 4 (3.25%) of patients (Table 3).

Table 1

External manifestations of liver cirrhosis

External signs	n (%)	Age groups (years)	*p*-value
Icterus	98 (79.67)	61–70	0.002
Spider nevi	67 (54.47)	51–60	0.014
Palmar erythema	45 (36.58)	51–60	0.027
Gynecomastia	23 (18.69)	51–60	0.032
Parotid swelling	10 (8.13)	61–70	0.041
Dupuytren's contracture	2 (1.62)	no significant difference	0.089
Lacquer tongue	2 (1.62)	no significant difference	0.091

n – number.

Table 2

Distribution of external manifestations by age

External signs	Age groups (years)				*p*-value
	< 50	51–60	61–70	> 71	
Icterus	10 (55.60)	32 (71.10)	43 (87.80)	13 (100.00)	0.002
Spider nevi	12 (66.70)	29 (64.40)	21 (42.90)	5 (45.50)	0.014
Palmar erythema	8 (44.40)	22 (48.90)	12 (24.50)	3 (27.30)	0.027
Gynecomastia	2 (11.10)	12 (26.70)	7 (14.30)	2 (18.20)	0.032
Parotid swelling	0 (0.00)	2 (4.40)	5 (10.20)	3 (27.30)	0.041
Dupuytren's contracture	0 (0.00)	1 (2.20)	1 (2.00)	0 (0.00)	0.089
Lacquer tongue	0 (0.00)	0 (0.00)	1 (2.00)	1 (9.10)	0.091

All values are given as numbers (percentages).

Table 3

Comorbidities in patients according to age

Comorbidities	n (%)	Age groups (years)	*p*-value
Diabetes mellitus	24 (19.51)	61–70	0.009
CHF	22 (17.88)	61–70	0.015
CKD	14 (11.38)	61–70	0.021
Peptic ulcer disease	7 (5.69)	< 60	0.038
COPD	6 (4.87)	51–60	0.033
HCC	5 (4.06)	no significant difference	0.086
Cerebrovascular diseases	4 (3.25)	61–70	0.048
Peripheral vascular diseases	4 (3.25)	61–70	0.048
Rheumatologic diseases	3 (2.43)	no significant difference	0.073
Myocardial infarction	1 (0.81)	> 71	0.061

n – number; CHF – congestive heart failure; CKD – chronic kidney disease; COPD – chronic obstructive pulmonary disease; HCC – hepatocellular carcinoma.

Table 4

Distribution of comorbidities by age

Comorbidities	Age groups (years)				*p*-value
	< 50	51–60	61–70	> 71	
Diabetes mellitus	2 (11.10)	6 (13.30)	12 (24.50)	4 (36.40)	0.009
CHF	2 (11.10)	5 (11.10)	10 (20.40)	5 (45.50)	0.015
CKD	1 (5.60)	4 (8.90)	7 (14.30)	2 (18.20)	0.021
Peptic ulcer disease	2 (11.10)	4 (8.90)	1 (2.00)	0 (0.00)	0.038
COPD	1 (5.60)	3 (6.70)	2 (4.10)	0 (0.00)	0.033
HCC	0 (0.00)	2 (4.40)	2 (4.10)	1 (9.10)	0.086
Cerebrovascular diseases	0 (0.00)	1 (2.20)	2 (4.10)	1 (9.10)	0.048
Peripheral vascular diseases	0 (0.00)	1 (2.20)	2 (4.10)	1 (9.10)	0.048
Rheumatologic diseases	1 (5.60)	0 (0.00)	1 (2.00)	1 (9.10)	0.073
Myocardial infarction	0 (0.00)	0 (0.00)	0 (0.00)	1 (9.10)	0.061

For abbreviations, see Table 3.

All values are given as numbers (percentages).

Comorbidities showed varying distributions across age groups. DM and CHF were significantly more prevalent in older patients ($p = 0.009$ and $p = 0.015$, respectively). CKD was most frequent in patients over 61 years ($p = 0.021$), while COPD was primarily observed in the 51–60 age group ($p = 0.033$). The presence of HCC was evenly distributed across age categories without significant statistical differences ($p = 0.086$) (Table 4).

Discussion

In discussing ALC, it is crucial to emphasize its significant impact on global health, as it represents the final stage of chronic liver disease resulting from excessive alcohol consumption. Data shows that ALC accounts for approximately 1.32 million deaths annually, highlighting the severity of this health issue. Furthermore, the variation in alcohol consumption across regions complicates public health strategies. For instance, while countries like Sudan and Luxembourg have minimal alcohol consumption, Russia reports exceptionally high levels, presenting specific challenges for prevention and treatment in different regions [1, 2, 14].

In recent years, the growing prevalence of alcohol use and its association with liver diseases have become subjects of intense research, as ALC leads to progressive liver dysfunction, which manifests through various clinical signs and comorbidities. These external symptoms, including jaundice, spider nevi, palmar erythema, and gynecomastia, often serve as indicators of liver damage and can reflect underlying systemic changes in the body. These signs are frequently associated with hormonal imbalances, alterations in bilirubin metabolism, and vascular changes, making them crucial for assessing the severity of the disease and its systemic effects [4, 6, 15].

Interestingly, ALC is particularly prevalent in developed countries, such as those in Europe and the United States, where it remains one of the leading causes of premature death among adults [3]. This trend suggests that, despite advances in healthcare, alcohol consumption continues to have a significant negative impact on public health. Therefore, further research is necessary to improve the prevention, diagnosis, and treatment of ALC, as well as to develop public health initiatives that could help reduce the number of alcohol-related deaths associated with this condition [9–11].

The demographic and clinical findings from this study provide significant insights into the characteristics and external manifestations of decompensated ALC. Our cohort aligns with previous studies, which suggest that ALC predominantly affects middle-aged and older men. In addition, our data show that the largest percentage of patients falls within the 51–70 age group [15–19]. Interestingly, while cirrhosis is more commonly seen in patients over 50, decompensated ALC was noted in a considerable number of patients younger than 50, underlining the importance of early identification and management in this group.

External manifestations in liver cirrhosis are important indicators of chronic liver damage and are linked to systemic complications. In our cohort, the most common external sign was icterus, observed in 79.67% of patients. This is consistent with the well-established association between jaundice and liver failure resulting from impaired bilirubin metabolism. A significant age-related trend was observed for icterus, as it predominantly affected patients over 61 years of age ($p = 0.002$).

Spider nevi, another hallmark of cirrhosis, were found in 54.47% of patients, particularly in those younger than 60 years ($p = 0.014$). This result corresponds with prior literature that associates spider nevi with estrogenic effects, commonly seen in younger cirrhosis patients [9, 19–22]. Palmar erythema, another frequent manifestation (36.58%), also demonstrated a significant association with patients under 60 years ($p = 0.027$).

Gynecomastia, a manifestation of hormonal imbalance, was present in 18.69% of patients, with the highest prevalence observed in the 51–60 age group ($p = 0.032$). Parotid swelling was present in only 8.13% of the cohort, with a notable increase in patients over 71 years ($p = 0.041$), likely indicating advanced disease and its systemic effects. Dupuytren's contracture and lacquer tongue were observed in only a small percentage of patients (1.62%), with no significant association to any particular age group. This suggests that while these signs are recognized, they are less commonly seen in the cohort.

The study revealed several comorbid conditions associated with decompensated ALC. DM was present in 19.51% of patients, particularly in those over 61 years ($p = 0.009$). This is consistent with known associations between chronic liver disease and metabolic disorders, such as diabetes, which often results from hepatobiliary dysfunction and insulin resistance seen in cirrhosis [12, 13].

CHF was another prominent comorbidity found in 17.88% of patients. The prevalence of CHF increased with age, with the highest rates observed in patients over 61 years ($p = 0.015$). The pathophysiological link between heart failure and liver cirrhosis, known as cirrhotic cardiomyopathy, may contribute to this association [23–24]. CKD, another comorbidity, was observed in 11.38% of patients and was more prevalent in older patients ($p = 0.021$), reflecting the complexity of multiorgan involvement in advanced cirrhosis.

PUD and COPD were seen in smaller proportions of the sample (5.69% and 4.87%, respectively), with the latter being more common in the 51–60 age group ($p = 0.033$). HCC was diagnosed in 4.06% of the cohort, with no significant age-related variation ($p = 0.086$). This highlights the ongoing risk of HCC development in cirrhosis patients, although the relatively low percentage may be indicative of the cohort's limited follow-up duration or the stage of disease at the time of diagnosis.

Cerebrovascular and peripheral vascular diseases were found in 3.25% of patients, predominantly in those over 61 years ($p = 0.048$), further supporting the multifactorial nature of cirrhosis and its systemic complications. Rheumatologic diseases (2.43%) and myocardial infarction (0.81%) were observed in fewer patients. However, these findings highlight the broad spectrum of comorbid conditions that can be associated with cirrhosis, potentially complicating management and outcomes.

The distribution of external manifestations across different age groups further emphasizes the association between age and the presence of certain signs. For instance, icterus and parotid swelling were more frequent in older age groups, while spider nevi, palmar erythema, and gynecomastia were more common in younger patients. This suggests that the degree of liver dysfunction and systemic involvement may influence the manifestation of specific signs [15, 16, 20, 21].

The study highlights a high prevalence of external signs and comorbidities in male patients with decompensated ALC. Jaundice was the dominant external manifestation, with a significantly higher occurrence in older patients. Comorbidities such as DM and CHF were frequent, particularly in the elderly population. The findings underscore the systemic impact of ALC and the importance of comprehensive clinical assessment in affected patients.

Similarly, the distribution of comorbidities by age group illustrates a tendency for certain conditions, such as DM, CHF, and CKD, to increase with age, reflecting the overall burden of disease in older cirrhosis patients. Interestingly, PUD and COPD were more common in younger patients, possibly reflecting lifestyle factors or earlier onset of disease.

Conclusion

The findings of this study highlight the multifaceted nature of decompensated alcoholic liver cirrhosis, with a high prevalence of external signs and comorbidities closely linked to age. Icterus, spider nevi, and palmar erythema emerged as the most common external manifestations, with significant age-related variations. Similarly, diabetes mellitus, congestive heart failure, and chronic kidney disease were the most frequently encountered comorbidities. These results emphasize the need for comprehensive management strategies that address both liver disease and its associated systemic complications, particularly in older patients. Further studies are warranted to investigate the long-term outcomes and survival rates in this patient population, as well as to identify potential interventions to mitigate the impact of comorbidities on overall health.

Conflict of interest

The author declares that there is no conflict of interest related to this research. The study was conducted without any commercial or financial influences, ensuring the objectivity and integrity of the research process and findings.

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National survey on the use of telepsychology in the work of psychologists in Serbia

Nacionalna anketa o korišćenju telepsihologije u radu psihologa u Srbiji

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Abstract

Background/Aim. In recent decades, telepsychology has gradually developed due to the increasing availability of digital technologies. However, its expansion was led by the pandemic and the introduction of social distancing rules. The aim of this study was to determine the level of computer and internet use by psychologists in Serbia in their everyday work. **Methods.** A hybrid research design was employed, combining survey methodology with content analysis. The survey consisted of 70 questions related to the use of digital technology in the practice of psychologists. The national survey included 340 graduate psychologists of both sexes and all age categories. The participants worked in various regions of Serbia and covered nearly all areas of psychological practice, including healthcare, education, industry, the military, and private practice. **Results.** The questions addressed to psychologists when a respondent is sent for tests assessment most often related to personality exploration, diagnostic/neuropsychological assessments, and psychotherapy-related evaluations. In the total sample, as many as 51% of psychologists in Serbia conducted online therapy, while a significantly smaller number of respondents (17%) performed test assessments *via* the Internet. In assessments, the most frequently used tests were domestically produced and have been in use for a long time, while a smaller number of psychologists used new psychological instruments. The survey revealed that younger psychologists and those from Belgrade valued telepsychology more positively compared to older psychologists and those living and working in other areas. The therapeutic orientation of the respondents was not a significant factor in assessing the success of telepsychology. **Conclusion.** The paper provides an overview of the current situation in the area of personality assessment and shows that the use of digital technology and telepsychology in Serbia is more developed than the professional regulations and education of psychologists regarding this modality. For the widespread application of telepsychology in the work of psychologists, it is necessary to introduce topics related to this specific modality into the curricula of university courses, both globally and in Serbia.

Key words:
digital technology; mental health teletherapy; personality tests; psychological tests; serbia.

Apstrakt

Uvod/Cilj. Poslednjih decenija, telepsihologija se postepeno razvijala zahvaljujući sve većoj dostupnosti digitalnih tehnologija. Međutim, njena ekspanzija bila je podstaknuta pandemijom i uvođenjem pravila socijalnog distanciranja. Cilj rada bio je da se utvrdi nivo korišćenja računara i interneta u svakodnevnom radu psihologa u Srbiji. **Metode.** Primenjen je hibridni istraživački dizajn, istraživanje anketnog tipa sa analizom sadržaja. Anketa je imala 70 pitanja koja su se odnosila na korišćenje digitalne tehnologije u praksi psihologa. Nacionalna anketa obuhvatila je 340 diplomiranih psihologa oba pola i svih starosnih kategorija. Učesnici su radili u različitim regionima Srbije, pokrivajući skoro sve delatnosti psihološke prakse, uključujući zdravstvo, prosvetu, industriju, vojsku, i privatnu praksu. **Rezultati.** Pitanja koja se upućuju psiholozima kada se ispitanik šalje na procenu testovima najčešće su se odnosila na eksploraciju ličnosti, dijagnostičke/neuropsihološke procene i procene vezane za psihoterapiju. U ukupnom uzorku, čak 51% psihologa u Srbiji radilo je *on-line* terapiju, dok je značajno manje anketiranih (17%) radilo procenu testovima putem interneta. U proceni su najčešće korišćeni testovi domaće proizvodnje, koji

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su već dugo u upotrebi, a manji broj psihologa koristio je nove psihološke instrumente. Anketa je pokazala da su mlađi psiholozi i psiholozi iz Beograda pozitivnije vrednovali telepsihologiju u poređenju sa starijim psiholozima i psiholozima koji su živeli i radili u drugim sredinama. Terapijska orijentacija anketiranih nije bila značajna za procenu uspešnosti telepsihologije. **Zaključak.** Rad daje pregled aktuelnog stanja u oblasti procene ličnosti i pokazuje da je korišćenje digitalne tehnologije i telepsihologije u Srbiji razvijenije od profesionalne regulative i edukacije psihologa o tom modalitetu. Za široku primenu telepsihologije u radu psihologa potrebno je da se u nastavni plan i program predmeta na fakultetima i u svetu i kod nas uvedu teme vezane za taj specifični modalitet.

Ključne reči:
tehnologija, digitalna; mentalno zdravlje, teleterapija; ličnost, testovi; psihološki testovi; srbija.

Introduction

The development of digital technology and the coronavirus disease 2019 (COVID-19) pandemic have contributed to the growing use of telepsychology in the work of psychologists. Telepsychology is broadly defined as the provision of psychological services through telecommunication technologies, such as telephone, mobile devices, interactive video conferences, e-mail, chatting, text messaging, and the Internet, as well as self-help websites, blogs, and social media platforms [1]. A specific area within telepsychology is teleassessment, i.e., working with a client through the administration of test materials, processing of test data, and report writing using digital technology.

The number of papers dedicated to telepsychology shows that psychotherapy has been the quickest to adopt this new medium of work [2]. As for personality test assessment, computer technology is utilized in various ways within this area of psychology: the use of digital test formats in direct contact with the client or online without direct client interaction, the use of software for the quantitative processing of test results and computerized textual interpretation, the development of software for writing psychological reports, experimental models of personality assessment (the term is broadly defined and encompasses the evaluation of all aspects of personality, including cognitive functions) through virtual reality, and, in behavioral psychology – psychobiological monitoring, where psychological reactions of respondents are recorded digitally throughout the day [3].

Today, it is both ethically and professionally accepted that the use of computers in psychology, including personality test assessment and computer-generated test results, is considered ethically and professionally correct, provided they are used exclusively by trained psychologists who must also be aware of the limitations associated with the use of computers [4].

Clients and psychologists report numerous benefits of telepsychology, which are described in the text that follows. Psychological services have become more accessible to clients living in rural areas or abroad. Telepractice also allows clients to communicate in their native language with a psychologist or examiner who shares their cultural background, an important aspect in modern psychology [5]. The cost of services can be lower because there is no need to rent office space, the participants are in their personal environments, and the sessions take place in virtual spaces. It is more comfortable for the client as there is no need to travel to the psychologist, no waiting in waiting rooms, and no need to take time off work. During the pandemic, both users of psychological services and professionals themselves feel safer in terms of health [6]. Clients find it easier to approach a psychologist because they perceive the situation as less intimidating and less obligatory. Clients have the impression that they have control over the communication, so they are less anxious [7]. Sometimes, the physical distance from the examiner makes respondents feel more relaxed, leading them to provide more honest answers to items involving intimate content during computer-based testing compared to interviews. Computer-assisted testing is faster, and test administration is standardized using publishers' platforms, which increases the validity of results. The use of computers also enables more precise measurement of respondents' reactions and allows the testing process to be adapted to individual respondents based on their answers [8].

The use of virtual reality in personality assessment holds particular significance [1], which allows performance-based tests with greater ecological validity to be used instead of self-reporting [9]. The application of virtual reality generates new ideas in the field of psychological science and inspires a large number of new studies. There are already attempts to examine various phenomena using virtual reality [10], most commonly cognitive functions such as memory [11, 12] and executive functions [13, 14], but also social interactions [15], anxiety, and social phobias [16].

Clients and psychologists frequently cite technical difficulties that disrupt communication as a weakness of telepsychology. Issues such as slow Internet speed and interruptions, poor video quality, echoing sound, and challenges in presenting test materials on a monitor can disrupt communication. If technical conditions are good, clients often prefer teleassessment; however, if the conditions are poor, they prefer face-to-face assessment [17].

The following observations are also noted as weaknesses of telepractice. Communication occurs differently, being less spontaneous and flexible compared to face-to-face interaction. Observing the client *via* monitor is limited; without personal contact and non-verbal cues, it becomes more challenging to make accurate judgments about the client and diagnostic assessments [18]. Testing the client's honesty and openness becomes more demanding, as does working to develop trust and alliance with the client. Clients who are dishonest or have ulterior motives, such as those involved in legal proceedings, may find it easier to manipulate the process in the absence of face-to-face contact. Telepsychology is not

suitable for certain categories of respondents: acutely psychotic individuals, clients with severe depression, those prone to acting-out behavior, and self-harming individuals [19]. The likelihood of mutual misunderstanding or the client misinterpreting the information provided by the psychologist (e.g., assessment results or recommendations) increases. There are still no specific training programs for providing psychological services online, and psychologists generally have less experience in virtual environments, which increases the likelihood of errors [3].

Globally, there is an increasing number of works focused on the topics of telepsychology, raising questions about the situation in Serbia, especially in the field of personality assessment. Personality test assessment is a fundamental activity for psychologists with a long-standing tradition. Both globally and in Serbia, it is mostly carried out face-to-face and using paper-and-pencil tests [20]. The introduction of the digital paradigm has gradually transformed test administration, blurring the line between traditional and computerized testing. Today, almost all psychologists use computers at some stage of the assessment process, which contributes to the validity and efficiency of testing.

The aim of the study was to include as many psychologists as possible working from various fields to collect, for the first time, data on the extent to which telepsychology and online test assessments are used by psychologists in Serbia. Additionally, the study analyzed the frequency of computer and specialized software use in their work, as well as psychologists' perceptions of the advantages and disadvantages of working with modern technologies.

Methods

Participants and procedure

A hybrid research design was employed, combining survey methodology with content analysis. The survey was conducted in 2023 and 2024 and included 70 questions related to the use of digital technology in psychological practice (e.g., psychotherapy, online teaching), along with 39 questions specifically focused on personality assessment. The study analyzed the use of computers in psychologists' daily work, particularly in personality assessment and writing psychological reports. The study was approved by the Ethics Committee of the Academy for Human Development, Belgrade, Serbia (approval No. 7/21). The study adheres to the ethical guidelines and requirements outlined in the Declaration of Helsinki.

The survey was completed by 340 psychologists living and working in Serbia. A convenience sampling method was used, as the survey was distributed electronically to over 1,000 psychologists *via* e-mail. Recipients included members of professional associations or psychotherapy organizations, professors at faculties of psychology, scientific workers at institutes, and psychologists advertising their services on social media or websites. The applied survey was designed for the purposes of this research. After a brief sociodemographic questionnaire, which included questions regarding age, years of work experience, education level, workplace, and place of residence, respondents answered specific questions related to the use of computers and the Internet in their professional context (e.g., whether they perform personality assessments online, use data processing programs, conduct psychotherapy online, etc.). Additionally, the survey included questions regarding the frequency of use of various psychological measurement instruments (answered on a Likert scale from 1 to 3, where 1 means "never" and 3 means "often"), the therapeutic modalities they apply in practice, and the types of psychological reports they most commonly write. Finally, the survey contained specific items regarding the experience and satisfaction using the Internet in the context of assessment and psychotherapy (answered on a Likert scale from 1 to 5, where 1 means "I am not satisfied at all" and 5 means "I am fully satisfied"). There was also an option for open-ended responses, which were subject to qualitative analysis in order to explore the advantages and challenges of online work in the context of assessment and psychotherapy.

Statistical analysis

Descriptive analyses were conducted on the survey responses. Additionally, scale measures obtained from the survey were standardized to facilitate comparison both between age groups and between psychologists working in smaller towns. Finally, in order to determine differences in satisfaction with telepsychology based on gender, place of residence, years of professional experience, and applied therapeutic modality, the Chi-square test was used. Data analysis was conducted using IBM SPSS version 26.0.

Results

Of the total respondents, the majority were female, reflecting the fact that women are more represented among psychology students and in the profession as a whole. In the sample, there was an equal number of respondents younger (50.6%) and older than 40 years (49.4%) (Table 1).

Table 1

Gender and age of psychologists in the total sample (n = 340)

Parameters	Values
Gender	
men	40 (11.8)
woman	300 (88.2)
Age (years)	
less than 30	42 (12.4)
31–40	130 (38.2)
41–50	67 (19.7)
over 50	101 (29.7)

Values are given as numbers (percentages).

Half of the surveyed psychologists (50.0%) were from Belgrade, 12.9% were from Novi Sad, and 5.5% were from Niš. While most psychologists resided and worked in Serbia's largest cities, the sample's diversity was enhanced by the fact that 22.4% of the surveyed psychologists lived in 52 smaller towns across Serbia.

In terms of work experience, 50.3% of respondents had between 11 and 30 years of professional experience. Among the respondents, 37.9% had less than 10 years of work experience, and 11.8% had more than 31 years of experience.

In terms of education, the sample characteristics are as follows: 48% of respondents completed basic studies (four-year), 44% held a master's degree, and 8% held a doctoral degree. Additionally, 20.1% of respondents completed specialization programs, most commonly in medical psychology (18.3%), which prepare them for clinical assessment.

The survey included psychologists working in various fields, as shown in Table 2. One-fifth of the respondents (20.3%) have experience working in different contexts concurrently. Among the survey participants, 14.4% work exclusively in private practice.

Of the 340 psychologists surveyed, 332 (97.7%), as expected, reported regularly or occasionally using computers in their work. Table 3 shows the primary reasons psychologists used computers and the Internet.

Most of the surveyed psychologists worked with clients: adults (82%), adolescents (59.6%), and children (33.3%). Only 2.4% of respondents did not work with clients; instead, they worked solely as professors, researchers, or in fields such as marketing or ministries.

Table 2

Fields of work among psychologists (n = 340)

Parameters	Values
Private practice	120 (35.3)
psychologists working only in private practice	49 (14.4)
psychologists working in multiple fields	69 (20.3)
other (they did not answer)	2 (0.6)
Schools and boarding schools	58 (17.0)
Centers for social work and other social protection institutions (center for family placement and adoption, palliative care, day care for children with developmental disabilities)	55 (16.2)
Health facilities (without psychiatry)	54 (15.9)
Faculties and scientific research organizations	42 (12.4)
Psychiatric institutions	38 (11.2)
National Employment Service	20 (5.9)
Work organizations/human resources	14 (4.1)
Non-governmental organizations/civil sector	11 (3.2)
Army	7 (2.1)
Marketing agencies	6 (1.8)
Prison and prison hospital	5 (1.5)

Values are given as numbers (percentages).
***Note*: Psychologists could indicate that they work in more areas.**

Table 3

Reasons for using computers or the Internet for work ([1]n = 339)

Parameters	Values
Searching for references and information relevant to the job	273 (80.3)
Written part of work: writing reports and scientific papers, making presentations, work plans, and reports	257 (75.6)
Online education and training	210 (61.8)
Online psychotherapy and counseling	148 (43.5)
Using a personal computer (and software developed by home psychologists) to calculate test scores	142 (41.8)
Conducting research (sending surveys, processing data, etc.)	147 (43.2)
Entering patient/client data into the electronic record	59 (17.4)
An electronic form of the test that is completed by the examinee during face-to-face testing	57 (16.8)
Using the service of a domestic test publisher for data processing (without textual interpretation)	48 (14.1)
Test assessment of clients *via* the Internet (interview and test assignment)	46 (13.5)
Using software programs (foreign test publishers) that have textual interpretations of test results	44 (12.9)
Teaching at the university	31 (9.1)
Other: taking notes during the session, communicating with colleagues and clients online or through emails, marketing campaigns, writing for social networks	29 (8.5)

Values are given as numbers (percentages).
***Note*: [1] One respondent did not answer the questions. Respondents could answer multiple questions.**

In the total sample of 340 psychologists, 173 (50.9%) regularly conducted test assessments, 99 (29.1%) conducted them occasionally, and 68 (20.0%) did not use psychological instruments for personality assessment. Out of 272 psychologists who performed assessments, 57 (21.0%) reported that they often or occasionally assessed clients based solely on interviews, without using tests.

In further analyses, only a portion of the sample was used, specifically the surveyed psychologists involved in a personality assessment. Reasons for referral for an appropriate psychological assessment are presented in Table 4.

Table 5 shows the psychological instruments most often used by psychologists in the tested sample. Respondents were allowed to select multiple tests they use in their practice. Instruments reported by fewer than five respondents are not included in the table, nor is the Wechsler Adult Intelligence Scale (WAIS)-IV test, which had only recently begun to be implemented in Serbia at the time of the survey.

According to assessment guidelines, all instruments used should be documented in the report. The majority of respondents (63.2%) consistently followed this rule; however, a substantial proportion of Serbian psychologists reported doing so only occasionally (24.5%) or never (12.3%). Most respondents (42.2%) never included test scores in their reports, while 36.8% reported doing so occasionally, and only 2.9% of those surveyed included them constantly.

An analysis of the subset of respondents involved in test assessment (n = 272) revealed that only 46 (16.9%) respondents regularly or frequently used the personality assessment online. In the total sample, 51% of those

Table 4

Reasons for referral for a psychological assessment ([1]n = 272)

Parameters	Values
Personality exploration	189 (69.5)
Intelligence assessment	160 (58.8)
Assessments related to psychotherapy	136 (50.0)
Diagnosis assessment	114 (41.9)
Professional orientation and training for the unemployed	93 (34.2)
Personnel selection	82 (30.2)
Assessments and training of already employed personnel	72 (26.5)
Assessment for various types of social assistance	68 (25.0)
Assessment of the child's problems in the school system	60 (22.1)
Monitoring treatment progress	59 (21.7)
Neuropsychological assessment	58 (21.3)
Assessment of work ability	58 (21.3)
Forensic assessments	33 (12.1)
Assessment of maturity for starting school	32 (11.8)
Resolving a hold-up in any treatment	32 (11.8)
Assessment of the ability to drive	21 (7.7)
Assessment of the ability to carry weapons	20 (7.4)

Values are given as numbers (percentages).
***Note*: [1] Respondents could mark multiple answers. Not all respondents answered all questions.**

Table 5

Psychological instruments used in psychological assessment ([1]n = 272)

Parameters	Values
Drawing (human figures, families, trees, houses)	228 (83.8)
Wechsler Individual Intelligence Test, Serbian version	175 (64.3)
Modified Minnesota Multiphasic Personality Inventory (MMPI 201 or MMPI 202)	161 (59.2)
Test of Incomplete Sentences or Basic Supports of Personality	156 (57.3)
Revised NEO Personality Inventory [(NEO PI-R/NEO Five Factor Inventory (NEO-FFI)]	145 (53.3)
Child assessment instruments	137 (50.4)
Personality Assessment Inventory (PAI)	119 (43.7)
The Big Five Plus Two Personality Inventory (BF + 2)	114 (41.9)
Neuropsychological tests	110 (40.4)
Rorschach method	82 (30.1)
Sixteen Personality Factor Questionnaire (16 PF)	73 (26.8)
Millon® Clinical Multiaxial Inventory (MCMI-any form)	69 (25.3)
Thematic Apperception Test (TAT)	49 (18.0)
Cybernetic battery of conative tests (KON-6); battery of intelligence tests (KOG-3)	16 (5.9)
Temperament And Character Inventory-Revised (TCI-R)	14 (5.1)
Emotions Profile Index (EPI)	14 (5.1)
Test of Professional Interests (TPI)	12 (4.4)

Values are given as numbers (percentages).
***Note*: [1] Not all respondents answered all questions.**

surveyed reported providing online therapy either consistently or occasionally.

As shown in Table 6, psychologists utilized computers in multiple ways for processing and interpreting test results.

Half of the respondents (55.6%) regularly gave oral feedback to clients about the assessment results, 25.9% frequently did so, and 18.5% of the surveyed psychologists rarely or never provided such feedback. The report was primarily written for the commissioning party, with only 0.2% of respondents indicating that they wrote it directly for the client.

To examine whether satisfaction with telepsychology differed by gender, age, place of residence, years of professional experience, and therapeutic modality used, a Chi-squared test was conducted. A total of 226 respondents answered this question. For easier interpretation, the variable "satisfaction with telepsychology" was summarized into three categories: respondents who rated it as 1 or 2 were grouped as "dissatisfied with telepsychology," those who gave a rating of 3 were grouped as "neither satisfied nor dissatisfied," while those who rated it as 4 or 5 were grouped as "satisfied with telepsychology."

The Chi-squared test showed that psychologists differed in the level of satisfaction with telepsychology concerning age [$\chi^2(6) = 44.17$, $p = 0.00$], place of residence [χ^2 (2) = 31.21, $p = 0.00$], and years of professional experience [χ^2 (6) = 36.93, $p = 0.00$], while there were no differences in the levels of satisfaction concerning gender [χ^2 (2) = 0.23, $p = 0.89$] or therapeutic modality which was used, where cognitive-behavioral therapists were compared to therapists of other modalities [χ^2 (2) = 2.44, $p = 0.30$].

Table 7 presents the number and percentage representation of dissatisfied, indecisive, and satisfied psychologists regarding their age group. Standardized residuals are also presented, allowing us to determine the "source" of statistically significant differences. It can be observed that younger respondents were significantly more satisfied with telepsychology compared to older ones, while almost half of psychologists over the age of 50 were either partially or entirely dissatisfied with telepsychology.

When considering the respondents' place of residence, it can be seen that psychologists from Belgrade were significantly more satisfied with telepsychology compared to psychologists from other places (Table 8).

Table 6

Using a computer in processing the results of psychological assessment ([1]n = 272)

Parameters	Values
The domestic publisher's platforms are used for test application and score processing, based on the Serbian standardization	37 (13.6)
The services (for grading and graphical presentation of results) of the domestic test publisher, based on the Serbian standardization, are used	122 (44.9)
Software programs from foreign publishers that have textual interpretations are used (they are not based on the results of domestic standardizations)	44 (16.2)
Electronic versions of assignments and scores created by local psychologists, which have not been research-verified, are used	37 (13.6)
Data processing programs are used on personal computers, created by local psychologists, and have not been verified by research	142 (52.2)
The results are scored and interpreted without the use of a computer	83 (30.5)

Values are given as numbers (percentages).
***Note*: Not all respondents answered all questions. [1]Respondents could mark multiple answers.**

Table 7

Satisfaction with telepsychology by age ([1]n = 226)

Satisfaction levels	Under 30 years		31–40 years		41–50 years		Over 50 years	
	n (%)	adj. res	n (%)	adj. res	n (%)	adj. res	n (%)	adj. res
Dissatisfied	4 (15)	-0.5	3 (4)	-3.7	6 (10)	-2.1	29 (43)	6.1
Indecisive	3 (11)	-1.5	15 (21)	-0.2	18 (30)	1.6	14 (21)	-0.4
Satisfied	20 (74)	1.7	52 (74)	3.1	37 (61)	0.3	25 (37)	-4.5

n – number; % – percentage; ajd. res. – adjusted standardised results.
***Note*: [1] 226 respondents answered the question about the possible difference in the level of satisfaction with telepsychology considering gender, age, place of residence, years of professional experience, and therapeutic modality used.**

Table 8

Assessment of satisfaction with telepsychology by workplace ([1]n = 226)

Satisfaction levels	Belgrade		Other places	
	n (%)	adj. res.	n (%)	adj. res.
Dissatisfied	9 (7.4)	-4.6	33 (31.4)	4.6
Indecisive	21 (17.4)	-1.9	29 (27.6)	1.9
Satisfied	91 (75.2)	5.2	43 (41.0)	-5.2

For abbreviations, see Table 7.
***Note*: [1] Explanation of the number of respondents is given in the legend of Table 7.**

Table 9

Assessment of satisfaction with telepsychology by work experience ([1]n = 226)

Satisfaction levels	Up to 10 years		11–20 years		21–30 years		Over 30 years	
	n (%)	adj. res.	n (%)	adj. res.	n (%)	adj. res.	n (%)	adj. res.
Dissatisfied	7 (8.4)	-3.0	6 (8.5)	-2.7	15 (34.9)	3.1	14 (48.3)	4.4
Indecisive	17 (20.5)	-0.5	18 (25.4)	0.8	10 (23.3)	0.2	5 (17.2)	-0.7
Satisfied	59 (71.1)	2.7	47 (66.2)	1.4	18 (41.9)	-2.6	10 (34.5)	-2.9

For abbreviations, see Table 7.

***Note*: [1] Explanation on the number of respondents is given in the legend of Table 7.**

Finally, Table 9 shows that as the years of work experience increased, satisfaction with telepsychology decreased.

Discussion

The use of computers is an integral part of psychologists' daily professional activities, ranging from writing scientific papers and reports to education, business communication, and digital presentation of test materials. In this regard, Serbian psychologists do not differ from their colleagues around the world [21]. Based on the survey results, it can be concluded that psychologists in Serbia, much like their counterparts worldwide, have accepted the innovations brought by telepsychology, with this adoption process accelerating during the pandemic. It can also be concluded that telepsychotherapy is far more prevalent among psychologists in Serbia than personality test assessment conducted *via* the Internet. As for online work with clients, it seems that psychotherapists in Serbia have quickly embraced online or hybrid forms of psychotherapy, primarily because they are cost-effective and allow for culturally adapted work with distant clients [2].

Although the number of surveyed psychologists using online personality assessments is not large, those who do report several perceived benefits. These include increased access to clients living in remote areas, shorter sessions, faster data processing, time savings, and the ability to test a larger number of clients. Additionally, telepractice reduces travel time for clients and reduces service costs because there are no expenses for business premises. However, Serbian psychologists also report numerous drawbacks of teleassessment. These include the following: greater difficulty in making accurate diagnostic judgments; the psychologist is more worried about making an oversight because there is less information about the client; concerns are raised about accurately verifying the client's identity; it is more difficult to establish trust; there is a possibility for the client to record the test material and abuse it; the client can interrupt sessions at their convenience; services are more difficult to charge. There are no cultural specificities in the responses of Serbian psychologists. A qualitative analysis of the responses revealed patterns and concerns similar to those identified by the authors referenced in the introductory section of this paper.

The results show that half of the surveyed psychologists use platforms and services from local publishers, and this number is not higher due to economic reasons. The habit of scoring test data manually, without the use of computers or programs created by psychologists, is also a reason why official platforms are not used. In each of these cases, the software in use has not been validated through research. Modern psychological instruments are sophisticated, and it is not economical to process results by counting scores with the help of templates and plotting graphs, especially when working with large groups of respondents. Another reason is that these services often offer textual interpretations that are not based on domestic standardization, which makes the interpretations questionable. Given that these services are charged, the number of users can be considered large, and the credit goes mostly to state institutions that are ready to pay for these services for their employed psychologists. In the area of personality assessment, changes are being introduced significantly slower in Serbia because they require more material resources, such as investment in new forms of tests, technologically improved computers that make it easier to monitor test performance online, investment in platforms, software, or services from publishers that provide scoring or textual interpretation of test results.

The number of studies comparing the adequacy of norms for online and classic test applications remains limited [22], and such research has not yet been published in our region, primarily due to the limited use of online testing. Another reason for the slow changes in this area is the restraint of psychologists. Personality assessment should provide clear and measurable results, which are presented in a written report in an explicit manner, so they may be subjected to later criticism or reevaluation. This leads to the fact that psychologists are more cautious in their work and are aware that they have a smaller range of information in online assessments than in face-to-face testing.

As in the case of the previous survey [23], we can see that psychologists in Serbia are still using older forms of instruments, many of which have not been (re)standardized, and they are not sufficiently educated on new-generation instruments. The main reason for such a situation is that the application of new instruments requires material investments in purchasing tests, education, and the provision of economic data processing. Secondly, for many psychologists, habits and previously acquired practical experience are more important than the question of whether the instrument has been proven valid and standardized in research. When they start their practice, Serbian psychologists usually continue to use the instruments for which they were trained during their studies. Therefore, it is essential to introduce new-generation

instruments into university curricula as soon as possible so that innovations can be implemented more quickly into practical work. Ethical, legal, and practical issues necessitate the introduction of more clear-cut recommendations and regulations [24], and Serbian professional associations should regulate various aspects of telepractice.

The survey provided data showing that some psychologists conduct assessments without using tests, fail to specify the applied instruments in their reports, or create their own electronic versions for assigning instruments or processing data, which have not been research-verified. Another unfavorable indicator is the frequent use of the interview as the sole assessment technique. The survey also indicated that a number of psychologists do not provide feedback to clients regarding assessment results, and reports written solely for the client are extremely rare, even though referral questions in our local community are similar to referral patterns observed globally [25]. Serbian psychologists are not sufficiently informed about the principles of collaborative-therapeutic assessment, which are now considered a standard in the field. The psychologist's professional obligation is to provide the client with information about the results of the test assessment, the only exception being judicial assessments. The modern point of view is that the analysis of the results should be balanced (stating the client's strengths, not only problems and weaknesses), and the assessment should be collaborative and therapeutic – integrated with brief psychotherapy and allowing the client to think about the results and use them in further therapeutic treatment. All these are work principles that deserve attention, and during their studies, students should be educated about the rules and modern trends in the work of psychologists. These survey results suggest that it would be beneficial to introduce theoretical and practical training during studies on how to give both oral and written information about the assessment results. It is part of the therapeutic-collaborative assessment, which is today considered a rule in the area of personality assessment [3].

Clients are referred to psychologists with referral questions that are identical to those found everywhere else in the world [20]. Their review highlights the growing involvement of psychologists across numerous fields and the increasing diversity of questions they are expected to address. In recent decades, there has been an increasing offer of psychological services and society's awareness of the contributions psychologists can make, which will likely continue to strengthen the role of psychologists in many decisions and therapeutic activities important for clients. The pandemic period, as well as major social traumas (bombing, wars, mass murders), have undoubtedly contributed to the increasing involvement of psychologists in the community and outside of traditional institutions.

The survey showed that older and more experienced psychologists are less satisfied with telepsychology, which can be interpreted as a result of their professional habits, i.e., that they have already developed their own way of working with clients and are reluctant to introduce novelties and modify their tried and successful methods. Since they have more work experience, they may perceive more delicately the differences between online work and personal contact with clients. On the other hand, younger psychologists grew up with digital technologies and consider such forms of communication more natural than older generations. Their better command of technique may also be the reason for such survey data. This finding is possibly culturally specific, as other authors [19] have found that older respondents were more satisfied with telepsychology. The authors believe that greater experience and training help older psychologists cope better with problems in telepsychology. In contrast, our more experienced psychologists, despite their greater experience, still value face-to-face work with clients more.

The results also showed that psychologists from Belgrade value telepsychology more than psychologists from other places. Similar reports have been obtained in other studies, possibly because the functionality of internet services is better and more accessible compared to the technical possibilities in smaller towns. Additionally, there are more institutions and workplaces in the cities that allow psychologists to use specialized computer programs for administering test materials, test publisher platforms, and results processing services; therefore, the experiences of psychologists are more positive.

The survey results indicate that the practice of telepsychology in Serbia is ahead of theory and legal regulations. Psychologists already apply telepractice, so our psychological community should formulate ethical and professional working principles in this specific area. The assumption is that a psychologist must be an expert in the field of personality assessment in order to apply that knowledge in teleassessment, which, by its nature, reduces the amount of available information and limits perception and communication with the respondent. Telepsychology requires an experienced psychologist and is particularly challenging due to the limited information about the client's identity and pathology. Furthermore, it is not a simple transposition of assessment into a new medium, but it has specific features that require the psychologist's competencies to be expanded. Therefore, it would be beneficial for university curricula to include new-generation instruments that are also applied in the world, as well as theoretical topics and practical skills related to the use of computers and the Internet in the work of psychologists [26]. Serbian psychologists should be informed during their studies about how the introduction of a digital paradigm into psychology leads to fundamental changes in psychology science, and the innovative use of virtual reality in experimental psychology, personality assessment, and psychotherapy is particularly important.

Limitations of the study

Psychologists in Serbia do not have a chamber, and thus no unified database on the number of psychologists in the country and their territorial distribution, so we can only assume that our sample represented the current situation quite well. Serbian Psychological Society, as the only professional association, gathers slightly more than 600

psychologists, while the actual number of psychologists in Serbia is probably two or three times higher. The survey was sent online to over 1,000 addresses, but only a third of the responses were received. Such a comprehensive survey has not been conducted in our community so far, and we believe that every ten years, a survey should be conducted to monitor the development of psychology in Serbia.

Conclusion

Overall, the findings suggest that the introduction of the digital paradigm into psychological science has sparked the development of new ideas in the field. Psychologists in Serbia have begun to implement telepsychology in practice; however, there remains a need for greater educational, theoretical, and research support to enhance its effective use. The obtained reports provide an overview of the current state of psychological practice in Serbia and indicate that the implementation of telepsychology is ahead of research, ethical, and professional regulations. The widespread application of telepsychology in our country requires the inclusion of topics related to this specific modality of psychological work in university curricula.

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Surgical treatment of pleurocutaneous fistula in a patient 20 years after radiation therapy and breast-conserving surgery

Hirurško lečenje bolesnice sa pleurokutanom fistulom 20 godina posle poštedne operacije dojke i zračne terapije

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Abstract

Introduction. Radiation therapy after breast-conserving surgery substantially reduces the risk of local disease recurrence and moderately reduces the risk of cancer-related death. However, long-term side effects are related to numerous factors, including patient age, comorbidities, total radiation dose, fraction size, and extent of disease. **Case report.** We present the case of a 61-year-old female patient who developed an ulceration at the center of a postoperative scar, accompanied by progressive tissue destruction that eventually reached 6 × 3 cm in size, resulting in rib exposure. Twenty years ago, she had a conservative surgery of the right breast due to invasive ductal carcinoma with postoperative radiation therapy to the right chest wall and the draining lymphatics. A simple mastectomy with operative removal of all sclerotic and calcified tissue, with reconstruction of the right thoracic wall, was performed by a multidisciplinary team comprising a surgical oncologist, thoracic surgeon, and plastic surgeon. The right rib cage defect of 12 × 12 cm was reconstructed with Mersilene® mesh. A large 21 × 15 cm island musculocutaneous *latissimus dorsi* flap was raised and transposed for reconstruction of the soft tissue defect. One thoracic and one wound drain were set in place. The postoperative course was uneventful, and the patient was discharged on the 12th postoperative day. She had no complications after a six-month follow-up. **Conclusion.** Long-term complications of radiation therapy are rare, but they are usually accompanied by severe consequences that require a multidisciplinary approach in complex surgical treatment.

Key words:
breast neoplasms; fistula; radiotherapy; surgical procedures, operative.

Apstrakt

Uvod. Radioterapija nakon poštedne operacije dojke smanjuje rizik od ponovnog lokalnog pojavljivanja bolesti i umereno smanjuje rizik od smrti izazvane rakom. Međutim, njeni dugoročni neželjeni efekti povezani su sa mnogobrojnim faktorima, uključujući starost bolesnika, komorbiditete, ukupnu dozu zračenja, veličinu frakcije, i stepen bolesti. **Prikaz bolesnika.** Prikazujemo bolesnicu staru 61 godinu koja je razvila ulceraciju u sredini postoperativnog ožiljka, praćenu progresivnom destrukcijom tkiva koja je na kraju dostigla veličinu 6 × 3 cm, što je rezultiralo ekspozicijom rebra. Pre dvadeset godina imala je poštednu operaciju desne dojke zbog invazivnog duktalnog karcinoma, sa postoperativnom terapijom zračenjem desnog zida grudnog koša i pripadajućih limfnih čvorova. Jednostavnu mastektomiju sa operativnim uklanjanjem svih sklerotičnih i kalcifikovanih tkiva, sa rekonstrukcijom desnog torakalnog zida, uradio je multidisciplinarni tim sačinjen od onkohiruga, torakalnog hirurga i plastičnog hirurga. Defekt desnog rebarnog luka 12 × 12 cm rekonstruisan je Mersilene® mrežicom. Za rekonstrukciju defekta mekog tkiva podignut je i premešten „ostrvski“ muskulokutani *latissimus dorsi* režanj veličine 21 × 15 cm. Postavljeni su jedan torakalni dren i jedan dren za ranu. Postoperativni tok protekao je uredno i bolesnica je otpuštena 12. postoperativnog dana. Nije imala komplikacije nakon šestomesečnog praćenja. **Zaključak.** Kasne komplikacije terapije zračenjem su retke, ali su obično praćene teškim posledicama koje zahtevaju multidisciplinarni pristup u kompleksnom hirurškom lečenju.

Ključne reči:
dojka, neoplazme; fistula; radioterapija; hirurgija, operativne procedure.

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Introduction

Radiation therapy (RT) after breast-conserving surgery substantially reduces the risk of local disease recurrence and moderately reduces the risk of cancer-related death. RT also affects healthy tissue, causing early or long-term side effects. The latter ones mostly engage superficial skin layers and seldom progress to deeper structures, causing fistula with the respiratory tract, with only a few patients described [1].

Late consequences of radiation occur in a small percentage of patients with breast cancer who were irradiated and operated on, most often after more than 10 years from the radiation therapy [2]. The frequency of rib fractures is 1.8%, while tissue necrosis occurs in only 0.18% of patients who had tissue necrosis with a breast defect and also received chemotherapy before radiation [3].

Case report

We present a 61-year-old woman with pleurocutaneous fistula (PCF) after RT. Initially, in 1997, she underwent conservative surgery on the right breast due to invasive ductal carcinoma. The patient was followed up for the first 5 years after radiation and surgery, and she did not come for check-ups after that period. Postoperatively, she was treated with right chest wall (CW) and draining lymphatics RT. The telecobalt unit was used in a tangential field technique on the CW and parallel opposed fields to the supraclavicular and axillary regions. She was prescribed a dose of 40 Gy in 15 fractions over 15 days and an additional 10 Gy in 4 fractions. Bolus 0.3 mm was used to treat the CW. The axilla and supraclavicular regions were treated on the second day using two parallel opposing fields, prescribing a mid-plane dose of 40.0 Gy in 15 fractions over 15 days. After RT, she developed sclerosis of the right breast and paresis of the right brachial plexus.

Twenty years later, in July 2017, a small ulceration developed in the middle of the postoperative scar with progressive tissue destruction. By the time of admission to the Institute for Oncology and Radiology of Serbia, the lesion had reached a size of 6 × 3 cm, with rib exposure, and the remaining breast tissue was extremely sclerotic (Figure 1). The histopathology report of the ulceration biopsy showed no sign of malignancy.

Head, spine, pelvis, abdominal, chest X-ray, ultrasonography, and multislice computed tomography (MSCT) showed no sign of metastatic disease. Chest MSCT revealed fractures of the third to sixth ribs (Figure 2A), along with large breast calcifications and a partial pneumothorax (Figure 2B). The patient had no other comorbidities. Bacterial and fungal culture tests were negative.

Fig 1 – Ulceration in the middle of the postoperative scar with tissue destruction and rib exposure.

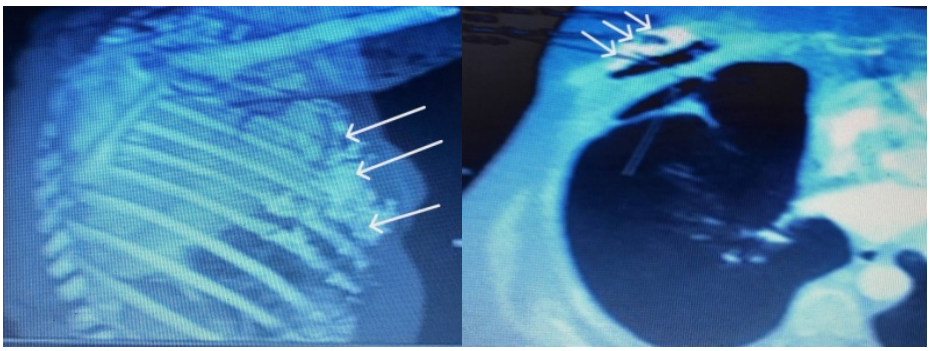
A)  **B)**

Fig 2 – Multislice computed tomography of the thorax shows A) fractures of the third to sixth ribs (indicated by white arrows), B) a calcified breast (indicated by white arrows) with a partial pneumothorax.

Operation plan

A simple mastectomy with the removal of calcified and sclerotic tissue was planned. In order to reconstruct thoracic wall defects, several options were discussed. The pectoralis major muscle could not be utilized due to *en bloc* adhesions with fibrotic and calcified breast tissue. The use of a transverse rectus abdominis muscle flap was precluded by the patient's obesity, and free flap reconstruction was not feasible due to the lack of technical support. A pedicled *latissimus dorsi* (LD) myocutaneous flap was planned for tissue defect coverage, while a Prolene™ mesh was selected for reconstruction of the thoracic wall defect.

Intraoperative finding

The procedure was performed by a multidisciplinary team that included a surgical oncologist, thoracic surgeon, and plastic surgeon. During breast excision, extensive areas of calcified tissue were identified, predominantly in the central and lower lateral poles. Parts of the pectoral major and minor muscles were also resected due to severe fibrosis (Figure 3A, B). Calcification and fractures were identified in the proximal portions of the excised third to sixth ribs, as well as part of the seventh rib (Figure 4A, B). No evidence of communication with pulmonary tissue was observed. Histopathological analysis of the excised fibrotic parietal pleura (Figure 5) revealed necrotic

A)   B)

Fig. 3 – A) and B) a simple mastectomy and resection of calcificated and fibrotic tissue.

A)   B)

Fig. 4 – A) and B) the resection of calcified ribs.

Fig. 5 – Excision of parietal pleura.

granulation tissue. The right rib cage defect measuring 12 × 12 cm was reconstructed using a polyester fiber mesh (Figure 6). A large island musculocutaneous LD flap, measuring 21 × 15 cm, was raised and transposed to reconstruct the soft tissue defect. One thoracic and one wound drain were placed accordingly (Figure 7A, B).

The postoperative course was uneventful, and the patient was discharged on the 12th postoperative day. A six-month follow-up showed no complications (Figure 8).

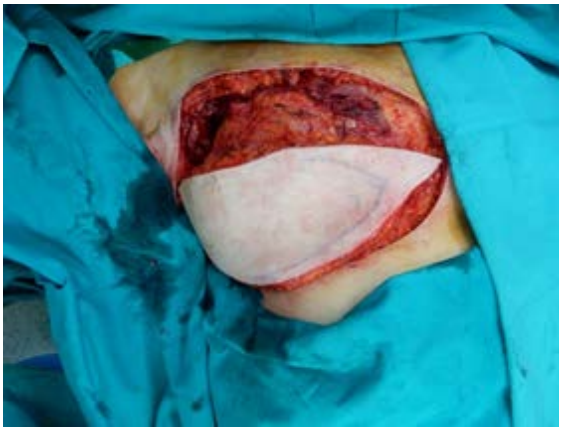
Discussion

PCF is a very rare late RT complication (after mastectomy) [4]. Our patient is the first case in the literature of PCF as a late RT complication after breast-conserving surgery.

RT is the most commonly used therapy for local disease control after breast-conserving surgery. Twenty years ago, when our patient was treated, a high daily radiation dose

Fig. 6 – Reconstruction of 12 × 12 rib cage defect with polyester fiber mesh.

A)



B)

Fig 7 – A) and B) reconstruction with pedicled myocutaneous *latissimus dorsi* flap.

Fig. 8 – The patient's appearance 6 months after the operation.

regimen was employed, consisting of 40 Gy delivered in 15 fractions, with an additional 10 Gy administered to younger and higher-risk patients. Overlapping of tangential fields that irradiate the chest or breast and the supraclavicular fields that radiate the lymphatics may occur. In the presented case, the tissue defect was located at the overlap of the supraclavicular and tangential fields, suggesting that it resulted from an excessive radiation dose delivered to the tissue. The importance of fraction size [1, 5] became apparent over time. Large fractions are no longer considered acceptable for this type of radiation for patients. Nevertheless, this therapy was routinely applied for many years in the past, and late side effects can manifest months, years, or even decades after RT. Pulmonary fibrosis, along with pleural and parenchymal changes, is recognized as a late effect of RT; however, these findings are mostly asymptomatic and are typically detected only on computed tomography scans [6]. In the presented case, RT left consequences on both skin and pleura, with additional calcifications that may represent a late manifestation of RT effects [7, 8]. Different responses to RT can be related to age, menopause, blood pressure, or smoking, but genetic differences are likely to represent most of the variations seen among patients [9].

Surgical treatment of such a patient was challenging due to the extensive CW defect. The challenge is not only the reconstruction of the CW defect but also the restoration of CW rigidity with minimal deformity, preservation of pulmonary mechanics, and prevention of air leakage and herniation of pulmonary tissue [10]. The type of reconstruction is determined by the size, location, and complexity of the defect, the viability of surrounding tissues affected by prior surgery and RT, as well as the patient's comorbidities, taking also in consideration the experience of the multidisciplinary team. In the current literature, there is no consensus regarding the indications for CW reconstruction. One might expect that reconstruction depends mainly on the extension and location of the full-thickness defect. Defects > 5 cm in diameter or including ≥ 4 ribs, situated in the anterolateral part of the CW, that cause paradoxical motion and have a high risk of lung herniation should be reconstructed. In contrast, reconstruction may not be necessary for some large apicoposterior defects, up to 10 cm in size, due to support provided by the scapula and shoulder girdle [11].

The first step in CW reconstruction is to re-establish skeletal stability. The ideal material should be flexible enough to conform to the shape of CW yet rigid enough to prevent paradoxical motion. Additionally, it should be biologically inert and radiolucent [12]. Today, we have a large number of different synthetic, biological, and metallic materials, but none of them have proven to be clearly superior. In our case, we used polyester fiber mesh. Following the restoration of CW stability, soft tissue and mesh coverage must be achieved. The best type for soft tissue reconstruction is the flap, which contains muscle in its composition. A muscle flap provides a good blood supply that can prevent possible infection of the prosthetic material, bulkiness for dead space obliteration, and, when necessary, contributes to the restoration of both structure and function. Although various options are available in the era of microsurgery, technical limitations in our setting made microsurgical reconstruction unfeasible, making flap selection particularly challenging. The optimal solution was the use of a pedicled LD myocutaneous flap, often considered the workhorse of CW reconstruction. It is the largest single flap that can be harvested. It can be tailored to any size and shape, while its long vascular pedicle makes the flap especially suitable for large skin defects [13].

Necrosis of the chest as a late effect of radiation therapy after breast cancer occurs most often on the skin or cartilage and can rarely appear as a fistula that communicates with the lungs or bronchus [14, 15]. In those cases, ST of the fistula is very challenging. Azuma et al. [4] demonstrated an effective approach for managing fistulas, where the defect was reconstructed using an omental graft. In their report, they presented a patient who, 25 years earlier, underwent a Halsted mastectomy and was irradiated postoperatively, and subsequently developed a bleeding fistula. A free omental flap with vascular anastomosis and a skin graft was implanted in the neck region, successfully closing the pulmonary cutaneous fistula. Postoperatively, emphysema remained inside the omentum, but it resolved spontaneously over time [16].

There are different techniques of flap surgery using healthy tissues, but each has some disadvantages. Han et al. [16] described cases in which the defect was reconstructed using a muscle-sparing vertical LD incision. In two patients with bronchopleural fistulas, reconstruction was performed using a muscle-sparing vertical LD flap, and thus, there was no need to change the patient's position.

A chest defect is often accompanied by a chronic infection. In their paper, Wang et al. [17] showed how they solved the operative fistula with chronic osteomyelitis. In the first act, a resection of the fistula, ribs, and clavicle was performed, as well as a partial resection of the sternum, and then the infection was repaired. In the second act, a reconstruction was made by myocutaneous incision with pedicles, with a satisfactory clinical effect. All these studies indicate various possibilities for solving chest defects, as well as the complications and risks that accompany surgical treatment. New techniques have enabled a greater choice of treatment and improved efficiency in treatment.

Conclusion

The treatment of radiation therapy complications is challenging due to extensive tissue destruction, engagement of other chest organs and structures, and severe tissue sclerosis. Surgical solutions should be individually tailored based on a multidisciplinary team assessment.

Conflict of interest

The authors declare no conflict of interest.

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CASE REPORT

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Accessory breast – an anomaly to live with: a case report and literature review

Akcesorna dojka – anomalija sa kojom se živi: prikaz slučaja i pregled literature

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Abstract

Introduction. During normal fetal development, between the second and third months of gestation, the thoracic ridge forms from the milk line on the front part of the chest at the level of the fourth intercostal space, while the remaining parts involute. If this tissue fails to involute, ectopic breast tissue develops, with or without the nipple-areola complex. **Case report.** We present a 50-year-old female patient who was examined for pain in the right breast region one month after a traffic accident. The performed clinical examination, ultrasound, and mammography confirmed the presence of an accessory breast along the left inframammary milk line without any pathological changes. Throughout her life, the patient has experienced no issues with her breasts or other organ systems, except for cholecystitis, for which she underwent gallbladder surgery several years ago. Given that the patient has had no problems so far, she was advised to have a breast ultrasound in one year and a mammogram in two years. **Conclusion.** An accessory breast is a congenital anomaly of breast tissue that clinicians should consider in patients presenting with marked subcutaneous tissue tumefaction in the mammary line region, along with an appropriate clinical picture. Since all diseases affecting normal breast tissue can also affect accessory breasts, their timely clinical recognition and treatment are necessary.

Key words:
breast; congenital abnormalities; diagnosis.

Apstrakt

Uvod. Tokom normalnog razvoja fetusa, između drugog i trećeg meseca gestacije, od „mlečne linije" se na prednjem delu grudnog koša, u nivou četvrtog međurebarnog prostora, formira grudni greben, dok preostali delovi involuiraju. Ukoliko ne dođe do involucije tog tkiva, dolazi do razvoja ektopičnog tkiva dojki, sa ili bez kompleksa bradavice-areole. **Prikaz bolesnika.** Prikazana je pacijentkinja stara 50 godina, koja je pregledana zbog bola u regiji desne dojke, mesec dana posle saobraćajne nesreće. Učinjenim kliničkim pregledom, ultrazvukom i mamografijom dokazano je prisustvo akcesorne dojke duž leve „mlečne linije", inframamilarno, bez patoloških promena. Osim holecistitisa, zbog čega joj je operisana žučna kesa pre više godina, pacijentkinja ranije nije imala druga oboljenja, niti bilo kakav problem sa dojkama. S obzirom na to da pacijentkinja ranije nije imala tegobe, savetovan joj je ultrazvučni pregled dojki za godinu dana i mamografija za dve godine. **Zaključak**. Akcesorna dojka je urođena anomalija tkiva dojke, koju kliničari treba da razmotre kod pacijenata koji imaju izražen otok potkožnog tkiva u regionu „mlečne linije", uz postojanje odgovarajuće kliničke slike. S obzirom na to da sva oboljenja koja se javljaju u normalnom tkivu dojke mogu zahvatiti i akcesorne dojke, neophodno je njihovo pravovremeno kliničko prepoznavanje i tretman.

Ključne reči:
dojka; anomalije; dijagnoza.

Introduction

Breasts in women represent mammary glands, which are the largest skin glandular tissue in a woman's body. Breast development begins in the fifth week of gestation when ectodermal folds appear on the ventral side of the fetus that extend from the axilla to the groin and are called milk lines [1, 2]. During the normal development of the fetus,

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in the period from the second to the third month of gestation, a thoracic ridge is formed from this line on the front part of the chest at the level of the fourth intercostal space (orthotopic breast), and the remaining parts involute. If the involution of this tissue does not occur, the development of ectopic breast tissue [polymastia, accessory breast (AB)] occurs, with or without the nipple-areola complex (NAC). This tissue has the characteristics of the primordial mammary gland and is, therefore, under the influence of hormonal changes that occur in a woman's body both during puberty and pregnancy [3, 4].

The prevalence of AB ranges from 0.4% to 6% in women, while in men, it ranges from 1% to 3%. The highest prevalence has been shown among Asians, especially Japanese [5, 6].

Localization of AB is predominantly in the axilla region, at the very beginning of the milk line, and it can also occur in other places (chest part of the thoracic wall, back, inguinal region, vulva, foot, etc.) [7–11]. In addition to the AB, congenital anomalies of the breast include excessive nipple (polythelia), absence of breast tissue (amastia), presence of a nipple without breast tissue (amasia), hyperplasia, hypoplasia, congenital inversion of the nipple, as well as Poland's syndrome, which consists of unilateral hypoplasia of the breast, hemithorax, and pectoral muscles. In addition to congenital, there are also acquired breast anomalies, which are most often of traumatic or iatrogenic origin [12].

Case report

A 50-year-old female patient was examined at the Radiology Department of the University Hospital Medical Center "Bežanijska Kosa" in Belgrade, Serbia, in February 2024 due to permanent pain in the area of the right breast, and one month after a traffic accident in which she suffered a blow to the chest region. The patient was a menopausal woman with a history of two childbirths and gallbladder surgery with a scar on the skin in a typical place (Figure 1).

On inspection, the clinical examination revealed symmetrical breast structure, with no skin retraction or nipple discharge. On the left, there was AB with a formed nipple in the plane of the milk line below the left breast. On palpation, no tumor changes or macrocysts were identified. Lymph nodes were not palpable. The patient was referred for breast and axilla ultrasound exams.

Breast and axillary ultrasound (Samsung V6) revealed the presence of AB tissue located along the mammary line, be-low the left breast. The structure of the breast was heterogeneous. No suspicious solid tumor changes or macrocysts were observed in the breasts. The cutis, subcutis, and pectoral fascia were intact. The retroareolar ducts were not dilated, and no pathologically altered axillary lymph nodes were observed. Findings were classified as Breast Imaging Reporting and Data System (BI-RADS) category 2 (benign) in the second area of the right breast (DD2), second area of the left breast (LD2), and in a third breast region (Figure 2).

a) **b)**

Fig. 1 – Frontal (a) and sagittal (b) view of a supernumerary breast scar on the skin of the anterior abdominal wall, under the right breast, that represents a usual condition after gallbladder surgery performed earlier.

Given that the last mammogram was performed seven years ago, the examination was supplemented with a mammogram of all three breasts, yielding the following findings:

"Mammography of all three breasts in craniocaudal and mediolateral oblique projections (PLANMED NUANCE EXCEL, 35kV/140mA): no suspicious tumor shadows or microcalcifications observed. BI-RADS category 2 for all three breasts. A follow-up breast ultrasound is recommended in one year, and a repeat mammography in two years (Figures 3–5)."

For the purpose of breast comparison, we took additional measurements and compared the nipple sizes of all three breasts, as well as the distances from each nipple to the submammary sulcus. We concluded that the formed nipple on the supernumerary breast was smaller compared to the nipples of the other two breasts and that the distance from the areola to the submammary sulcus of the supernumerary breast was smaller compared to the same distances of the other two breasts (Figures 6–9).

After one year, the patient came for a control ultrasound examination, where it was reported that there were no pathological changes in the breasts. Therefore, annual ultrasound examinations and mammography every two years were advised according to the screening recommendations.

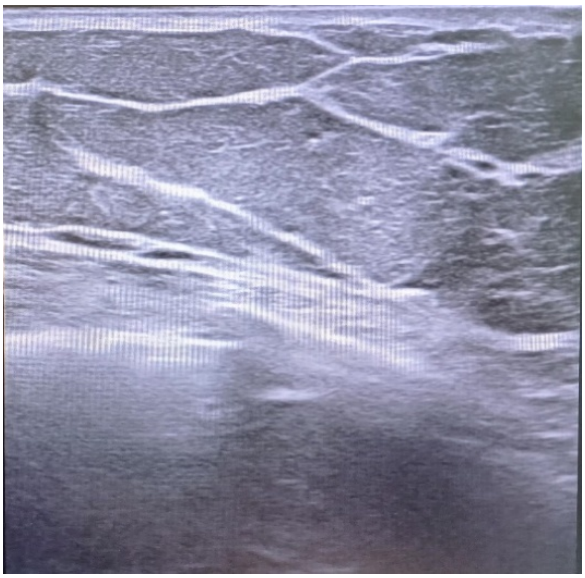
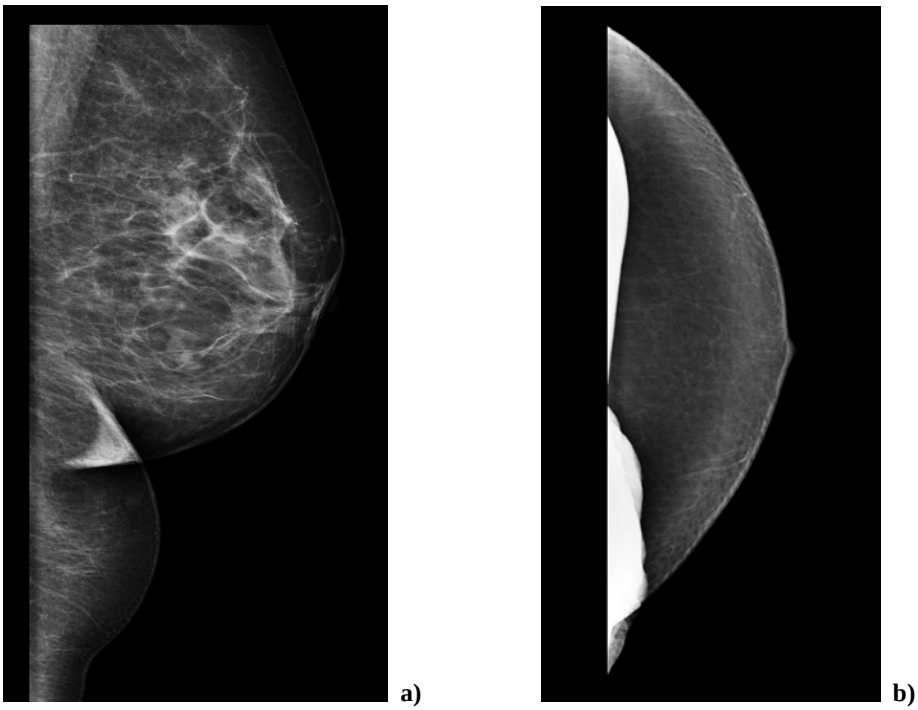
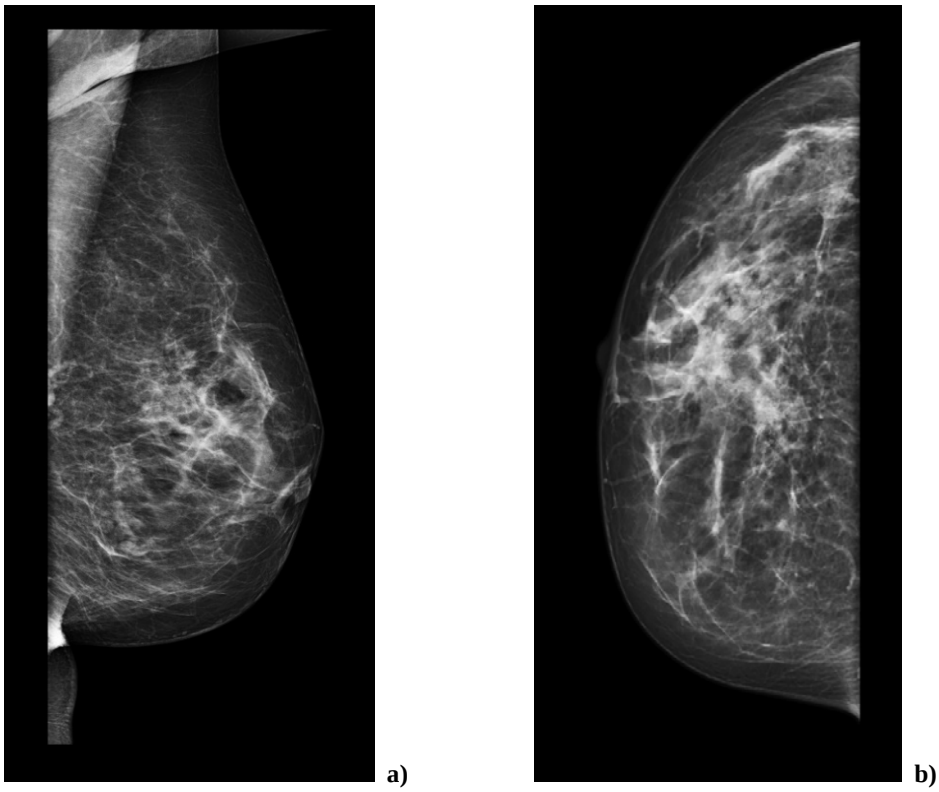


Fig. 2 – Ultrasound image of supernumerary breast tissue.



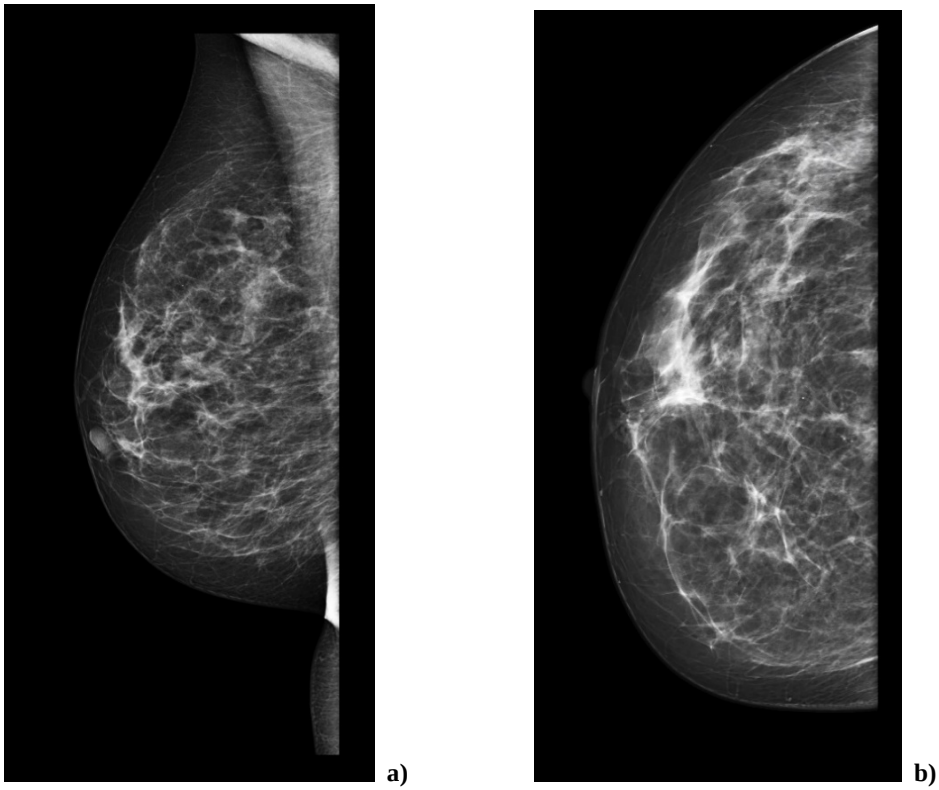
a) **b)**

Fig. 3 – Mammography images of the supernumerary breast in a) mediolateral oblique view and b) craniocaudal view.



a) **b)**

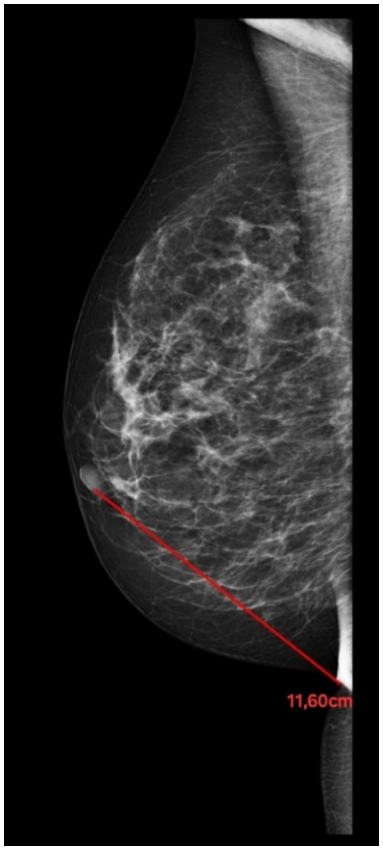
Fig. 4 – Mammography images of the left breast in a) mediolateral oblique view and b) craniocaudal view.



a) **b)**

Fig. 5 – Mammography images of the right breast in a) mediolateral oblique view and b) craniocaudal view.

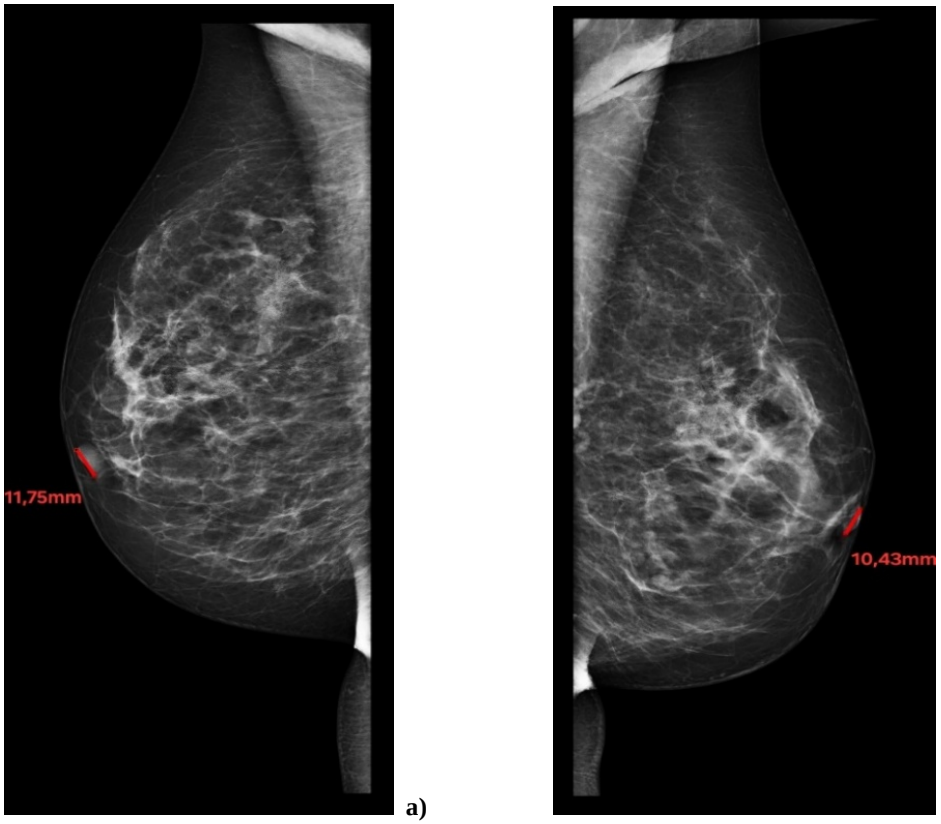
Fig. 6 – Distance from the mamilla of the supernumerary breast to the submammary sulcus.



a)

b)

Fig. 7 – Distance from the mamilla to the submammary sulcus of a) the right breast and b) the left breast.



a) **b)**

Fig. 8 – Mamillary dimension of a) the right breast; b) the left breast.

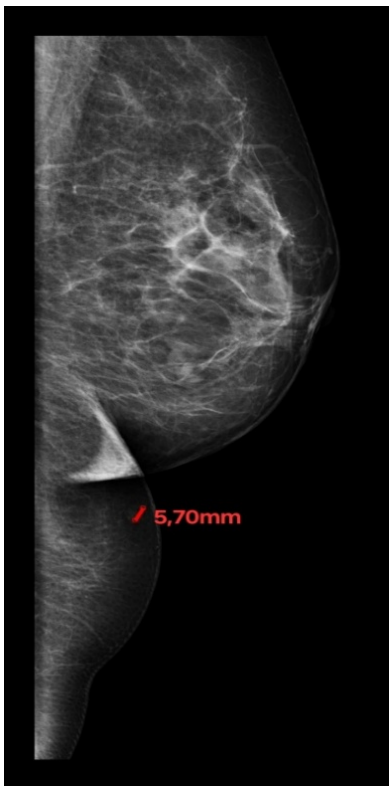


Fig. 9 – Mamillary dimension of the supernumerary breast.

Written informed consent has been obtained from the patient for the publication of this case report and the accompanying images.

Discussion

Congenital anomalies are, by definition, structural or functional defects in tissues or organs that occur during morphogenesis and are visible immediately after birth or later in life. The incidence of congenital anomalies ranges from 0.4–6%, with a lower percentage in Caucasians and a higher percentage in other races [3]. As part of this phenomenon, it is clear that the incidence of minor congenital anomalies, which represent only an aesthetic problem, is unknown in our geographical area. A review of the literature shows that a smaller number of papers describe more clinical cases of ABs [13, 14]. The problems accompanying anomalies like ABs are differential diagnosis, often a non-specific clinical picture, association of ectopic breasts with other anomalies, and involvement of ectopic breast tissue by some pathological process. An AB can be the same shape and size as a normal breast, making the diagnosis straightforward. If the AB has NAC, diagnosis should not be a problem; however, the incidence of NAC in AB is low, so early diagnosis and further treatment may be challenging. If AB is smaller in size and does not have a nipple, it usually does not represent a big problem, so people rarely consult a doctor [15]. The differential diagnosis of this anomaly is particularly challenging when the tissue is localized in the axilla, as it is often mistaken for an enlarged lymph node or, if unilateral, a benign soft tissue tumor [16]. An AB can be associated with various other malformations and may therefore be considered a marker for additional anomalies. If it occurs in men, it can be related to various organic malformations where certain organs grow faster than other parts of the body, both prenatally and postnatally [3]. AB is associated with anomalies in the development of the urinary tract, which is explained by the parallel development of the breast and the genitourinary system. AB is also associated with anomalies of the heart and central nervous system [17]. It is important to emphasize that the AB tissue is susceptible to all diseases that affect "normal" breasts. The same pathological conditions, inflammations, and various benign or malignant diseases that can be found in normal breast tissue can also be seen in ectopic tissue. To date, cases of benign cysts, adenomas, fibroadenomas, schwannomas, and AB carcinomas have been published [18–20]. Malignant tumors arising in ABs are rare but should not be ignored as a possibility. According to literature data, the incidence of AB cancer is 0.2–0.6%, and diagnosis and treatment are identical to the case of localization of the pathological process in normal breast tissue [21]. Cases of AB with galactorrhea have also been described in the literature [22].

In addition to the physical aspects, AB is also a psychosocial problem, both during puberty, when it is most often recognized as the breast tissue begins to swell, and during pregnancy and lactation, when the breasts usually enlarge. Interestingly, our patient had no problems during the generative period of life. Surgical treatment is the method of choice in treating AB. Given the association of this congenital malformation with other anomalies, a thorough examination of all organ systems, especially the urogenital system, is indicated. Since AB can be affected by various pathological processes, it is necessary to perform a preoperative ultrasound or, if needed, a mammography or magnetic resonance imaging. After radiological diagnostic procedures, a biopsy is performed, and only after histopathological verification is surgical excision with extirpation performed. Liposuction can sometimes be one of the modalities in the treatment of AB. Complex reconstructive procedures are usually not required after surgical intervention, which has been shown to be best done before pregnancy, considering that pregnancy, due to hormonal changes, can lead to *de novo* complaints or worsening of the existing ones [4, 22, 23]. Patients who do not want surgical treatment should be clinically monitored from time to time and educated about the risks they are exposed to.

Many published papers report a manifested pathological process in the AB tissue, most often cancers of various pathohistological structures. However, in the past 17 years, only two papers have been published in Serbia – one was related to pathological changes in the AB tissue [24], and the other was related to surgical correction of the AB tissue without pathological changes [25]. Aside from that paper, there are no other published studies in Serbia on the topic of AB without pathological changes that did not cause somatic or psychological issues in the patient during her life. Our report is unique precisely because it shows a patient who lived most of her life, including the entire generative period, with an AB but without problems and also without pathological changes in the other organ systems.

Conclusion

An accessory breast is a congenital anomaly of breast tissue that clinicians should be aware of in patients with marked tumefaction of the subcutaneous tissue in the mammary line region. Additional breast-related symptoms such as cyclic changes of ectopic tissue (swelling, pain) during menstruation, tissue enlargement in pregnancy, postpartum milk secretion from accessory nipples, as well as the specific location, should guide the clinician towards the diagnosis of accessory breast. Early diagnosis of accessory breast and proper surgical treatment with histopathological examination of ectopic tissue represents the gold standard that should lead to optimal results and a high degree of patient satisfaction. Since all diseases that can affect normal breast tissue may also occur in accessory breasts, timely clinical recognition and treatment are necessary, along with regular annual monitoring.

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C O R R I G E N D U M

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I. In the original article by **Nataša Tomić, Saša Vukmirović, Stanislav Sabo, Arsen Uvelin, Radmila Popović, Sanja Vicković, Ljiljana Tomić, Zdenko Tomić: Compliance of extended infusion of piperacillin/tazobactam with the desired pharmacokinetic/pharmacodynamic index in septic patients** (Usklađenost produžene primene infuzije piperacilina/tazobaktama sa željenim farmakokinetičkim/farmakodinamskim indeksom kod septičnih bolesnika).
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This article was corrected Online [1] .

1. **Nataša Tomić, Saša Vukmirović, Stanislav Sabo, Arsen Uvelin, Radmila Popović, Sanja Vicković, Ljiljana Tomić, Zdenko Tomić. Compliance of extended infusion of piperacillin/tazobactam with the desired pharmacokinetic/pharmacodynamic index in septic patients. Vojnosanit Pregl 2025; 82(5): 263–271**. (https://doi.org/10.2298/VSP241213029T).
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3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

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

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

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

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

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

Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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