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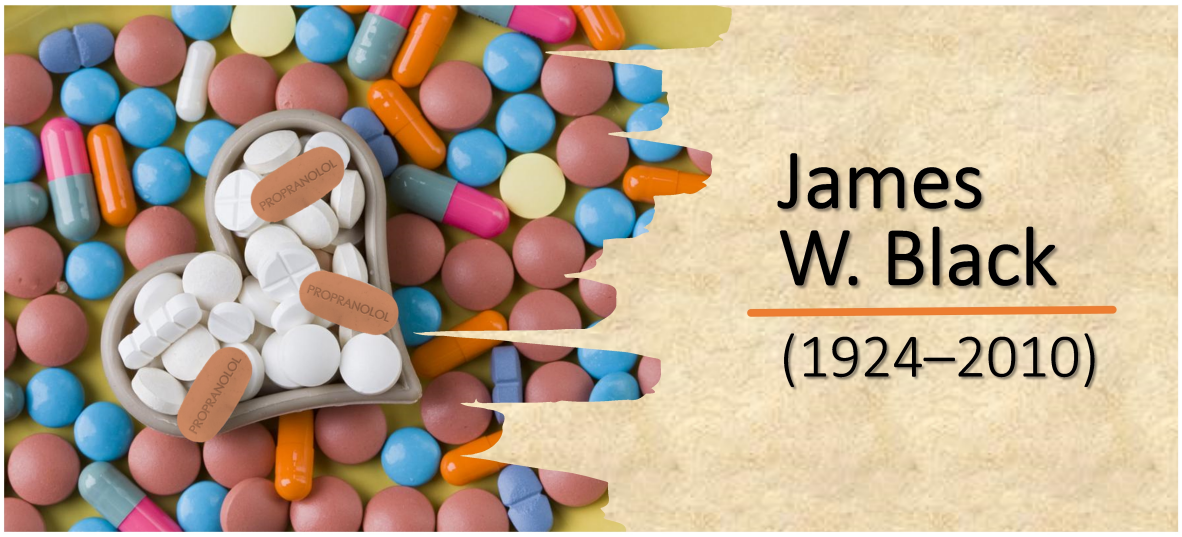

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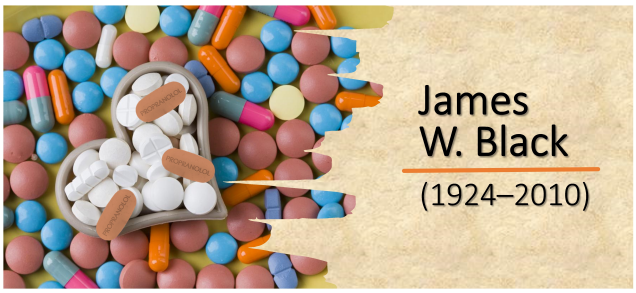

In June this year, we celebrate the 100th birth anniversary of Sir James W. Black (1924–2010), a physician and pharmacologist. Among his key discoveries were the drugs propranolol, the first successful beta blocker, and cimetidine, the first histamine-2 receptor antagonist. Together with Gertrude B. Elion and George H. Hitchings, he won the Nobel Prize in Physiology or Medicine for their discoveries of important principles for drug treatment (1988).

U junu ove godine obeležavamo 100 godina od rođenja ser Džejmsa V. Bleka (1924-2010), lekara i farmakologa. Među njegovim ključnim otkrićima bili su lekovi propranolol, prvi uspešni beta blokator, i cimetidin, prvi antagonista receptora histamina-2. Zajedno sa Gertrudom B. Elion i Džordžom H. Hičinsom, dobitnik je Nobelove nagrade za fiziologiju ili medicinu za njihova otkrića važnih principa u lečenju medikamentima (1988. godine).

ORIGINAL ARTICLES
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Early rehabilitation challenges of surgical patients with COVID-19 infection – a single-arm study

Izazovi u ranoj rehabilitaciji hirurških bolesnika sa COVID-19 infekcijom – *single-arm* studija

Dušica Simić-Panić*†, Ksenija Bošković*‡, Slobodan Pantelinac*†, Aleksandar Knežević*†, Predrag Jovićević‡, Apostolos Kozios*, Nataša Janjić*§, Nikola Nikolić‖, Larisa Vojnović*†, Snežana Tomašević-Todorović*†

***University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; University Clinical Center of Vojvodina, †Medical Rehabilitation Clinic, §Department of Orthopedic Surgery and Traumatology, ‖Department of Abdominal and Endocrine Surgery, Novi Sad, Serbia; ‡Special Hospital for Rheumatic Diseases, Novi Sad, Serbia**

Abstract

Background/Aim. A very limited amount of data regarding the rehabilitation outcome of surgical patients with COVID-19 is available in the current literature. The aim of this study was to point out the characteristics of early rehabilitation of these patients and determine the predictors of rehabilitation outcomes. **Methods**. The study was designed as a prospective clinical trial. It included patients who had surgical treatment from April 1, 2022, to March 31, 2023, at the University Clinical Center of Vojvodina, Serbia and either had positive results for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) preoperatively or developed coronavirus disease 2019 (COVID-19) within 72 hrs after surgery. The rehabilitation program was planned for each patient according to the type of surgical treatment, age, clinical presentation and severity of the COVID-19, length of immobilization, and comorbidities. Rehabilitation treatment (RT) started with a minimum of one 30-minute daily session, up to three 30-minute sessions daily. Patients were assessed at the beginning of RT and discharge. Outcomes were assessed with the Modified Borg Scale (MBS) for dyspnoea, Barthel index (BI) for activities of daily living, Six-Minute Walk Test (6MWT) for exercise tolerance, and Timed up and Go (TUG) test for balance and lower limb mobility. **Results.** A total of 81 patients were included in the study. RT was successful for 42 patients (24 female and 18 male) with an average age of 62.10 ± 20.07 years. These patients exhibited significant functional improvement, which was measured by all tests that assessed rehabilitation outcome at discharge: BI ($p < 0.001$), MBS ($p < 0.001$), 6MWT ($p < 0.001$), and TUG test ($p < 0.001$). The remaining 31 patients had unsuccessful RT. The binary logistic regression analysis has shown that age ($p = 0.009$), cardiovascular disease ($p = 0.017$), and malignancy ($p = 0.022$) were significant predictors of rehabilitation outcome. **Conclusion**. Results of the present study implicate that individually tailored RT during the acute phase of COVID-19 in surgical patients is very challenging. Advanced age, cardiovascular disease, and malignancy are predictors of unfavorable outcomes, and careful consideration is needed when planning the treatment for these patients.

Key words:
covid-19; general surgery; rehabilitation; treatment outcome.

Apstrakt

Uvod/Cilj. U aktuelnoj literaturi dostupno je veoma malo podataka o ishodu rehabilitacije hirurških bolesnika obolelih od COVID-19. Cilj istraživanja bio je da ukaže na karakteristike rane rehabilitacije ovih bolesnika i da se utvrde prediktori ishoda rehabilitacije. **Metode.** Studija je bila osmišljena kao prospektivno kliničko ispitivanje i obuhvatila je bolesnike koji su bili hirurški lečeni od 1. aprila 2022. do 31. marta 2023. godine u Univerzitetskom kliničkom centru Vojvodine, Srbija, a koji su bili pozitivni na *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) preoperativno ili su razvili koronavirusnu bolest 2019 (COVID-19) u roku od 72 sata nakon operativnog tretmana. Program rehabilitacije planiran je za svakog bolesnika pojedinačno, shodno vrsti hirurškog lečenja, uzrastu, kliničkoj slici i težini COVID-19, dužini imobilizacije i komorbiditetima. Tretman rehabilitacije (TR) je počinjao sa najmanje jednom sesijom od 30 minuta dnevno, do tri dnevne sesije od po 30 minuta. Bolesnici su procenjivani na početku TR i pri otpustu iz bolnice. Za procenu ishoda korišćene su modifikovana Borgova skala

Correspondence to: Dušica Simić-Panić, University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21 000 Novi Sad, Serbia.
E-mail: dusica.simic-panic@mf.uns.ac.rs

(MBS) za dispneju, Bartelov indeks (BI) za aktivnosti svakodnevnog života, Šestominutni test hoda (*Six-Minute Walk Test*-6MWT) za toleranciju vežbanja i test Ustani i kreni (*Timed up and Go*-TUG), za ravnotežu i pokretljivost donjih ekstremiteta. **Rezultati.** U studiju je bilo uključeno ukupno 81 bolesnika. TR je bio uspešan kod 42 bolesnika (24 žene i 18 muškaraca) prosečne starosti 62,10 ± 20,07 godina. Ovi bolesnici su pokazali značajno funkcionalno poboljšanje koje je izmereno svim testovima za procenu ishoda rehabilitacije, pri otpustu: BI ($p < 0,001$), MBS ($p < 0,001$), 6MWT ($p < 0,001$) i test TUG ($p < 0,001$). Preostalih 31 bolesnika nije imalo uspešan TR. Binarna logistička regresiona analiza pokazala je da su starost ($p = 0,009$), kardiovaskularne bolesti ($p = 0,017$) i malignitet ($p = 0,022$) značajni prediktori ishoda rehabilitacije. **Zaključak.** Rezultati ove studije ukazuju da je individualno prilagođen TR tokom akutne faze COVID-19 kod hirurških bolesnika veoma izazovan. Starije životno doba, kardiovaskularne bolesti i malignitet su prediktori nepovoljnog ishoda i potrebno je pažljivo razmatranje prilikom planiranja lečenja ovih bolesnika.

Ključne reči:
covid-19; hirurgija, opšta; rehabilitacija; lečenje, ishod.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease 2019 (COVID-19) pandemic has placed healthcare systems throughout the world under considerable strain, with a substantial effect on patients who require surgical care. Furthermore, surgical treatment of patients diagnosed with COVID-19 increases the risk of perioperative morbidity and mortality. Doglietto et al. [1] in their study demonstrated that the 30-day mortality risk for patients with COVID-19 undergoing surgery, as well as the odds for pulmonary and thrombotic complications, were significantly higher compared with patients without COVID-19. So far, very limited data are available in the current literature regarding the outcome of patients who undergo surgical intervention and either have positive results for COVID-19 or develop positive results soon after surgery.

Early respiratory rehabilitation is of vital value for hospitalized COVID-19 patients recovering from surgery to improve dyspnoea, prevent complications, decrease morbidity, reduce anxiety and depression, and prevent muscle weakness and physical performance impairment [2]. Rehabilitation treatment (RT) needs to be individually tailored for each patient according to the type of surgery, age, comorbidities, and respiratory status. Evaluation and monitoring of the patient need to be performed at all times during the RT. However, early respiratory rehabilitation is often delayed in critically ill patients while their condition is unstable and in progressive decline [3]. Furthermore, respiratory manifestations, complications of intensive care and hospitalization, and neurological sequelae necessitate the need for early RT in surgical COVID-19 patients [4].

The aim of this study was to point out the challenges of early rehabilitation of surgical patients with COVID-19, to determine the outcome of the treatment and functional status (FS), and to ascertain the predictors of rehabilitation outcome. That would enable us to gain insight into which patients most benefit from the RT, when the best moment is to begin, how to conduct the treatment, and for how long.

Methods

Patients who received surgical treatment from April 1, 2022, to March 31, 2023, at the University Clinical Center of Vojvodina, Serbia, and either had positive results for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) preoperatively or developed coronavirus disease 2019 (COVID-19) within 72 hrs after surgery were included in the study. Patients received early RT postoperatively, after team evaluation by a surgeon, physiatrist, and specialist of internal medicine. The research was conducted as a prospective study; all patients gave written consent at the beginning of the RT, and the study was approved by the Ethics Committee of the University Clinical Center of Vojvodina, in Novi Sad, Serbia (No. 600-66, from March 25, 2022).

Inclusion criteria were surgical treatment, positive nasopharyngeal swab test for SARS-CoV-2 obtained by real-time reverse transcription polymerase chain reaction (RT-PCR) method preoperatively or within 72 hrs after surgery, ability to participate in early RT defined by oxygen saturation (SpO_2) over 94% on admission, body temperature under 37.5°C, and clinical stability defined as the ability to perform active bedside mobilization with $SpO_2 > 92\%$. Patients who underwent minor procedures such as lumbar puncture, tracheostomy, minor gynecological interventions, and suturing of superficial wounds were excluded from the study. Other exclusion criteria were age below 18, SARS-CoV-2-positive surgical patients who were treated non-operatively, patients who had lethal outcomes, patients who were unable to tolerate RT due to clinical instability, reduction of SpO_2 lower than 94% on admission and moderate and severe heart failure (New York Heart Association classes III and IV), and impaired cognitive function.

Data collection

The following data were recorded for all patients: gender, age, medical comorbidities, pathology and type of surgery, length of hospital stay, the beginning of RT, and the duration of RT. For all patients, evidence of SARS-CoV-2 infection was recorded, as well as chest radiograph or computerized tomography (CT) findings. The need for oxygen therapy and type of oxygen therapy (nasal cannula or oxygen mask, continuous positive airway pressure, high flow nasal cannula, or mechanical ventilation) was also noted for each patient.

Rehabilitation treatment

The rehabilitation program was planned for each patient individually, according to the type of surgical treatment, age,

clinical presentation and severity of the disease, length of immobilization, and comorbidities. Treatment started from a minimum of one 30-minute daily session and up to three 30-minute daily sessions. RT began with patient positioning, breathing exercises, and postural drainage. It was followed by a range of motion exercises to preserve the mobility of the upper and lower extremities adjusted to the individual needs of the patient. At the beginning of each session, the patient was positioned either in a lying supine position with legs bent at the knees or in a semi-sitting or sitting position, depending on type of the surgery and respiratory status. Patients were instructed to relax their neck and shoulder muscles. Training in diaphragmatic breathing was used to improve breathing control, reduce the energy needed to breathe, and enhance lung ventilation. The patients were instructed to do deep inspiration through the nose, followed by a passive prolonged exhale through the half-open mouth. Expiration was prolonged, so it was two to three times longer than the inspiration, leading to a decrease in respiratory rate. Forced expiration techniques were included for patients to help eliminate secretions from airways. Breathing exercises were followed by exercises for peripheral circulation and range of motion exercises for upper and lower extremities lasting for 10–20 minutes. Depending on the clinical stability of the patient and type of surgery, mobilization exercises were applied, as well as walking with or without aid and balance exercises. Subjects were assessed every day so that the type and intensity of the treatment could be adjusted to the patient's condition. SpO_2, respiratory rate, heart rate, and blood pressure were measured before and after each session. Rehabilitation sessions would be ended if patients complained of any chest discomfort, palpitations or dyspnea scored as 4 or above on a Modified 10-point Borg Scale [5, 6] (MBS), shortness of breath, blurred vision, or dizziness.

Measurement of outcome

At the beginning of the RT and before discharge from the hospital, patients underwent a functional and physical evaluation by a specialist in physical and rehabilitation medicine. Functional assessment was performed by measurement of the following outcome parameters, along with all the safety procedures and appropriate personal protective equipment: MBS for dyspnea assessment was used to determine the patient's subjective exertion and provide feedback for exercise intensity. It consists of ten numerical values ranging from 0 to 10 (0 – no breathlessness, 10 – maximum breathlessness) [5, 6]. Independence in activities of daily living (ADL) was measured by the Barthel index (BI). The total BI score ranges from 0 (which represents the maximum level of dependency) to 100 (indicating complete autonomy). A score lower than 70 is considered to correspond to severe disability [7].

Six-Minute Walk Test (6MWT) was used to assess exercise tolerance as well as cardiovascular and respiratory function [8]. The patients were instructed to walk on a flat surface for six minutes and the walking distance was recorded. Patients who were unable to perform the test were given a value of 0 for analysis. Timed up and Go test (TUG) was administered to evaluate balance and lower limb mobility [9].

Statistical analysis

Statistical analysis was performed using SPSS 20.0 (IBM, USA). Continuous variables were reported as mean ± standard deviation, and categorical variables were presented as frequencies and percentages. Comparisons between baseline and discharge values of numerical variables were performed using a *t*-test for paired samples or a Wilcoxon signed-rank test where appropriate. Differences in categorical variables among the two groups were determined with the Chi-square (χ^2) test. An independent sample *t*-test or Mann-Whitney *U* test was used for comparing continuous variables between the two groups. Binary logistic regression was used to assess the predictive significance of various factors/parameters for the success of RT. All *p*-values < 0.05 were considered statistically significant.

Results

The study flow chart is shown in Figure 1. Out of 81 patients who were assessed for eligibility, 42 completed the rehabilitation program with successful outcomes. Eight patients refused to participate in the study. The remaining 31 patients had unsuccessful rehabilitation outcomes: 5 patients

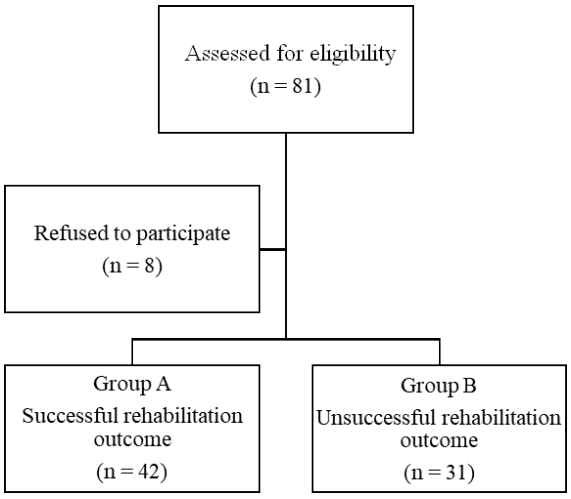

Fig. 1 – The flow chart of the study.

died, 12 patients had medical complications that prevented further RT, and 14 patients were unable to participate in the RT due to worsening of their clinical status and inability to tolerate physical exertion. Patients were divided into two groups according to the rehabilitation outcome: group A – successful rehabilitation and group B – unsuccessful rehabilitation. Group A included 42 patients, 24 (57.1%) women and 18 (42.9%) men, with an average age of 62.10 ± 20.07 years. Patients in group B were significantly older, with a mean age of 75.87 ± 6.40 years. No significant difference according to gender was found between the two groups.

The most common comorbidities in group A were hypertension (54.76%), cardiovascular diseases (16.67%), and diabetes (11.90%). There were significant differences regarding comorbidities between the groups. Patients in group B had a higher incidence of hypertension, cardiovascular disease, diabetes, and comorbidities in general. Chest radiographs and CT findings imply that bilateral and multiple lobe lesions were seen in most surgical patients with COVID-19. No significant differences were found between the two groups concerning radiological characteristics. In group A postoperatively, 10 (23.80%) patients required invasive mechanical ventilation (IMV), 5 (11.90%) received high-flow oxygen or non-invasive ventilation (NIV), and 28 (66.67%) needed oxygen support *via* an oxygen mask or nasal cannula. In group B, a significantly higher number of patients required oxygen support *via* NIV or high-flow nasal cannula, and 25 (80.65%) and 20 (64.52%) patients needed IMV. The demographics and clinical characteristics of the patients are shown in Table 1.

Most patients (16; 38.10%) in group A underwent abdominal surgery procedures. In comparison, 9 (21.43%) patients had orthopedic interventions, 7 (16.67%) had vascular surgery treatment, 6 (14.29%) had neurosurgical procedures, 2 (4.76%) had gynecological interventions, 1 (2.38%) had a urological procedure, and 1 (2.38%) patient had thoracic surgery. In group B, the most common interventions were orthopedic in 8 (25.81%) patients, while 7 (22.58%) patients had abdominal surgery procedures, 5 (16.13%) had vascular procedures, another 5 (16.13%) had neurosurgical interventions, 2 (6.45%) had urological procedures, another 2 (6.45%) had thoracic surgery, and 2 (6.45%) patients had cardiosurgical interventions. Types of surgery and underlying pathologies are presented in Table 2. One patient in group A had two types of operation: endovascular coiling and evacuation of intracranial hemorrhage. No significant differences were found between the groups regarding underlying pathology and type of intervention.

The mean length of hospital stay for the patients in group A was 21.74 ± 16.02 days, and the mean duration of RT was 13.67 ± 11.64 days. In group B, patients had longer hospitalization, with a mean value of 29.13 ± 13.46 days; rehabilitation onset was delayed, with a mean onset of 11.77 ± 6.66 days; the duration of RT was shorter, with a mean dura-

Table 1

Demographic and clinical characteristics of the patients

Variable	Group A (n = 42)	Group B (n = 31)	Statistics
Age, years	62.10 ± 20.07	75.87 ± 6.40	t = -3.680; p = 0.000
Gender			
female	18 (42.86)	13 (41.94)	χ^2 = 0.006; p = 0.937
male	24 (57.14)	18 (58.06)	
Comorbidity			
hypertension	23 (54.76)	24 (77.42)	χ^2 = 3.993; p = 0.046
cardiovascular disease	7 (16.67)	15 (48.39)	χ^2 = 8.524; p = 0.004
diabetes	5 (11.90)	11 (35.48)	χ^2 = 5.794; p = 0.016
cerebrovascular disease	4 (9.52)	7 (22.58)	χ^2 = 2.376; p = 0.123
malignancy	3 (7.14)	7 (22.58)	χ^2 = 3.596; p = 0.058
COPD	2 (4.76)	5 (16.13)	χ^2 = 2.658; p = 0.103
chronic kidney disease	1 (2.38)	4 (12.90)	χ^2 = 3.095; p = 0.079
No comorbidity	11 (26.19)	0 (0.00)	χ^2 = 9.560; p = 0.002
Radiological characteristics			
unilateral pneumonia	3 (7.14)	5 (16.13)	χ^2 = 1.476; p = 0.224
bilateral pneumonia	26 (61.90)	18 (58.06)	χ^2 = 0.110; p = 0.740
single lung lobe	4 (9.52)	1 (3.23)	χ^2 = 1.109; p = 0.292
multiple lung lobes	28 (66.67)	14 (45.16)	χ^2 = 3.376; p = 0.066
ground glass opacity	18 (42.86)	10 (32.26)	χ^2 = 0.847; p = 0.357
patchy shadows	7 (16.67)	9 (29.03)	χ^2 = 1.594; p = 0.207
normal radiological findings	13 (30.95)	8 (25.81)	χ^2 = 0.230; p = 0.631
Postoperative oxygen support			
nasal cannula or oxygen mask	28 (66.67)	28 (90.32)	χ^2 = 5.587; p = 0.018
duration, days	10.11 ± 8.55	13.39 ± 8.26	U = 278.5; p = 0.062
NIV or high-flow nasal cannula	5 (11.90)	25 (80.65)	χ^2 = 34.815; p = 0.000
duration, days	7.60 ± 2.88	7.76 ± 3.05	U = 47.5; p = 0.019
IMV	10 (23.80)	20 (64.52)	χ^2 = 12.209; p = 0.000
duration, days	4.10 ± 5.38	5.55 ± 3.38	U = 47.50; p = 0.019

COPD – chronic obstructive pulmonary disease; NIV – non-invasive ventilation; IMV – invasive mechanical ventilation; *U* – Mann-Whitney *U* test; *t* – Student's t-test; χ^2 – Chi-square test.
Data are presented as numbers (percentages) or mean ± standard deviation.

Table 2

Descriptive statistics of preoperative features and type of operation

Variable	Group A (n = 42)	Group B (n = 31)	Statistics
Pathology			
acute appendicitis	9 (21.43)	0 (0.00)	
femoral fracture	6 (14.29)	6 (19.35)	
ischemia of the lower limb	3 (7.14)	2 (6.45)	
acute cholecystitis	3 (7.14)	1 (3.23)	
gastric cancer	2 (4.76)	1 (3.23)	
subdural hematoma	2 (4.76)	2 (6.45)	
ruptured abdominal aortic aneurysm	2 (4.76)	1 (3.23)	
humeral fracture	2 (4.76)	1 (3.23)	
lower limb gangrene	2 (4.76)	2 (6.45)	
ruptured cerebral aneurysm	2 (4.76)	1 (3.23)	
uterine myoma	1 (2.38)	0 (0.00)	
gallbladder cancer	1 (2.38)	1 (3.23)	$\chi^2 = 22.725$
IMSCT	1 (2.38)	0 (0.00)	$p = 0.477$
endometrial carcinoma	1 (2.38)	0 (0.00)	
brain tumor	1 (2.38)	2 (6.45)	
bladder cancer	1 (2.38)	0 (0.00)	
intestinal occlusion	1 (2.38)	1 (3.23)	
tibia fracture	1 (2.38)	1 (3.23)	
lung cancer	1 (2.38)	1 (3.23)	
colon cancer	0 (0.00)	2 (6.45)	
rectal cancer	0 (0.00)	1 (3.23)	
breast cancer	0 (0.00)	1 (3.23)	
myocardial infarction	0 (0.00)	2 (6.45)	
prostate cancer	0 (0.00)	2 (6.45)	
Surgery procedure/intervention name *			
laparoscopic appendectomy	9 (21.43)	0 (0.00)	
femur fixation with a nail	4 (9.52)	2 (6.45)	
lower limb amputation	5 (11.90)	4 (12.9)	
hip hemiarthroplasty	2 (4.76)	4 (12.9)	
laparoscopic cholecystectomy	2 (4.76)	0 (0.00)	
open cholecystectomy	2 (4.76)	2 (6.45)	
subtotal gastrectomy	2 (4.76)	1 (3.23)	
evacuation of ICH	3 (7.14)	3 (9.68)	
aortic replacement	2 (4.76)	1 (3.23)	$\chi^2 = 25.704$
humerus fixation	2 (4.76)	1 (3.23)	$p = 0.315$
endovascular coiling	2 (4.76)	0 (0.00)	
total hysterectomy	2 (4.76)	0 (0.00)	
IMSCT resection	1 (2.38)	0 (0.00)	
craniotomy and tumor resection	1 (2.38)	2 (6.45)	
cystectomy	1 (2.38)	0 (0.00)	
adhesiolysis	1 (2.38)	1 (3.23)	
tibia fixation	1 (2.38)	1 (3.23)	
lobectomy	1 (2.38)	1 (3.23)	
open hemicolectomy	0 (0.00)	2 (6.45)	
hartmann procedure	0 (0.00)	1 (3.23)	
radical prostatectomy	0 (0.00)	2 (6.45)	
radical unilateral mastectomy	0 (0.00)	1 (3.23)	
CABG surgery	0 (0.00)	2 (6.45)	
Type of surgery procedure/intervention			
abdominal surgery	16 (38.10)	7 (22.58)	
vascular surgery	7 (16.67)	5 (16.13)	
neurosurgical procedures	6 (14.29)	5 (16.13)	
gynecological interventions	2 (4.76)	0 (0.00)	$\chi^2 = 7.177$
orthopedic	9 (21.43)	8 (25.81)	$p = 0.411$
urological procedures	1 (2.38)	2 (6.45)	
thoracic surgery	1 (2.38)	2 (6.45)	
cardiosurgical interventions	0 (0.00)	2 (6.45)	

IMSCT – intramedullary spinal cord tumor; ICH – intracranial hemorrhage; CABG – coronary artery bypass graft; χ^2 – Chi-square test. Data are presented as numbers (percentages).

***one patient in group A had two types of operation: endovascular coiling and evacuation of ICH.**

tion of 6.77 ± 4.71 days. Data regarding the duration of treatment and discharge destination are shown in Table 3. The length of hospital stay is shown in Figures 2 and 3, and the duration of RT is shown in Figures 4 and 5.

The physical condition of our patients at the beginning of RT was severe, judging by the baseline values of the assessed outcome parameters. The baseline value of BI in group A was 35.60 ± 18.88, which indicates the patients' severe disability in all areas of ADL. Rehabilitation led to an increase in BI at discharge 76.09 ± 21.12, with most patients exhibiting only mild or moderate disability in ADL. In group B, the baseline value of BI was significantly lower (26.29 ± 13.16, $p = 0.016$), which indicates an even more pronounced disability. The comparison of the values of outcome parameters at the beginning of RT between groups is shown in Table 3. Furthermore, a comparison between baseline values of outcome

Table 3

Clinical characteristics related to rehabilitation

Parameter	Group A (n = 42)	Group B (n = 31)	Statistics
Length of hospital stay, days			
min–max	4.0–74.0	7.0–68.0	t = -2.082
median	15.00	29.00	$p = 0.041$
mean ± SD	21.74 ± 16.02	29.13 ± 13.46	
Onset of rehabilitation, days			
min–max	2.0–25.0	3.0–27.0	t = -2.716
median	6.00	10.00	$p = 0.008$
mean ± SD	7.93 ± 5.43	11.77 ± 6.66	
Duration of rehabilitation, days			
min–max	2.0–51.0	2.0–21.0	$U = 358.0$
median	10.00	6.00	$p = 0.001$
mean ± SD	13.67 ± 11.64	6.77 ± 4.71	
Discharge destination, n (%)			
home	30 (71.43)	14 (45.16)	$\chi^2 = 9.373$
other healthcare facility	12 (28.57)	12 (38.71)	$p = 0.009$
death	0 (0.00)	5 (16.13)	
Outcome test results at the beginning of rehabilitation treatment, mean ± SD			
MBS (0–10)	2.76 ± 0.43	2.90 ± 0.30	$U = 559.0$; $p = 0.121$
BI (0–100)	35.60 ± 18.88	26.29 ± 13.16	t = 2.480; $p = 0.016$
6MWT (m)	210.62 ± 144.02	29.52 ± 45.07	t = 7.657; $p = 0.000$

MBS – Modified Borg Scale; BI – Barthel Index; 6MWT – Six-Minute Walk Test; SD – standard deviation; *U* – Mann-Whitney *U* test; t – Student's *t*-test; χ^2 – Chi-square test.

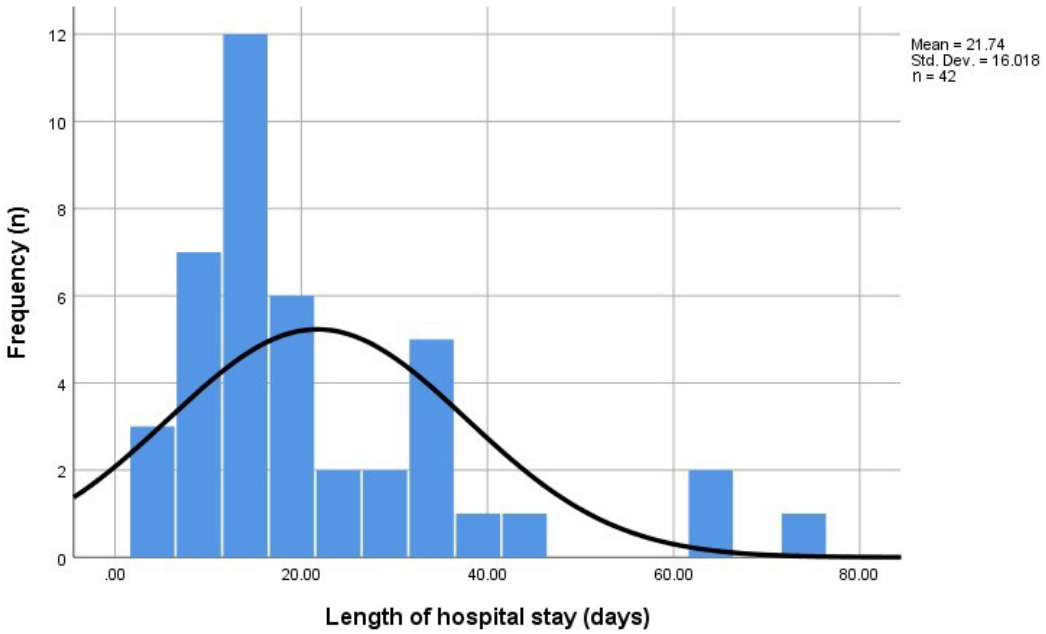

Fig. 2 – Length of hospital stay for group A (successful rehabilitation outcome). n – number.

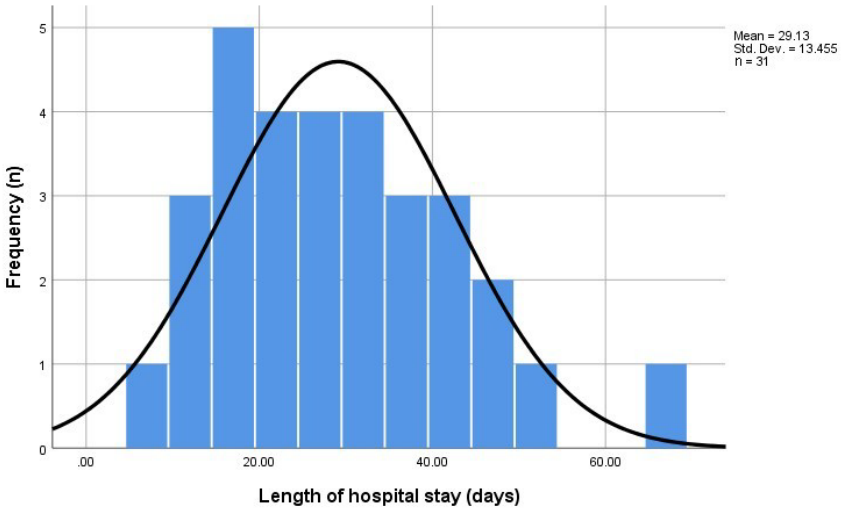

Fig. 3 – Length of hospital stay for group B (unsuccessful rehabilitation outcome). n – number.

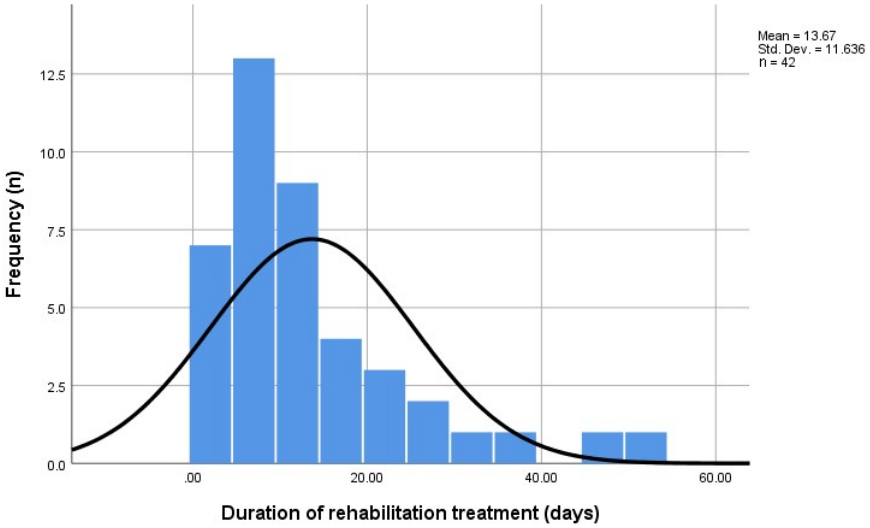

Fig. 4 – Duration of rehabilitation treatment for group A (successful rehabilitation outcome). n – number.

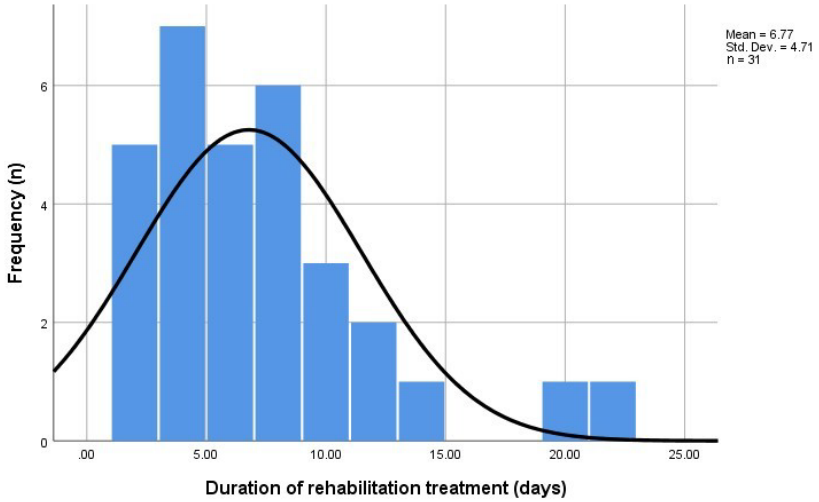

Fig. 5 – Duration of rehabilitation treatment for group B (unsuccessful rehabilitation outcome). n – number.

Table 4

Outcome test results at the beginning of rehabilitation treatment (T1) and discharge (T2) for patients with successful rehabilitation (group A)

Variable	Time point		*p*-value
	T1	T2	
MBS (0–10)	2.75 ± 0.86	0.78 ± 0.68	< 0.001*
6MWT (m)	210.04 ± 46.67	307.79 ± 74.32	< 0.001**
TUG (sec)	35.37 ± 7.27	22.78 ± 54.04	< 0.001**
BI (0–100)	35.60 ± 18.88	76.09 ± 21.12	< 0.001**

TUG – Timed Up and Go. For other abbreviations, see Table 3. Data are presented as mean ± standard deviation. *Paired Student's *t*-test; ** Wilcoxon signed-rank test.

Table 5

Results of binary logistic regression analysis for predicting rehabilitation outcome – model significance and partial contribution of predictors

Parameter	B	SE	Wald	Sig	Exp(B) / OR	95% CI for Exp(B)	
						LL	UL
Age (years)	0.08	0.03	6.77	**0.009**	1.08	1.02	1.15
Hypertension	0.32	0.74	0.19	0.666	1.38	0.32	5.90
CVD	1.80	0.75	5.74	**0.017**	6.04	1.39	26.28
Diabetes	1.11	0.83	1.82	0.178	3.05	0.60	15.38
Malignancy	2.19	0.96	5.21	**0.022**	8.92	1.36	58.39
LOS (days)	0.01	0.03	0.11	0.741	1.01	0.95	1.07
Onset of RT (days)	0.05	0.08	0.36	0.550	1.05	0.90	1.23
BI on the beginning of RT	0.01	0.03	0.22	0.641	1.01	0.96	1.07
Constant	-8.46	2.93	8.35	0.004	0.00	/	/

CVD – cardiovascular disease; LOS – length of hospital stay; RT – rehabilitation treatment; BI – Barthel Index; B – coefficient for the constant (also called the "intercept") in the null model; SE – standard error; Wald – Wald test; Sig – significance (*p*-value); OR – odds ratio; Exp(B) – exponentiation of the B coefficient (prognostic values for each predictor); CI – confidence interval; LL – lower limbs; UL – upper limbs.

Bolded values are statistically significant.

and values of outcome measured at discharge for patients with successful rehabilitation (MBS, BI, 6MWT, and TUG) shows a significant improvement after RT ($p < 0.001$ for all variables). The values of outcome parameters at the beginning of RT and discharge are shown in Table 4.

The last step in statistical analysis was to evaluate which clinical variables best predict the success of the RT of surgical patients with COVID-19 infection. For this purpose, logistic regression was conducted. Given the limited sample size, those variables that showed significant relationships with the criterion of rehabilitation outcome at the univariate level were included in the final list of predictors. The group of predictors consisted of the patient's age, comorbidities: hypertension, cardiovascular disease, diabetes, and malignancy, length of hospital stay, the onset of RT, and BI at the beginning of RT. The forced entry method was used. The test of the final model compared to zero proved to be statistically significant [$\chi^2(8) = 32.766$, $p < 0.001$, Nagelkerke $R^2 = 0.486$], so it can be concluded that the model significantly contributes to the prediction of the treatment outcome. The model corresponds with the data [$\chi^2(8) = 5.347$, $p = 0.720$). The prediction success rate, based on the model, was 81%. Based on the Wald's indicator, the following predictors were statistically significant: age [$p = 0.009$, odds ratio (OR) = 1.08; 95% confidence interval (CI): 1.02–1.15], cardiovascular disease ($p = 0.017$, OR = 6.04; 95% CI: 1.39–26.28) and malignancy ($p = 0.022$, OR = 8.92; 95% CI: 1.36–58.39). Results of binary logistic regression analysis for the prediction of rehabilitation outcome, model significance, and partial contribution of predictors are shown in Table 5. The surface area under the receiver operating characteristic (ROC) curve was AUC = 0.866 ($p < 0.001$), which shows that the tested final model contributes well to the prediction of the outcome of RT (successful vs. unsuccessful). The ROC curve of the treatment success prediction model is shown in Figure 6.

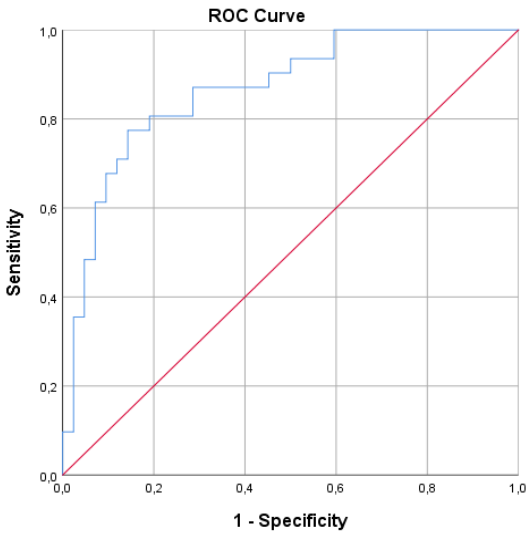

Fig. 6 – Receiver operating characteristic (ROC) curve of the treatment success prediction model.

Discussion

Recent studies have shown that COVID-19 patients who underwent surgical procedures had higher mortality rates and more complications compared to patients who had negative results for SARS-CoV-2 [1, 10, 11]. However, there is currently no data available on the effect of RT on surgical patients with COVID-19. Our results imply that COVID-19 surgical patients had prolonged hospital stays and severe dependence on ADL at the beginning of RT. Lei et al. [12], in their study regarding outcomes of patients undergoing surgeries during the incubation period of COVID-19, also reported prolonged hospitalization due to numerous complications such as acute respiratory distress syndrome-ARDS, arrhythmia shock, and acute cardiac injury.

Severe disability in COVID-19 patients is often the result of muscle weakness caused by prolonged immobility, post-intensive care myopathy and polyneuropathy, nutritional status, and underlying health conditions [4, 13]. Results from the current studies on the role of acute rehabilitation in COVID-19 patients suggest that rehabilitation has a beneficial effect on respiratory and FS [14–16]. In our research, we report a significant improvement in the independence in ADL measured by BI and exercise tolerance and cardiovascular and respiratory function evaluated by the 6MWT after completing early RT. Zampogna et al. [2], in their research on the effect of pulmonary rehabilitation in patients recovering from COVID-19, report a similar increase in the values of BI and 6MWT after RT. However, patients in our study had lower baseline results, which can be attributed to the additional effect of surgical treatment. However, a study by Curci et al. [17] on 32 post-acute COVID-19 patients showed that BI was not significantly increased after RT. Patients in this study were significantly older than patients in our research, which can explain the difference in our findings. In a study on patients who suffered from severe and critical COVID-19 pneumonia, Güler et al. [18] showed that patients gained significant functional independence during RT (mean BI improved from 44.8 to 88.4). We also noted significant improvement in lower limb function at the end of RT (mean TUG values reduced from 35.37 at baseline to 22.78 ± 54.04 at discharge). Similar results were obtained in a study by Rodrigues et al. [19], who reported a significant increase in lower limb and respiratory muscle strength, balance, and exercise capacity measured by TUG, 6MWT, and Functional Independence Measure. These findings underline the need for early rehabilitation for functional recovery of surgical COVID-19 patients.

In our study, patients with unsuccessful rehabilitation outcomes were significantly older than patients with successful rehabilitation, while they also had a higher overall incidence of comorbidities, in particular hypertension, cardiovascular disease, diabetes, and malignancy. Bellou et al. [20], in their random-effects meta-analysis of 263 studies, found that female gender, obstructive sleep apnoea, history of venous thromboembolism, coronary heart disease, cancer, chronic liver disease, chronic obstructive pulmonary disease-COPD, dementia, peripheral arterial disease, and rheumatological disease were associated with adverse outcomes in patients with COVID-19. Barbieri et al. [21], in their retrospective cohort study, reported a significant decrease in the level of disability in both motor and cognitive functioning after a multidisciplinary patient-tailored rehabilitation program. However, neither motor and nutritional characteristics nor comorbidities played a significant role in predicting the overall positive change registered after rehabilitation. Furthermore, our research showed that patients with adverse rehabilitation outcomes more often required oxygen support *via* nasal cannula, oxygen mask, high-flow nasal cannula, NIV, and IMV. Paneroni et al. [22], in their cross-sectional study on the sample of 184 patients, determined that the predictors for impaired FS in patients with COVID-19 were age, previous disability, comorbidity, and use of both IMV and NIV. Similarly to our results, a study by SeyedAlinaghi et al. [23] showed that patients who required IMV due to COVID-19 had a prolonged hospitalization duration and poor outcomes. In their research, Piquet et al. [24] demonstrated that grip strength was negatively correlated with the number of days spent in the intensive care unit-ICU, both on admission and at discharge. However, their findings suggest that an ICU or longer acute stay did not hamper responsiveness to rehabilitation. Our results imply that patients with unsuccessful rehabilitation had lower values of the BI and 6MWT at the beginning of RT. In their study, Trevisson-Redondo et al. [25] found that the BI total results could potentially be used to predict the related quality of life after recovering from COVID-19. In our research, after binary logistic regression analysis was performed, age, cardiovascular disease, and malignancy emerged as factors with predictive significance of rehabilitation outcome. Ikebuchi et al. [26], in their research on a sample of 57 patients with the severe form of COVID-19, determined that predictive factors for mobility after early rehabilitation were chronic lung disease, renal impairment, heart disease, and the presence of cerebrovascular disorder. Based on the results of this study, early rehabilitation of surgical patients with COVID-19 is very challenging due to underlying pathology, which often leads to severe disability, impaired respiratory function, comorbidities, and age of the patients. Although most patients achieved significant functional improvement at the end of RT, caution is needed when planning RT for patients with advanced age, cardiovascular comorbidity, and malignancy. Patients with successful rehabilitation had an earlier onset of RT, implying that the treatment should be started as soon as the patient is stable enough to prevent possible complications that can lead to adverse outcomes. Patients with favorable rehabilitation outcomes also had longer durations of RT, which can account for their significant functional gain.

This study has several limitations. As a single-arm study, it is methodologically insufficient in proving the effectiveness of RT because there are no results of a similar control group of surgical patients with COVID-19 who were not included in RT for comparison. However, because of the pandemic, a clinical trial of a rehabilitation group as opposed to a 'sham rehabilitation' control group was not considered ethical. The follow-up period is limited (only during hospi-

talization), and only early outcomes can be investigated. Moreover, the cohort of patients is small and does not allow detailed subpopulation analyses. To address these limitations, further controlled trials with a larger sample size at multiple centers are required to understand better the role of RT in the long-term recovery of surgical patients with COVID-19.

Conclusion

Results of the present study have indicated that individually tailored RT during the acute phase of COVID-19 in surgical patients is very challenging. At the end of RT, most patients achieved significant improvement in their FS, greater independence in the activities of daily living, better balance, and lower limb mobility. In addition to functional benefits and reduction of disability, patients demonstrated a significant decrease in dyspnoea and better tolerance of physical exertion. However, a significant number of patients had unfavorable outcomes. Binary logistic regression analysis has shown that age, cardiovascular disease, and malignancy are predictors of unfavorable outcomes and that caution is needed when devising a RT for these patients.

REFERENCES

1. *Doglietto F, Vezzoli M, Gheza F, Lussardi GL, Domenicucci M, Vecchiarelli L*, et al. Factors Associated with Surgical Mortality and Complications Among Patients With and Without Coronavirus Disease 2019 (COVID-19) in Italy. JAMA Surg 2020; 155(8): 691–702.
2. *Zampogna E, Paneroni M, Belli S, Aliani M, Gandolfo A, Visca D,* et al. Pulmonary Rehabilitation in Patients Recovering from COVID-19 2021; 100(5): 416–22.
3. *Halpin SJ, McIvor C, Whyatt G, Adams A, Harvey O, McLean L*, et al. Postdischarge symptoms and rehabilitation needs in survivors of COVID-19 infection: A cross-sectional evaluation. J Med Virol 2021; 93(2): 1013–22.
4. *Imamura M, Mirisola AR, Ribeiro FQ, De Pretto LR, Alfieri FM, Delgado VR,* et al. Rehabilitation of patients after COVID-19 recovery: An experience at the Physical and Rehabilitation Medicine Institute and Lucy Montoro Rehabilitation Institute. Clinics (Sao Paulo) 2021; 76: e2804.
5. *Mahler DA, Horowitz MB*. Perception of breathlessness during exercise in patients with respiratory disease. Med Sci Sports Exerc 1994; 26(9): 1078–81.
6. *Mador MJ, Rodis A, Magalang UJ*. Reproducibility of Borg scale measurements of dyspnea during exercise in patients with COPD. Chest 1995; 107(6): 1590–7.
7. *Pan L, Wang H, Cao X, Ning T, Li X, Cao Y*. A Higher Postoperative Barthel Index at Discharge is Associated with a Lower One-Year Mortality After Hip Fracture Surgery for Geriatric Patients: A Retrospective Case-Control Study. Clin Interv Aging 2023; 18: 835.
8. *Klanidhi KB, Chakrawarty A, Bhadouria SS, George SM, Sharma G, Chatterjee P,* et al. Six-minute walk test and its predictability in outcome of COVID-19 patients. J Educ Health Promot 2022;11: 58.
9. *Kowal M, Morgiel E, Winiarski S, Gieysztor E, Madej M, Sebastian A,* et al. Effect of COVID-19 on Musculoskeletal Performance in Gait and the Timed-Up and Go Test. J Clin Med 2023; 12(13): 4184.
10. *Cheung ZB, Forsh DA*. Early outcomes after hip fracture surgery in COVID-19 patients in New York City. J Orthop 2020; 21: 291–6.
11. *Ross SW, McCartt JC, Cunningham KW, Reinke CE, Thompson KJ, Green JM,* et al. Emergencies do not shut down during a pandemic: COVID pandemic impact on Acute Care Surgery volume and mortality at a level I trauma center. Am J Surg 2022; 224(6): 1409–16.
12. *Lei S, Jiang F, Su W, Chen C, Chen J, Mei W,* et al. Clinical characteristics and outcomes of patients undergoing surgeries during the incubation period of COVID-19 infection. EClinicalMedicine 2021; 21: 100331.
13. *Simpson R, Robinson L*. Rehabilitation after critical illness in people with COVID-19 infection. Am J Phys Med Rehabil 2020; 99(6): 470–4.
14. *Filipović T, Gajić I, Gimigliano F, Backović A, Hrković M, Nikolić D,* et al. The role of acute rehabilitation in COVID-19 patients. Eur J Phys Rehabil Med 2023; 59(3): 425–35.
15. *Li Z, Zheng C, Duan C, Zhang Y, Li Q, Dou Z*, et al. Rehabilitation needs of the first cohort of post-acute COVID-19 patients in Hubei, China. Eur J Phys Rehabil Med 2020; 56(3): 339–44.
16. *Al Chikhanie Y, Veale D, Schoeffler M, Pépin JL, Verges S, Hérengt F*. Effectiveness of pulmonary rehabilitation in COVID-19 respiratory failure patients post-ICU. Respir Physiol Neurobiol 2021; 287: 103639.
17. *Curci C, Pisano F, Bonacci E, Camozzi DM, Ceravolo C, Bergonzi R,* et al. Early rehabilitation in post-acute COVID-19 patients: data from an Italian COVID-19 Re-habilitation Unit and proposal of a treatment protocol. Eur J Phys Rehabil Med 2020; 56(5): 633–41.
18. *Güler T, Yurdakul FG, Acar Sivas F, Kiliç Z, Adigüzel E, Yaşar E,* et al. Rehabilitative management of post-acute COVID-19: clinical pictures and outcomes. Rheumatol Int 2021; 41(12): 2167–75.
19. *Rodrigues M, Costa AJ, Santos R, Diogo P, Conçalves E, Barroso* D, et al. Inpatient rehabilitation can improve functional outcomes of post-intensive care unit COVID-19 patients – a prospective study. Disabil Rehabil 2023; 45(2): 266–76.
20. *Bellou V, Tzoulaki I, van Smeden M, Moons KGM, Evangelou E, Belbasis L*. Prognostic factors for adverse outcomes in patients with COVID-19: a field-wide systematic review and meta-analysis. Eur Respir J 2022; 59(2): 2002964.
21. *Barbieri V, Scarabel L, Bertella L, Scarpina F, Schiavone N, Perucca L,* et al. Evaluation of the predictive factors of the short-term effects of a multidisciplinary rehabilitation in COVID-19 survivors. J Int Med Res 2022; 50(11): 3000605221138843.
22. *Paneroni M, Vogiatzis I, Bertacchini L, Simonelli C, Vitacca M*. Predictors of Low Physical Function in Patients With COVID-19 With Acute Respiratory Failure Admitted to a Subacute Unit. Arch Phys Med Rehabil 2021; 102(6): 1228–31.
23. *SeyedAlinaghi S, Abbasian L, Solduzian M, Ayoobi Yazdi N, Jafari F, Adibimehr A,* et al. Predictors of the prolonged recovery period in COVID-19 patients: a cross-sectional study. Eur J Med Res 2021; 26(1): 41.
24. *Piquet V, Luczak C, Seiler F, Monaury J, Martini A, Ward AB,* et al. Do patients with COVID-19 benefit from rehabilitation? Functional outcomes of the first 100 patients in a COVID-19 rehabilitation unit. Arch Phys Med Rehabil 2021; 102(6): 1067–74.
25. *Trevissón-Redondo B, López-López D, Pérez-Boal E, Marqués-Sánchez P, Liébana-Presa C, Navarro-Flores E,* et al. Use of the

Barthel Index to Assess Activities of Daily Living before and after SARS-COVID-19 Infection of Institutionalized Nursing Home Patients. Int J Environ Res Public Health 2021; 18(14): 7258.

26. *Ikebuchi M, Ohta Y, Minoda Y, Toki A, Nakatsuchi T, Terai H,* et al. Efficacy of Early Rehabilitation for Severe Coronavirus Disease 2019 Pneumonia: Factor Analysis Using Machine Learning. Prog Rehabil Med 2023; 8: 20230027.

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Evaluation of clinical, biohumoral, and morphological findings of the thyroid gland as possible predictors of risk for cancer in patients with atoxic nodular and multinodular goiter

Evaluacija kliničkih, biohumoralnih i morfoloških nalaza tiroidne žlezde kao mogućih prediktora rizika od karcinoma kod bolesnika sa atoksičnom nodoznom i multinodoznom strumom

Dragana Malović*, Tamara Dragović*†, Saša Kiković*, Petar Ristić*†, Dejan D. Marinković*†, Zorana Djuran*, Snežana Kuzmić-Janković*, Stevan Jovanović*, Jelena Rakočević‡, Zoran Hajduković*†

***Military Medical Academy, Clinic for Endocrinology, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ‡University of Belgrade, Faculty of Medicine, Institute of Histology and Embryology, Belgrade, Serbia**

Abstract

Background/Aim. Thyroid nodules are usually asymptomatic and may occur in 68% of the general population. In most cases, they are discovered incidentally. As malignancy is proven in 10–15% of cases, a rational diagnostic approach is necessary. The aim of this retrospective study was to examine and grade different characteristics of patients with nodular and multinodular atoxic goiter in order to identify the potential predictors for the assessment of thyroid cancer risk. **Methods**. The study included 275 patients with nodular and multinodular atoxic goiter hospitalized at the Clinic for Endocrinology of the Military Medical Academy, Belgrade, Serbia, from January 1, 2017, to October 1, 2022, for preoperative preparation. The most relevant clinical, biohumoral, and pathomorphological characteristics were analyzed. **Results.** Patients with multiple thyroid nodules were older (57.21 ± 13.16 vs. 49.36 ± 15.83 years, $p < 0.001$) and had higher body mass index (29.12 kg/m^2 vs. 26.50 kg/m^2, $p = 0.004$) compared to patients with one nodule. On the other hand, patients with one thyroid nodule had a higher level of the thyroid-stimulating hormone than patients with multiple nodules (1.73 mIU/L vs. 1.21 mIU/L, $p < 0.0001$). Comparison of patients with and without proven thyroid cancer has shown a highly significant association between the higher categories of Bethesda classification and the presence of cancer (Bethesda IV–VI vs. Bethesda II–III, 52.2% vs. 22.3%, respectively, $p = 0.002$). **Conclusion**. Considering all the observed parameters, the cytological finding of fine needle aspiration biopsy emerged as the only one with predictive relevance for assessing thyroid cancer risk.

Key words:
biopsy, fine needle; goiter; neoplasm staging; thyroid neoplasms; thyroid nodule.

Apstrakt

Uvod/Cilj. Nodusi tiroidne žlezde (TŽ) su najčešće asimptomatski i mogu se naći u 68% opšte populacije. U većini slučajeva otkrivaju se slučajno. S obzirom na to da u 10–15% slučajeva bude dokazan malignitet, neophodan je racionalan dijagnostički pristup. Cilj ove retrospektivne studije bio je da se ispitaju i rangiraju različite karakteristike bolesnika sa nodoznom i polinodoznom atoksičnom strumom radi identifikacije faktora od prediktivnog značaja za procenu rizika od postojanja karcinoma TŽ. **Metode**. U studiju je bilo uključeno 275 bolesnika sa nodoznom i polinodoznom atoksičnom strumom, koji su bili hospitalizovani u Klinici za endokrinologiju Vojnomedicinske akademije, Beograd, Srbija, u periodu od 01. januara 2017. godine do 01. oktobra 2022. godine, radi pripreme za hirurško lečenje. Analizirane su najznačajnije kliničke, biohumoralne i patomorfološke karakteristike. **Rezultati.** Bolesnici sa više nodusa u TŽ bili su stariji (57,21 ± 13,16 godina vs. 49,36 ± 15,83 godina, $p < 0,001$) i imali su viši indeks telesne mase (29,12 kg/m^2 vs. 26,50 kg/m^2, $p = 0,004$) u poređenju sa bolesnicima sa jednim nodusom. Nasuprot tome, bolesnici sa jednim nodusom u TŽ imali su viši nivo tireostimulišućeg hormona u odnosu

Correspondence to: Dragana Malović, Military Medical Academy, Clinic for Endocrinology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: dr.malovic@gmail.com

na bolesnike sa dva i više nodusa (1,73 mIU/L vs. 1,21 mIU/L, $p < 0,0001$). Rezultati poređenja bolesnika sa i bez dokazanog karcinoma TŽ pokazali su da postoji značajna povezanost između viših kategorija Bethesda klasifikacije i pojave karcinoma (Bethesda IV–VI 52,2% vs. Bethesda II–III 22,3%, $p = 0,002$). **Zaključak.** Od svih posmatranih parametara citološki nalaz aspiracione biopsije tankom iglom izdvojio se kao jedini sa prediktivnim značajem za postojanje karcinoma TŽ.

Ključne reči:
biopsija tankom iglom; gušavost; neoplazme, određivanje stadijuma; tireoidna žlezda, neoplazme; tireoidni nodusi.

Introduction

Thyroid nodules are usually asymptomatic and may occur in 68% of the general population [1]. In most cases, they are discovered incidentally during diagnostic procedures that are not primarily targeted at the thyroid gland. Since malignancy is proven in 10–15% of cases [2], a rational diagnostic approach is needed. The evaluation of patients with thyroid nodules comprises case history, clinical examination, biohumoral testing, ultrasound (US) examination, and fine needle aspiration biopsy (FNAB). The clinical examination includes the assessment of the number and size of nodules, their consistency, mobility during swallowing, pain on palpation, and the presence of enlarged lymph nodes on the neck. Biohumoral testing includes the measurement of thyroid-stimulating hormone (TSH). TSH values below the lower reference threshold represent an indication for thyroid scintigraphy with Iodine-123, -131 (I-123, I-131) or Technetium -99m, as pertechnetate, in order to assess the functionality of the nodule ("hot nodule", functional adenoma). Considering the low incidence of medullary carcinoma and, at the same time, not ignoring its malignant potential and often falsely elevated calcitonin values, there is still a debate regarding routine calcitonin measurement in patients with newly discovered thyroid nodule [3]. The simplest, most accessible, and most informative radiological method for the visualization of a thyroid nodule is the US examination. US characteristics that indicate the benign nature of the nodule comprise a cystic appearance, iso- or hyperechoicity, clear boundaries, and a "halo" surrounding a nodule. On the other hand, the US findings of a hypoechoic nodule with vague boundaries, microcalcifications, dominant solid component, increased vascularization, pathologically altered neck lymph nodes, as well as the elastographic "solid" nodules are signs of suspected malignancy [4]. These characteristics imply the necessity of FNAB of the suspected nodule. In order to standardize the US findings and adequately assess the cancer risk, the Thyroid Imaging, Reporting and Data System (TI-RADS) was adopted. TI-RADS takes into account the composition, shape, edges, echogenicity, and presence of echogenic focuses [5]. The cytological findings are classified according to the last, third version of Bethesda classification published in 2023 [6]. In patients whose cytological findings indicate a benign lesion, clinical and US monitoring is indicated. The latest version of the Bethesda classification emphasizes the relevance of molecular testing in cases where cytological findings correspond to categories III-VI [7]. In order to preoperatively assess the risk of cancer and avoid unnecessary surgical treatment in patients with cytological findings of the Bethesda III category, it is necessary to consider molecular testing [8, 9]. Bethesda IV-VI categories are indicated for surgical treatment in order to make a definite pathohistological verification. In patients with Bethesda V and VI categories, molecular testing may help decide the extent of surgery [10]. Considerable compressive symptoms are also an indication for surgical treatment, regardless of the cytological findings [11]. The aim of this retrospective study was to examine and grade the clinical and biohumoral characteristics and morphological features of the thyroid gland in patients with nodular and multinodular atoxic goiter in order to identify the possible predictors for the assessment of thyroid cancer risk.

Methods

This retrospective study included 275 patients with nodular and multinodular atoxic goiter hospitalized at the Clinic for Endocrinology of the Military Medical Academy (MMA), Belgrade, Serbia, from January 1, 2017, to October 1, 2022, for preoperative preparation. The ethical principles for medical research involving human subjects stipulated in the Declaration of Helsinki (1964) and subsequent amendments of the declaration were applied. Indication for surgical treatment was established on the cytological findings, ultrasonographic features of the nodule extremely suspicious for malignant lesion, or on the presence of pronounced compressive symptoms.

The distinctive patients' epidemiological and clinical features comprised gender and age, body mass index, presence of compressive symptoms, smoking status, presence of comorbidities, family history of thyroid diseases, previously diagnosed primary hypothyroidism, and primary hyperparathyroidism. The analyzed biohumoral parameters included the concentration of thyroid stimulating hormone (TSH), thyroglobulin, calcitonin and thyroid-specific antibodies (Abs) – thyroid peroxidase (TPO) Abs and thyroglobulin (Tg) Abs. Most patients underwent FNAB, and the cytological findings were interpreted by cytologists specializing in the thyroid gland. The cytological findings were classified according to the Bethesda classification.

The pathohistological findings were interpreted by an experienced pathologist in the field of thyroid pathohistology and classified according to World Health Organization classification. The tumor stage was established according to TNM classification for malignancies, which

includes the size of the tumor (T), the extent to lymph nodes (N), and the presence of metastasis (M).

Data were presented as mean ± standard deviation or median with interquartile range, where appropriate. The between-the-group difference was calculated using the Chi-square test, Student's *t*-test for independent samples, and Mann-Whitney *U* test, where appropriate. All tests were performed as two-tailed, and a *p*-value of < 0.05 was considered statistically significant. Data analysis was performed in the statistical software package SPSS, version 25 (IBM Corporation, Armonk, NY, USA).

Results

The study included 275 patients with an average age of 54.2 ± 14.7 years, 196 (71.3%) women and 79 (28.7%) men. A total of 105 (38.2%) patients had one nodule, and 170 (62.8%) patients had two or multiple nodules, of which 48 (17.4%) had two nodules, and 122 (44.4%) had more than two nodules. FNAB was performed with 202 (73.5%) patients. Total thyroidectomy was done in 267 (97.1%) and lobectomy in 8 (2.9%) patients. The pathohistological findings indicated the benign nature of the nodules in 118 (42.9%) patients, whereas cancer was proven in the remaining 157 (57.1%) patients. Demographic and clinical characteristics and cytological and pathohistological findings of the thyroid gland of all patients are given in Tables 1 and 2.

The results obtained from comparing patients with one and two or more nodules are presented in Table 3. Gender, smoking status, Tg values, TPO Abs, Tg Abs, FNAB cytological findings, and pathohistological findings did not differ significantly between the two groups. On the other hand, patients with two or more nodules were significantly older than patients with one nodule (57.2 ± 13.1 years vs. 49.4 ± 15.8 years, $p < 0.001$) and had a significantly higher body mass index (29.1 kg/m^2 vs. 26.5 kg/m^2, $p = 0.004$). Multinodular goiter occurred significantly more often in patients older than 50 ($p < 0.001$). On the other hand, patients with one nodule had a significantly higher TSH level compared with the patients with multinodular goiter (1.73 mUI/L vs. 1.21 mUI/L, $p < 0.001$).

In an additional analysis, we compared patients with and without proven thyroid cancer (Table 4). Age, gender, smoking status, body mass index, TSH, TPO Abs, Tg Abs, Tg, number of nodules, and dimensions of the dominant nodule did not differ significantly between these two groups.

Table 1

Demographic and clinical characteristics of patients

Characteristics	Value
Age, years	54.2 ± 14.7
Female gender	196 (71.3)
Smoker	52 (32.9)
Former smoker	24 (15.2)
Non-smoker	82 (51.9)
No comorbidities	107 (39.1)
Diabetes	39 (14.2)
Cardiovascular diseases	96 (35.1)
Autoimmune diseases	9 (3.3)
Carcinoma	16 (5.8)
COPD	4 (1.5)
Pituitary adenoma	3 (1.1)
BMI, kg/m²	28.4 ± 5.3
Presence of compressive symptoms	86 (35.4)
Family history of thyroid nodes/carcinoma	54 (28.6)
1 node in thyroid gland	105 (38.2)
2 nodes in thyroid gland	48 (17.4)
> 2 nodes in thyroid gland	122 (44.4)
Dimension of dominant node, mm	28 (19–39)
Hypothyroidism	25 (9.1)
Primary hyperparathyroidism	13 (4.7)
TSH, mIU/L	1.42 (0.89–2.18)
TPO Abs, IU/mL	3.2 (0.8–38.28)
Tg Abs, IU/mL	0.3 (0.1–13.96)
Tg, ng/L	48 (17.35–160.9)
Calcitonin, pg/mL	2.0 (1.0–2.47)

COPD – chronic obstructive pulmonary disease; BMI – body mass index; TSH – thyroid stimulating hormone; TPO – thyroid peroxidase; Abs – antibodies; Tg – thyroglobulin.
Values are expressed as mean ± standard deviation or numbers (percentages), except for dimension of dominant node, TSH, TPO Abs, Tg Abs, Tg, and calcitonin, which are presented as median (interquartile range).
***Note:* reference ranges for TSH, Tg, calcitonin, TPO Abs, and Tg Abs are 0.34–5.60 mIU/L, 3.50–77.00 ng/L, 0.50–9.82 pg/mL, < 9.0 IU/mL, and 0.0–4.0 IU/mL, respectively.**

Table 2

Cytological and histopathological parameters

Parameters	Patients
FNAB not done	73 (26.5)
Bethesda classification	
I	13 (4.7)
II	27 (9.8)
III	46 (16.7)
IV	50 (18.2)
V	59 (21.5)
VI	7 (2.5)
Total thyroidectomy	267 (97.1)
Lobectomy	8 (2.9)
Pathohistological benign finding	118 (42.9)
Pathohistological malignant finding (carcinoma)	157 (57.1)
Type of carcinoma	
papillary	76 (48.4)
micropapillary	51 (32.5)
hurthle cell	10 (6.4)
follicular	8 (5.1)
medullary	7 (4.4)
poorly differentiated thyroid carcinoma	3 (1.9)
well-differentiated tumor with uncertain malignant potential	2 (1.3)
T staging	
T1a	64 (42.4)
T1b	28 (18.5)
T2	30 (19.9)
T3a	19 (11.9)
T3b	10 (6.6)
T4	1 (0.7)
N staging	
Nx	56 (37.1)
N0	74 (49)
N1a	13 (8.6)
N1b	8 (5.3)
Radioiodine therapy, yes	88 (56.8)

FNAB – fine needle aspiration biopsy; T – extent of the tumor according to Tumor Nodes Metastasis (TNM) classification; N – extent of spread to the lymph nodes according to TNM classification.
Results are expressed as numbers (percentages).

Table 3

Demographic and clinical characteristics of patients with different numbers of nodes in the thyroid gland

Characteristics	1 node (n = 105)	≥ 2 nodes (n = 170)	*p*-value
Age, years	49.4 ± 15.8	57.2 ± 13.1	< 0.001
Age ≥ 50 years	43	124	< 0.001
Age < 50 years	62	46	
Female	71	125	0.293
Male	34	45	
Smoker	28	48	0.732
Nonsmoker	28	54	
BMI, kg/m²	26.5 (22.9–30.0)	29.1 (25.4–32.9)	0.004
TPO Abs, IU/mL	4.60 (0.95–102.65)	3.00 (0.80–35.67)	0.280
Tg Abs, IU/mL	0.70 (0.10–22.35)	0.25 (0.10–10.00)	0.232
Tg, ng/L	42.12 (13.48–164.85)	52.00 (19.51–159.80)	0.214
TSH, mIU/L	1.73 (0.95–3.00)	1.21 (0.82–1.78)	< 0.001
Cytological finding			
Bethesda II–III	27	46	0.159
Bethesda IV–VI	55	61	
Carcinoma	62	95	0.645
No carcinoma	43	74	

For abbreviations, see Table 1.
Values are expressed as numbers or median (interquartile range), except for age which is presented as mean ± standard deviation.

Table 4

Demographic and clinical characteristics of patients with and without thyroid carcinoma

Characteristics	Thyroid carcinoma		*p*-value
	No (n = 118)	Yes (n = 15)	
Age, years (mean ± SD)	53.13 ± 14.86	55.67 ± 14.40	0.156
Age ≥ 50 years, n	78	89	0.114
Age < 50 years, n	40	68	
Female, n	91	105	0.063
Male, n	27	52	
Smoker, n	28	48	0.291
Nonsmoker, n	37	45	
BMI, kg/m² (Me, IQR)	28.44 (24.88–32.00)	28.00 (24.00–31.70)	0.693
TPO Abs, IU/mL (Me, IQR)	3.90 (1.10–34.38)	2.25 (0.80–40.67)	0.688
Tg Abs, IU/mL (Me, IQR)	0.20 (0.10–10.10)	0.55 (0.10–15.52)	0.344
Tg, ng/L (Me, IQR)	45.43 (15.32–130.25)	54.45 (19.30–216.25)	0.134
TSH, mIU/L (Me, IQR)	1.42 (0.81–2.23)	1.42 (0.91–2.14)	0.73
1 nodule in thyroid gland, n	43	62	
≥ 2 nodules in thyroid gland, n	75	95	0.606
Dimension of dominant node, cm (Me)	28.6	27.05	0.836
Dimension of dominant node, cm (n)			
≤ 1	4	8	
1–2	26	40	0.784
2–3	35	41	
> 3	48	67	
Cytological finding, n			
Bethesda II–III	38	35	
Bethesda IV–VI	34	82	0.002

SD – standard deviation; n – number; Me – median; IQR – interquartile range. For other abbreviations, see Table 1.

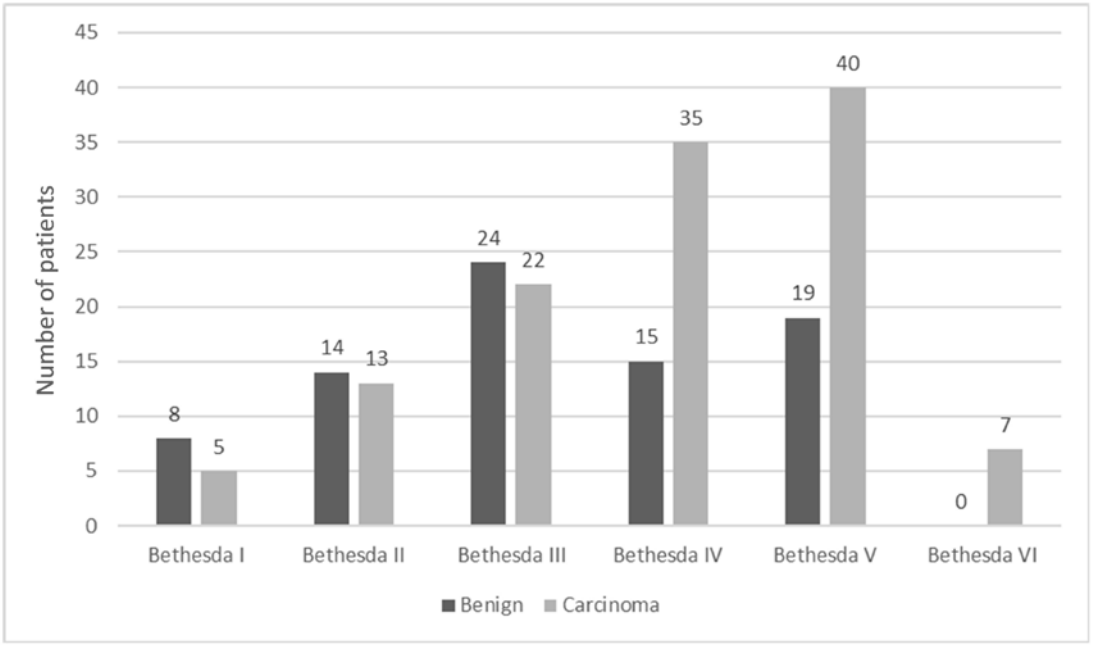

Fig. 1 – Distribution of benign and malignant findings in different categories of Bethesda classification.

The interpretation of FNAB cytological findings proved to be somewhat more complicated. However, there was a statistically significant association between the higher categories of the Bethesda classification and the cancer presence. Thyroid cancer was significantly more often diagnosed in patients with FNAB Bethesda category IV-VI compared to Bethesda II and III cytological findings (52.2% vs. 22.3%, $p = 0.002$). The distribution of diagnosed thyroid cancer for each Bethesda category is shown in Figure 1.

Discussion

Thyroid nodules are common and can be detected by palpation in 5% of women and 1% of men and/or in as many as 68% of the general population when thyroid imaging is applied [1, 12, 13]. The results of previously published studies show that nodules occur significantly more often in women [14, 15] and their incidence increases with age [16]. Previous studies showed that multinodular goiter is more

common than nodular goiter and that patients with thyroid nodules are usually euthyroid [15, 17]. These observations were in accordance with our results, where 25 of 275 patients were treated for hypothyroidism, and the remaining were euthyroid (9.1% vs. 90.9%).

Comparing the patients with nodular and multinodular goiter, we found that those with multinodular goiter were older and that multinodular goiter occurred significantly more often in patients older than 50 years, which was in accordance with the results of a study by Carlé et al. [18]. In the available literature, we did not find many studies that examined the above-mentioned parameters in relation to the number of thyroid nodes. A study by Elbalka et al. [17] showed that multinodular goiter was more common in women and that nodules in nodular goiter were larger than the dominant nodule within multinodular goiter.

Thyroid carcinomas are the most common endocrine cancers [19], and in the last 30 years, they have recorded a significant increase in incidence [16, 20, 21]. There is still an ongoing debate whether this is an actual incidence increase of thyroid cancer or a consequence of a more frequent use of US and cytological diagnostics [22, 23]. Surprisingly, a more precise diagnosis of thyroid cancers and their detection in early stages is not accompanied by a mortality rate reduction [20]. In order to avoid excessive and unnecessary diagnostics, there is ongoing work on the identification of predictive risk factors for the occurrence of thyroid cancers. However, the results of the earlier studies have not always been consistent.

It is generally considered that thyroid cancers occur more often in women [24] and at a particular age [25]. This was only partially confirmed by our study. Namely, cancer was more often diagnosed in women compared to men (66.88% vs. 33.12%, respectively). However, comparing patients with and without cancer did not prove a specific gender effect on cancer occurrence (p = 0. 063). When comparing the same groups by age, whether using the average age (p = 0.156) or the cut-off age of 50 (p = 0.114), the years of life showed no association with the increasing risk of developing thyroid cancer. Meta-analysis of prospective observational studies pointed to an increased risk of developing thyroid cancer with a higher body mass index [26], which was not observed in our results. A possible explanation might be the smaller sample size of our study population. Mack et al. [27] pointed to surprising results of a potentially protective effect of smoking against the development of thyroid cancers. A possible explanation for this finding was that smoking might decrease estrogen levels, which was considered responsible for increasing the risk of thyroid cancer and the incidence of cancer in women, especially in particular age groups [28]. In our study, body mass index (p = 0.693) as an indirect indicator of diet and smoking status (p = 0.291) were not shown to be important risk factors for developing thyroid cancer.

Although most of the patients with thyroid cancer were euthyroid, the results of some studies showed a rise in cancer risk with the increase in the TSH value within the reference limits [29]. Previous studies have not reached a uniform conclusion on the correlation between thyroid cancer occurrence and TPO Abs and Tg Abs [30, 31]. It is well-known that Tg values may be elevated in various thyroid diseases. Therefore, Tg is not a sufficiently sensitive and specific marker for thyroid cancer diagnosis [32]. Examining these biohumoral parameters in our study cohort, we found no association between TSH (p = 0.73), Tg (p = 0.134), TPO Abs (p = 0.688), and Tg Abs (p = 0.344) with the risk for thyroid cancer development.

Analyzing numerous US characteristics, Frates et al. [33] showed that the risk for thyroid cancer did not increase with the number and dimensions of the nodules, despite the generally accepted opinion that larger nodules are associated with a higher cancer risk [34]. A study by Kamran et al. [35] showed that the presence of nodules larger than 2 cm in diameter is associated with a higher risk of thyroid cancer. In our study, we compared the patients with and without thyroid cancer relative to the mean value of the dimensions of the dominant nodule (p = 0.836). We have also performed a subanalysis, classifying the nodules by size into four groups: < 1 cm, 1–2 cm, 2–3 cm, and > 3 cm. In both cases, it was shown that the nodule size did not affect the risk of cancer. Moreover, in our study, there was no difference in the frequency of cancer occurrence in patients with nodular vs. multinodular goiter (p = 0.645).

The FNAB is considered the most reliable diagnostic procedure with the best benefit-risk ratio for screening thyroid nodules for cancer [36, 37]. Of all the analyzed parameters in our study, the FNAB cytological finding was the only one showing a strong association with cancer occurrence. Namely, higher Bethesda classification categories were associated with a higher risk of developing cancer (p = 0.002), with a frequency of cancer in Bethesda categories V and VI of 67.8% and 100%, respectively, compared to 38.5%, 48.1%, 47.8%, and 70% in Bethesda categories I, II, III, and IV, respectively. Results for the Bethesda III category agreed with the results of previous studies where the frequency of 6–48% was an actual cancer ratio in this category [38, 39]. A more detailed subcategorization of the Bethesda III category could help estimate the risks of cancer occurrence better and avoid unnecessary surgical interventions [40]. In our study, cancer frequency in the Bethesda IV category was in concordance with the results of some previous studies [41, 42]. The high frequency of carcinoma in the Bethesda I category could be explained by not repeating the FNAB in previous studies, which would have otherwise delivered a better sample for a more precise classification of cytological findings.

The incidence of various types of thyroid cancer in our study was similar to the findings of previous major studies [5, 43]. In our study, papillary cancer was the most common type (48.4%), followed by micropapillary cancer present in 32.5% of patients. It was followed by oncocytic carcinoma, historically known as the Hurthle cell carcinoma (6.4%), follicular carcinoma (5.1%), medullary carcinoma (4,4%), poorly differentiated carcinoma (1.9%), and well-differentiated tumor of uncertain malignant potential (1.3%). Furthermore, cancer was diagnosed in the first stage in 60.9% of patients (T1a – 42.4%, T1b – 18.5), and less frequent in the

more advanced stages (T2 – 19.9%, T3a – 11.9%, T3b – 6.6%, T4a – 0.7%). Relying on these findings, it could be concluded that the indications for surgical treatment were well established, and the patient selection was properly made.

Several study limitations should be mentioned. First, patients from only one healthcare center were included, which can be potentially unrepresentative of the general population. Second, FNAB was performed only on the dominant nodule and/or ultrasonographically suspicious nodules, not on all existing nodules in patients with multinodular goiter. Finally, there are a few missing data on observed parameters.

Conclusion

Evaluation of thyroid nodules remains a diagnostic challenge in everyday clinical practice. Apart from clinical findings, biohumoral testing, and ultrasonographic characteristics, FNAB is necessary for nodules presenting with suspected malignant features on US examination. Our work demonstrated the importance of FNAB and Bethesda classification in the evaluation of thyroid nodules in patients with atoxic nodular and multinodular goiter, once again proving that the higher Bethesda categories imply a higher thyroid cancer risk.

R E F E R E N C E S

1. *Russ G, Leboulleux S, Leenhardt L, Hegedüs L.* Thyroid incidentalomas: epidemiology, risk stratification with ultrasound and workup. Eur Thyroid J 2014; 3(3): 154–63.
2. *Siegel RL, Miller KD, Jemal A.* Cancer statistics, 2018. CA Cancer J Clin 2018; 68(1): 7–30.
3. *Silvestre C, Sampaio Matias J, Proença H, Bugalho MJ.* Calcitonin Screening in Nodular Thyroid Disease: Is There a Definitive Answer? Eur Thyroid J 2019; 8(2): 79–82.
4. *Kiković S, Marinković DM, Ristić P, Karajović J, Kuzmic Janković S, Djuran Z,* et al. Role and importance of elastography in the diagnosis of differentiated thyroid carcinomas regarding the clinical, echosonographic, biohumoral and cytological examination and correlation of these results with definitive histopathological findings – A retrospective study. Vojnosanit Pregl 2021; 78(6): 589–98.
5. *Tessler FN, Middleton WD, Grant EG, Hoang JK, Berland LL, Teefey SA,* et al. ACR Thyroid Imaging, Reporting and Data System (TI-RADS): White Paper of the ACR TI-RADS Committee. J Am Coll Radiol 2017; 14(5): 587–95.
6. *Ali SZ, Baloch ZW, Cochand-Priollet B, Schmitt FC, Vielh P, VanderLaan PA.* The 2023 Bethesda System for Reporting Thyroid Cytopathology. Thyroid 2023; 33(9): 1039–44.
7. *Nishino M, Krane JF.* Role of Ancillary Techniques in Thyroid Cytology Specimens. Acta Cytol 2020; 64(1–2): 40–51.
8. *Livhits MJ, Zhu CY, Kuo EJ, Nguyen DT, Kim J, Tseng CH,* et al. Effectiveness of Molecular Testing Techniques for Diagnosis of Indeterminate Thyroid Nodules: A Randomized Clinical Trial. JAMA Oncol 2021; 7(1): 70–7.
9. *Lupo MA, Walts AE, Sistrunk JW, Giordano TJ, Sadow PM, Massoll N,* et al. Multiplatform molecular test performance in indeterminate thyroid nodules. Diagn Cytopathol 2020; 48(12): 1254–64.
10. *Hier J, Avior G, Pusztaszeri M, Krasner JR, Alyouha N, Forest VI,* et al. Molecular testing for cytologically suspicious and malignant (Bethesda V and VI) thyroid nodules to optimize the extent of surgical intervention: a retrospective chart review. J Otolaryngol Head Neck Surg 2021; 50(1): 29.
11. *Chen AJ, Bernet VJ, Carty SE, Davies TF, Ganly I, Inabnet WB 3rd,* et al. American Thyroid Association Statement on optimal surgical management of goiter. Thyroid 2014; 24(2): 181–9.
12. *Mazzaferri EL.* Management of a solitary thyroid nodule. N Engl J Med 1993; 328(8): 553–9.
13. *Tan GH, Gharib H.* Thyroid incidentalomas: management approaches to nonpalpable nodules discovered incidentally on thyroid imaging. Ann Intern Med 1997; 126(3): 226–31.
14. *Hegedüs L, Bonnema SJ, Bennedbaek FN.* Management of simple nodular goiter: current status and future perspectives. Endocr Rev 2003; 24(1): 102–32.
15. *Gharib H, Papini E, Paschke R, Duick DS, Valcavi R, Hegedüs L,* et al. AACE/AME/ETA Task Force on Thyroid Nodules. American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association Medical guidelines for clinical practice for the diagnosis and management of thyroid nodules: executive summary of recommendations. Endocr Pract 2010; 16(3): 468–75.
16. *Vaccarella S, Franceschi S, Bray F, Wild CP, Plummer M, Dal Maso L.* Worldwide Thyroid-Cancer Epidemic? The Increasing Impact of Overdiagnosis. N Engl J Med 2016; 375(7): 614–7.
17. *Elbalka SS, Metwally IH, Shetiwy M, Awny S, Hamdy O, Kotb SZ,* et al. Prevalence and predictors of thyroid cancer among thyroid nodules: a retrospective cohort study of 1,000 patients. Ann R Coll Surg Engl 2021; 103(9): 683–9.
18. *Carlé A, Krejbjerg A, Laurberg P.* Epidemiology of nodular goiter. Influence of iodine intake. Best Pract Res Clin Endocrinol Metab 2014; 28(4): 465–79.
19. *Bonnefond S, Davies TF.* Thyroid Cancer-Risks and Causes. Oncol Hematol Rev 2014; 10(2): 144–51.
20. *Davies L, Welch HG.* Current thyroid trends in the United States. JAMA Otolaryngol Head Neck Surg 2014; 140(4): 317–22.
21. *Davies L, Welch HG.* Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA 2006; 295(18): 2164–7.
22. *Kent WD, Hall SF, Isotalo PA, Houlden RL, George RL, Groome PA.* Increased incidence of differentiated thyroid carcinoma and detection of subclinical disease. CMAJ 2007; 177(11): 1357–61.
23. *Udelsman R, Zhang Y.* The epidemic of thyroid cancer in the United States: the role of endocrinologists and ultrasounds. Thyroid 2014; 24(3): 472–9.
24. *Yao R, Chiu CG, Strugnell SS, Gill S, Wiseman SM.* Gender differences in thyroid cancer: a critical review. Expert Rev Endocrinol Metab 2011; 6(2): 215–43.
25. *Kwong N, Medici M, Angell TE, Liu X, Marqusee E, Cibas ES,* et al. The Influence of Patient Age on Thyroid Nodule Formation, Multinodularity, and Thyroid Cancer Risk. J Clin Endocrinol Metab 2015; 100(12): 4434–40.
26. *Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M.* Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008; 371(9612): 569–78.
27. *Mack WJ, Preston-Martin S, Dal Maso L, Galanti R, Xiang M, Franceschi S,* et al. A pooled analysis of case-control studies of thyroid cancer: cigarette smoking and consumption of alcohol, coffee, and tea. Cancer Causes Control 2003; 14(8): 773–85.
28. *Derwahl M, Nicula D.* Estrogen and its role in thyroid cancer. Endocr Relat Cancer 2014; 21(5): T273–83.
29. *Boelaert K, Horacek J, Holder RL, Watkinson JC, Sheppard MC, Franklyn JA.* Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by

fine-needle aspiration. J Clin Endocrinol Metab 2006; 91(11): 4295–301.
30. *Wong SL, Grodski S, Yeung MJ, Serpell JW*. Anti-thyroid antibodies as a predictor of thyroid cancer. ANZ J Surg 2015; 85(11): 849–53.
31. *Azizi G, Keller JM, Lewis M, Piper K, Puett D, Rivenbark KM,* et al. Association of Hashimoto's thyroiditis with thyroid cancer. Endocr Relat Cancer 2014; 21(6): 845–52.
32. *Suh I, Vriens MR, Guerrero MA, Griffin A, Shen WT, Duh QY,* et al. Serum thyroglobulin is a poor diagnostic biomarker of malignancy in follicular and Hurthle-cell neoplasms of the thyroid. Am J Surg 2010; 200(1): 41–6.
33. *Frates MC, Benson CB, Doubilet PM, Kunreuther E, Contreras M, Cibas ES*, et al. Prevalence and distribution of carcinoma in patients with solitary and multiple thyroid nodules on sonography. J Clin Endocrinol Metab 2006; 91(9): 3411–7.
34. *Shin JJ, Caragacianu D, Randolph GW*. Impact of thyroid nodule size on prevalence and post-test probability of malignancy: a systematic review. Laryngoscope 2015; 125(1): 263–72.
35. *Kamran SC, Marqusee E, Kim MI, Frates MC, Ritner J, Peters H,* et al. Thyroid nodule size and prediction of cancer. J Clin Endocrinol Metab 2013; 98(2): 564–70.
36. *Hadi M, Gharib H, Goellner JR, Heerden JA*. Has fine-needle aspiration biopsy changed thyroid practice? Endocr Pract 1997; 3(1): 9–13.
37. *Wang SH, Arscott P, Wu P, Baker JR Jr.* No apparent damage in the thyroid of transgenic mice expressing antiapoptotic FLIP. Thyroid 2006; 16(1): 1–8.
38. *Ohori NP, Schoedel KE*. Variability in the atypia of undetermined significance/follicular lesion of undetermined significance diagnosis in the Bethesda System for Reporting Thyroid Cytopathology: sources and recommendations. Acta Cytol 2011; 55(6): 492–8.
39. *Ho AS, Sarti EE, Jain KS, Wang H, Nixon IJ, Shaha AR,* et al. Malignancy rate in thyroid nodules classified as Bethesda category III (AUS/FLUS). Thyroid 2014; 24(5): 832–9.
40. *Shrestha RT, Hennessey JV*. Cytologic subclassification of atypia of undetermined significance may predict thyroid nodules more likely to be malignant at surgery. Diagn Cytopathol 2016; 44(6): 492–8.
41. *Özdemir A, Uyan M, Kalcan S, Çolakoğlu MK, Pergel A.* Malignancy rates and risk factors in Bethesda category IV thyroid nodules: Is lobectomy enough in an endemic region? J Exp Clin Med 2022; 39(3): 749–54.
42. *Chirayath SR, Pavithran PV, Abraham N, Nair V, Bhavani N, Kumar H,* et al. Prospective Study of Bethesda Categories III and IV Thyroid Nodules: Outcomes and Predictive Value of $BRAF^{V600E}$ Mutation. Indian J Endocrinol Metab 2019; 23(3): 278–81.
43. *Miranda-Filho A, Lortet-Tieulent J, Bray F, Cao B, Franceschi S, Vaccarella S,* et al. Thyroid cancer incidence trends by histology in 25 countries: a population-based study. Lancet Diabetes Endocrinol 2021; 9(4): 225–34.

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Tracheostomy in infants: indications and outcomes

Traheostomija kod odojčadi: indikacije i ishodi

Aleksandar Sovtić*†, Bojana Gojšina*, Ivan Baljošević*‡, Stefan Popović*

***Institute of Mother and Child Health Care of Serbia "Dr. Vukan Čupic", Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia; ‡University of Business Academy, Faculty of Dentistry Pančevo, Novi Sad, Serbia**

Abstract

Background/Aim. Prolonged ventilation is the most common indication for pediatric tracheostomy. The aim of the study was to determine the indications, possible complications, and outcomes of tracheostomy in infants, as well as the association of patient phenotype with complications following tracheostomy. **Methods.** This retrospective study highlights the main indications, complications, and decannulation rates in tracheostomy pediatric patients treated at the Institute of Mother and Child Health Care of Serbia "Dr. Vukan Čupić", Belgrade, for three years. **Results.** A total of 38 infants were included in our retrospective study, 31 (81%) of whom underwent elective tracheostomy, and 7 (19%) underwent urgent tracheostomy due to acute respiratory distress and difficult intubation. The mean age was 5.4 ± 3.5 months, and the youngest participant was 36 hrs old. The primary indication for elective tracheostomy was prolonged mechanical ventilation in 13 (42%) patients, neuromuscular disorders in 5 (16%), airway obstruction in 10 (32%), craniofacial anomalies in 2 (5%), and pulmonary disease in 1 (3%) patient. Early complications (occurring within the first seven days after tracheostomy) were present in 4 (10.5%) patients, three of whom had air leaks (due to inappropriate cannula selection), whereas wound dehiscence was reported in one patient. Late complications (those occurring more than seven days after tracheostomy) were reported in 4 (10.5%) patients and they were peristomal granulations in three patients and tube obstruction in one patient. There were no deaths associated with tracheostomy, although overall mortality was 21% (8 patents). All of these patients died as a result of their primary diseases. Seventeen (44%) patients were successfully decannulated. **Conclusion.** Most patients required long-term treatment and tracheostomy retention due to the nature of their primary diseases, which coincided with low decannulation rates. Therefore, tracheostomies should preferentially be carried out in specialized pediatric centers with trained medical personnel ensuring adequate health care.

Key words:
infant, newborn; respiration, artificial; serbia; tracheostomy.

Apstrakt

Uvod/Cilj. Produžena ventilacija predstavlja najčešću indikaciju za pedijatrijsku traheostomiju. Cilj rada bio je da se utvrde indikacije, komplikacije i ishod traheostomije kod dece, kao i udruženost fenotipa i komplikacija koje prate traheostomiju. **Metode.** Retrospektivnom studijom procenjene su osnovne indikacije, komplikacije i stope dekanulacije kod dece sa traheostomom, lečene na Institutu za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić" u Beogradu, tokom trogodišnjeg perioda. **Rezultati.** Studijom je obuhvaćeno ukupno 38 novorođenčadi, od kojih je njih 31 (81%) bilo podvrgnuto elektivnoj traheostomiji, a 7 (19%) urgentnoj traheostomiji zbog akutnog respiratornog distresa i teške intubacije. Srednja životna starost novorođenčadi bila je 5,4 ± 3,5 meseci, a najmlađe je bilo staro 36 sati. Primarna indikacija za elektivnu traheostomiju bila je produžena mehanička ventilacija kod 13 (42%) bolesnika, neuromuskularni poremećaji kod 5 (16%) bolesnika, opstrukcija disajnih puteva kod 10 (32%), kraniofacijalne anomalije kod 2 (5%) i plućna bolest kod jednog (3%) bolesnika. Rane komplikacije (koje su se javljale tokom prvih sedam dana od traheostomije) bile su prisutne kod 4 (10,5%) bolesnika, od kojih je njih troje imalo curenje vazduha (zbog neodgovarajućeg izbora kanile), a dehiscenciju rane imao je jedan bolesnik. Kasne komplikacije (koje su se pojavile kasnije od sedam dana posle traheostomije) bile su prisutne kod 4 (10,5%) bolesnika i to peristomalna granulacija kod tri bolesnika i opstrukcija tubusa, kod jednog bolesnika. Nije bilo smrtnih ishoda kod bolesnika povezanih sa traheostomijom, mada je ukupan mortalitet bio 21% (8 bolesnika). Svi ovi bolesnici preminuli su usled primarnog oboljenja. Sedamnaest (44,7%) bolesnika je uspešno dekanilisano. **Zaključak.** Za većinu

Correspondence to: Ivan Baljošević, Institute of Mother and Child Health Care of Serbia "Dr. Vukan Čupić", Department of Otorhinolaryngology, Radoja Dakića 6, 11 070 Belgrade, Serbia. E-mail: ivan.baljosevic@gmail.com

bolesnika je, zbog prirode njihovih osnovnih oboljenja, bilo potrebno dugotrajno lečenje i retencija traheostomije što se podudaralo sa niskom stopom dekanulacije. Zbog toga bi traheostomije trebalo izvoditi prvenstveno u specijalizovanim centrima sa obučenim medicinskim osobljem, čime se obezbeđuje odgovarajuća zdravstvena zaštita.

Ključne reči:
novorođenče; disanje, veštačko; srbija; traheostomija.

Introduction

Tracheostomy is a potentially lifesaving surgical procedure performed in children for centuries [1]. The first reported surgical airway in adults dates back to Ancient Egypt [2], albeit the first pediatric tracheostomy was performed in 1620 by Nicholas Habicot on a 14-year-old boy who swallowed a bag of pistoles or gold coins to prevent their theft – the bag of coins lodged in the esophagus, compressing the trachea and causing subsequent airway obstruction [3]. Until as recently as the late 1970s, the primary indication for tracheostomy was upper airway obstruction secondary to acute infections such as *epiglottitis* and acute *laryngotracheobronchitis*, or croup [1]. Although the widespread use of antibiotics and the advent of vaccines have led to changes in the primary indications for pediatric tracheostomy in the last thirty years, the incidence of tracheostomy in children has risen due to prolonged survival in intensive care units (ICUs) [4]. Today, the most common indications are chronic respiratory failure with ventilator dependence, congenital or acquired upper airway anomalies, and neurological impairment [4, 5]. More than half of all pediatric tracheostomies are performed in infants, the majority of whom are born preterm with very low birth weights. In this population, mortality rates and health-care costs due to prolonged length of hospital stay (LHS) are higher [6].

Tracheostomy in children, especially in infants, can be more difficult to perform than in adults due to the size of the airway, a soft compliant trachea with greater lateral mobility and proximity of the cupula, and is frequently associated with complications such as deep incisional surgical site infection, scarring and stenosis, recurrent pneumonia, and sudden death. The majority of complications occur in neonates and children with cardiac anomalies and preoperative intraventricular hemorrhage [7].

The aim of this study was to identify the indications, possible complications, and outcomes of tracheostomy in infants, as well as the association of patient phenotype with complications following tracheostomy.

Methods

This retrospective review of medical records of all infants who underwent primary tracheostomy at the Institute of Mother and Child Health Care of Serbia "Dr. Vukan Čupić", Belgrade, Serbia has been implemented between January 1, 2020, and December 31, 2022. A total of 38 patients were identified, 31 of whom underwent elective tracheostomy under endotracheal anesthesia and seven underwent urgent tracheostomy due to acute respiratory distress and difficult intubation. Before tracheostomy, parental permission was obtained for each patient. The study was approved by the Ethics Committee of the Institute (No. 119/2024).

In cases of prolonged intubation, the date of elective tracheostomy was decided upon by pediatricians in the ICU in consultation with otorhinolaryngologists after acquiring parental permission. Data included gender, age, tracheostomy indications, early and late complications, underlying diseases, lower airway pathogens, flexible bronchoscopy findings, culture and sensitivity of tracheal aspirates before tracheostomy, overall LHS, and outcomes. Endotracheal intubation lasting at least four weeks was considered prolonged.

Early complications were defined as complications occurring within the first seven days after tracheostomy. Late complications were those occurring eight or more days after tracheostomy up until decannulation. Potential complications included accidental decannulation, tracheal tube occlusion, infection, pulmonary air-leak syndromes (including subcutaneous emphysema and pneumothorax), bleeding, tracheal or tracheostomy stenosis, and peristomal granulation.

Patients on mechanical ventilation (MV) who no longer required hospitalization were discharged after their parents or caregivers received adequate home care and ventilation education and underwent tracheostomy tube changes in the hospital every three weeks in an outpatient setting. Patients were decannulated when they no longer required MV once the original indication for tracheostomy was resolved after tube downsizing and a trial of daytime capping (24 hrs for 3–4 consecutive days without respiratory difficulties).

The relationship between patient age and the presence of complications and LHS before tracheostomy was examined as well. For this study, patients were divided into three age categories: 0–3 months, 3–6 months, and 6–12 months.

Surgical technique

Irrespective of whether an urgent or elective tracheostomy was performed, a midline vertical "Y" tracheal incision through the second, third, and fourth tracheal rings was used. Maturation sutures were then used where the edges of the skin incision were sutured to the edges of the tracheal incision using absorbable 4/0 vicryl. Uncuffed either polyvinyl chloride Tracoe or silicone Rusch® tracheostomy tubes were introduced into the tracheal stoma after endotracheal tube withdrawal, and the tracheal cannula flanges were securely tied around the neck using shoestring-style ties. In each patient, a tracheostomy tube of appropriate

dimensions was selected, and its proper position was determined by ventilator parameters and/or chest radiography.

Statistical analysis

Statistical data was determined using the Easy R software program. The Kolmogorov-Smirnov test was used to determine whether variables exhibited normal distribution. Variables with normal distribution were expressed as mean and standard deviation. Categorical data was compared using nonparametric tests – Pearson's Chi-square test and Fisher's exact test. Multivariable logistic regression analyses were used to calculate the odds ratio (OR) and corresponding 95% confidence intervals (CIs) to identify independent factors that may influence disposition. A p-value < 0.05 was considered statistically significant.

Results

The study included 38 infants, 20 (52.6%) male and 18 (47.4%) female. The majority of children, 18 (47.4%), were aged 6–12 months, 7 (18.4%) of them were 3–6 months old, and 13 (34.2%) were 0–3 months old. The mean age was 5.4 ± 3.5 months, and the youngest participant was 36 hrs old. Demographic characteristics are shown in Table 1.

Elective tracheostomy was performed in 31 (81%) infants, and urgent tracheostomy in 7 (19%). The most common indication for urgent tracheostomy involved airway anomaly or underdevelopment, and the mean age was 26.3 ± 16.4 days. There was no statistically significant difference in LHS after tracheostomy between these three groups (p = 1). Almost all of these infants had low or very low birth weights (71.4%), see Table 2.

The average ICU LHS was 44 days (ranging from 1 to 97 days), and the average LHS was 85 days (ranging from 21 to 227 days). An intubation time before tracheostomy of less than 15 days was seen in 9 (29%) patients, an intubation time of 15–30 days was seen in 4 (13%) patients, and 18 (58%) patients had an intubation time of more than 30 days.

Ventilator-associated respiratory failure (chronic respiratory failure with ventilator dependence) was the most common indication for elective tracheostomy seen in 14 (36.8%) patients, most of whom had complex congenital heart disease or neuromuscular disorders. In 41.7% of these patients, extubating was attempted more than twice before tracheostomy. The second most common indication was upper airway obstruction in 11 (28.9%) participants (tracheomalacia in four, subglottic hemangioma in two, lymphangioma in two, bilateral vocal cord paralysis in one, subglottic stenosis in two). Other indications are shown in Figure 1.

The most common pathogen isolated in tracheal aspirates before tracheostomy was *Pseudomonas aeruginosa* in 23.6% of participants, with the highest prevalence (30.8%) in the youngest age category. Chronic colonization of lower airways was associated with prolonged hospital stay (Pearson's Chi-square test, p = 0.03). The most frequent pathogens from tracheal aspirates are shown in Figure 2.

All tracheostomies were carried out by three surgeons with over 15 years of experience. High-flow oxygen therapy after tracheostomy was utilized in 29 (77%) patients.

Table 1

Demographic data of 38 studied infants

Variable	Values
Gender	
male	20 (52.6)
female	18 (47.4)
Mean age (months)	5.4 ± 3.5
Elective tracheostomy	31 (81)
Urgent tracheostomy	7 (19)
Mean ICU length of stay (days)	44 ± 23
Mean length of ET intubation (days)	28 ± 26.2
Early complications	4 (10.5)
Late complications	4 (10.5)
Mortality	8 (21.1)

ICU – Intensive Care Unit; ET – endotracheal.
All values are given as numbers (percentages) or mean ± standard deviation.

Table 2

Urgent tracheostomies in seven out of a total of 38 analyzed infants

Age (days)	Gender	Body weight (g)	Diagnosis
45	male	1,250	acquired tracheal stenosis
5	female	1,000	congenital tracheal stenosis
35	male	1,900	congenital tracheal stenosis
30	female	2,730	laryngeal membrane
2	male	1,800	hypopharyngeal tumor
37	female	2,300	choanal atresia
30	male	3,030	pertusis

All values are expressed as numbers.

Early complications (≤ 7 days after tracheostomy) were reported in 4 (10.5%) patients. An air leak was reported in three of these patients, each of whom was subsequently managed by tracheostomy tube upsizing (using a larger tube). One case of wound dehiscence was reported, which was treated conservatively using simple wound care, antibiotic coverage, and suture closure. Early complications were more common in the youngest age category (0–3 months) compared to the other age categories. In particular, air leaks were present in 65% of patients in the 0–3 months-old group (Fisher's exact test, $p = 0.01$).

Late complications (> 7 days after tracheostomy) were reported in 4 (10.5%) patients: peristomal granulations were reported in three children, and tracheostomy tube obstruction was reported in one child.

The ICU LHS post-tracheostomy lasted more than one month in 17 (44%) cases, less than one month in 9 (23%) cases, and less than seven days in 12 (33%) cases.

Total LHS lasted more than one month in 33 (87%) cases and less than one month in 5 (13%) cases.

Tracheostomy revision surgery was not reported in any of the 38 patients in this study.

There were no deaths associated with tracheostomy, although the total mortality was 21% (8 patients). These patients succumbed as a direct result of or from sequelae related to their primary diseases, all of them in the ICU, in four patients within 30 days after tracheostomy and in the remaining four cases within a year after tracheostomy. Six of these children had multiple congenital anomalies, and two had neuromuscular disorders. Mortality rates were higher among the youngest patients (73%, $p = 0.04$). Multivariable logistic regression was used to examine which variable predicted mortality the best – prolonged intubation, early complication occurrence, patient age, or emergency tracheostomy. Younger age was the most significant predictor of fatal outcome ($p = 0.03$, OR 0.295, 95% CI 0.032–0.97).

Of the 38 tracheostomized patients, 20 (64%) discharged patients required home MV. Parents received adequate education on home care before discharge, including home ventilation, airway management, suctioning, tracheostomy care, and emergency management. Tracheostomy tube changes were carried out for outpatients every three weeks in the hospital.

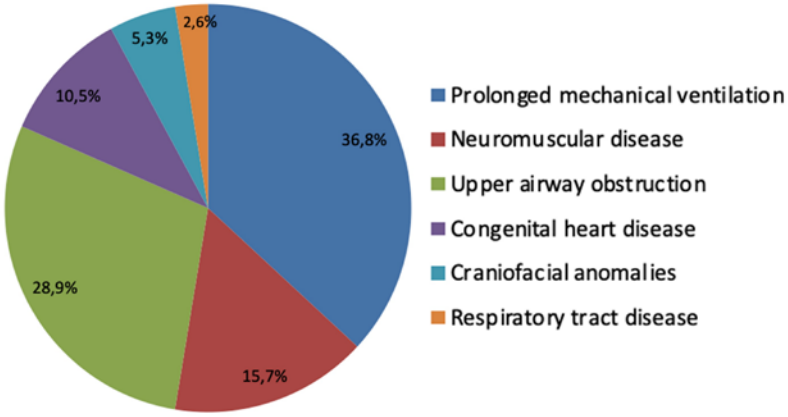

Fig. 1 – Indications for tracheostomy in 38 studied infants.

Fig. 2 – Tracheal aspirate cultures before tracheostomy in analyzed infants.
PA – *Pseudomonas aeruginosa*; SA – *Staphylococcus aureus*; KP – *Klebsiella pneumoniae*; MRSA – methicillin-resistant *Staphylococcus aureus*; G^- – Gram-negative.

Seventeen (44.7%) patients were successfully decannulated. One patient (in whom tracheostomy was carried out because of pertussis) was successfully decannulated within one month. The remaining 16 patients were decannulated 12 months or more after tracheostomy. Three children required tracheocutaneous fistula closure.

Discussion

In our study, the most common indication for tracheostomy in infants was ventilator-associated respiratory failure (or prolonged MV), which is in concordance with other published studies [8, 9]. Although variation exists in terminology and defined criteria for prolonged MV, in our study, an intubation time of a minimum of 28 days was considered prolonged. Upper airway obstruction was the second most common indication in almost 30% of patients, comparable to a study conducted by D'Souza et al. [10]. According to Gumussoy [11], about 80% of children undergoing tracheostomy have comorbidities that can complicate the procedure itself, as well as their postoperative care. In our study, 31 (81%) participants had associated comorbidities. These children often needed their tracheostomies to be retained for months or even years, requiring complex care in centers with expertise, as well as specific home care. Moreover, they spent long periods in the ICU or high dependency units before tracheostomy (44 ± 23 days), less than Dursun and Ozel [12] reported.

In all our infants, tracheostomy was performed using a vertical midline "Y" tracheal incision through the second, third, and fourth tracheal rings. Maturation sutures using absorbable 4/0 vicryl were then used, fixing the edges of the skin incision to the edges of the tracheal incision, allowing a more secure opening that tended to stay open even without the tube *in situ* (allowing ease of access to the airway in case of accidental decannulation) [10]. Some authors advise against the use of horizontal skin and tracheal incisions. One study found a significant correlation between bleeding and the use of horizontal skin incisions and between the incidence of subcutaneous emphysema (an early complication) and stomal infection (a late complication) and horizontal tracheal incisions [11]. Zenk et al. [13] showed good outcomes using a vertical incision between the second and third tracheal rings and a horizontal incision at either end (an "H cut"). A higher incidence of intraoperative bleeding was reported with surgery duration of more than 30 min or with surgeons who had less than five years of experience [11].

Tracheostomy-associated complications can be minor, including wound dehiscence and formation of granulation tissue, or major, including accidental decannulation, bleeding, tube obstruction, and death. Studies show that the frequency of early complications ranges from between 5% and 49%, which agrees with our results [10, 11, 14].

Accidental decannulation, a potentially life-threatening complication, has a reported incidence of approximately 2%, according to literature [12], whereas in our study, no cases were reported. In our study, early complications were reported in 10.5% of patients. There were no major complications. Minor complications occurring within the seven-day postoperative period included an air leak (retrograde flow of air around the tracheostomy tube due to inadequate tube size placement), which was treated by tracheostomy tube upsizing, and one case of wound dehiscence, which was conservatively treated with simple wound care, antibiotic coverage, and suture closure. The moist environment of the tracheostomy and proximity to oral secretions were thought to contribute to chronic skin inflammation around the stoma. In its early stages, wound dehiscence may be recognized by peristomal skin erythema and/or pallor, leading to overt ulceration and dehiscence. No patients developed air leak syndrome (pneumothorax, pneumomediastinum, or subcutaneous emphysema). Early complications were most common in the youngest age category (0–3 months). One author reported a higher incidence of early complications such as intraoperative bleeding with surgery duration of more than 30 min or surgeon experience of less than five years [11]. Some authors recommend early tracheostomy tube changing (up to four days after surgery) to prevent complications [15].

Late complications are mostly associated with postoperative wound care. In our study, 7.9% of patients developed tracheal-peristomal granulations, which is at a lower rate than reported by several other studies, with incidences of between 24% and 100% [14]. Medical care largely depends on the medical staff's support and ability to care for the surgical airway. Fear of accidental decannulation can lead to hesitation in manipulating cannulas, inadequate peristomal skin inspection, and wound care. This can lead to tracheostomy tube obstruction and the need for urgent tube replacement. Caregivers should be adequately trained in tracheal aspiration, surgical airway management, and tracheostomy tube manipulation in case of accidental decannulation and basic cardiopulmonary resuscitation. Proper caregiver training can help reduce mortality and the incidence of complications. Zenk et al. [13] reported a high tracheal stenosis rate (13%) in children with multiple diseases because of prolonged tracheostomy and frequent infection occurrence.

Overall tracheostomy-associated mortality has been steadily declining the past 30 years and is largely dependent on comorbidities. Relative mortality after tracheostomy is approximately 16% [16, 17]. Dursun and Ozel [12] reported a mortality rate of 59%. The highest mortality was recorded in children with neuromuscular diseases. Primary disease progression was the leading cause of death in children with tracheostomy in our study, which is comparable to other published studies [17]. There were no tracheostomy-associated deaths in our study; however, the overall mortality was 21%, and younger age was found to be an independent risk factor. These patients died as a result of their primary diseases, all of them in the ICU; four patients died within 30 days after tracheostomy, and the remaining four patients within a year after tracheostomy. Six of these children had multiple congenital anomalies, and two had neuromuscular diseases.

After recovery, 64% of patients were discharged from the hospital needing home MV. Parents received adequate

education on home ventilation before discharge. Adequate parent education may reduce tracheostomy-associated mortality. Patients underwent tracheostomy tube changes in the hospital every three weeks in an outpatient setting.

A shift in indications for tracheostomy coinciding with improvements in standards of pediatric intensive care facilities has highlighted two important outcome measures: length of tracheostomy retention and successful decannulation. Successful decannulation rates have decreased, whereas lengths of tracheostomy retention have more than doubled, with maximum figures ranging from 12 months to 24 months and above (decannulation was achieved in 22.5% of cases in the three years) [18, 19]. Decannulation depends primarily on comorbidities and secondarily on postoperative complications. Patients with neuromuscular diseases are rarely decannulated. Zenk et al. [13] reported patients with neuromuscular diseases retaining their tracheostomies the longest (17.1 years). In our study, 17 (44.7%) patients underwent successful decannulation. In all cases, tracheostomies were performed as temporary airway accompanying surgical procedures relieving upper airway anomalies or underdevelopment.

Conclusion

Tracheostomy in infants is a relatively safe procedure. The most common indication for tracheostomy is prolonged intubation and/or ventilator-associated respiratory failure. Only minor resolvable complications were reported in our study. There were no deaths associated with tracheostomy. The majority of patients had severe primary diseases that required multidisciplinary treatment over many years and longer tracheostomy retention periods, resulting in low decannulation rates. Therefore, tracheostomies should be carried out in specialized pediatric centers with experienced medical personnel where parents can receive adequate education on home care.

Conflict of interest

The authors of this paper certify that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

REFERENCES

1. *Roberts J, Powell J, Begbie J, Siou G, McLarnon C, Welch A,* et al. Pediatric tracheostomy: A large single-center experience. Laryngoscope 2020; 130(5): E375–80.
2. *Blomstedt P.* Tracheostomy in ancient Egypt. J Laryngol Otol 2014; 128(8): 665–8.
3. *Walsh J, Rastatter J.* Neonatal Tracheostomy. Clin Perinatol 2018; 45(4): 805–16.
4. *Gergin O, Adil E, Kawai K, Watters K, Mortiz E, Rahbar R.* Indications of pediatric tracheostomy over last 30 years: Has anything changed? Int J Pediatr Otorhinolaryngol 2016; 87: 144–7.
5. *Ogilvie LN, Kozak JK, Chiu S, Adderley RJ, Kozak FK.* Changes in pediatric tracheostomy 1982-2011: a Canadian tertiary children's hospital review. J Pediatr Surg 2014; 49(11): 1549–53.
6. *Chang J, Sidell DR.* Tracheostomy in Infants in the Neonatal Intensive Care Unit. Neoreviews 2020; 21(5): e323–34.
7. *Mahida JB, Asti L, Boss EF, Shah RK, Deans KJ, Minneci PC,* et al. Tracheostomy placement in children younger than 2 years: 30-day outcomes using the National Surgical Quality Improvement Program Pediatric. JAMA Otolaryngol Head Neck Surg 2016; 142(3): 241–6.
8. *Mahadevan M, Barber C, Salkeld L, Douglas G, Mills N.* Pediatric tracheotomy: 17-year review. Int J Pediatr Otorhinolaryngol 2007; 71(12): 1829–35.
9. *Atmaca S, Bayraktar C, Aşilioğlu N, Kalkan G, Ozsoy Z.* Pediatric tracheotomy: 3-year experience at a tertiary care center with 54 children. Turk J Pediatr 2011; 53(5): 537–40.
10. *D'Souza JN, Levi JR, Park D, Shah UK.* Complications Following Pediatric Tracheotomy. JAMA Otolaryngol Head Neck Surg 2016; 142(5): 484–8.
11. *Gumussoy M.* Pediatric Tracheotomy: Comparison of surgical technique with early and late complications in 273 cases. Pak J Med Sci 2019; 35(1): 247–51.
12. *Dursun O, Ozel D.* Early and long-term outcome after tracheostomy in children. Pediatr Int 2011; 53(2): 202–6.
13. *Zenk J, Fyrmpas G, Zimmermann T, Koch M, Constantinidis J, Iro H.* Tracheostomy in young patients: indications and long-term outcome. Eur Arch Otorhinolaryngol 2009; 266(5): 705–11.
14. *Jain KM, Patnaik S, Sahoo B, Mishra R, Behera JR.* Tracheostomy in Pediatric Intensive Care Unit: Experience from Eastern India. Indian J Pediatr 2021; 88(5): 445–9.
15. *Zebda D, Anderson B, Huang Z, Yuksel S, Roy S, Jiang ZY.* Early Tracheostomy Change in Neonates: Feasibility and Benefits. Otolaryngol Head Neck Surg 2021; 165(5): 716–21.
16. *Muller RG, Mamidala MP, Smith SH, Smith A, Sheyn A.* Incidence, epidemiology, and outcomes of pediatric tracheostomy in the United States from 2000 to 2012. Otolaryngol Head Neck Surg 2019; 160(2): 332–8.
17. *Teplitzky TB, Brown AF, Brooks RL, Bailey CH, Whitney C, Sewell A,* et al. Mortality among children with a tracheostomy. Laryngoscope 2023; 133(2): 403–9.
18. *Maunsell R, Avelino M, Caixeta Alves J, Semenzati G, Lubianca Neto JF, Krumenauer R,* et al. Revealing the needs of children with tracheostomies. Eur Ann Otorhinolaryngol Head Neck Dis 2018; 135(5S): S93–7.
19. *Fuller C, Wineland AM, Richter GT.* Update on pediatric tracheostomy: indications, technique, education, and decannulation. Curr Otorhinolaryngol Rep 2021; 9(2): 188–99.

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The pros and cons of suturing the ventral hernia defect using the intraperitoneal onlay mesh technique

Prednosti i mane suture defekta prilikom korišćenja intraperitonealne *onlay mesh* tehnike kod ventralnih kila

Bojan Jovanović, Dejan Rančić, Ljubiša Rančić, Miljan Krstović

University Clinical Center Niš, Center for Minimally Invasive Surgery, Niš, Srbija

Abstract

Background/Aim. Laparoscopic ventral hernia surgery, including intraperitoneal onlay mesh (IPOM), is as effective and safe as open surgery, with a lower recurrence rate. Some surgeons advocate laparoscopic primary fascial closure (PFC) with intraperitoneal mesh placement to reduce recurrence rates. The aim of this study was to compare the treatment outcome between two laparoscopic techniques: the PFC technique and mesh placement without suture closure (IPOM technique) for defects under 4 cm in patients with ventral hernias. **Methods.** The study sample was comprised of 50 patients who underwent laparoscopic ventral hernia surgery from January 1, 2018, until December 31, 2020. Half of the patients underwent only the IPOM technique (group of patients without the suture), while in others, this was preceded by the closure of the hernial ring (group of patients with the suture). All hernias were midline and all defects were under 4 cm. The studied groups were homogeneous according to gender and age. Comorbidities, complications, and postoperative comfort were monitored. **Results.** The most common (76%) hernias were primary, and the most common comorbidity was arterial hypertension (28%). One (2%) patient had intraoperative bleeding, and the most common postoperative complication was pain in 7 (14%) patients. After a three-year follow-up, there were 10 (20%) patients with complications – one hernia recurred, while 9 (18%) patients died. There was no difference in the types of occurrence of hernias, comorbidities, and intraoperative complications. The distribution of postoperative complications differed significantly ($p = 0.007$) between the groups. Pain was statistically significantly more prevalent in patients with sutures. During the first three months postoperatively, significantly more patients with sutures had chronic pain ($\chi^2 = 8.140$; $p = 0.004$). **Conclusion.** We recommend the application of the PFC technique in selected ventral hernia repair cases, although it can lead to more frequent postoperative pain (which, fortunately, is easily treated).

Key words:
fascia; hernia, ventral; laparoscopy; postoperative period; quality of life; surgical mesh; surgical procedures, operative; suture techniques.

Apstrakt

Uvod/Cilj. Laparoskopska operacija ventralne kile, uključujući intraperitonealnu *onlay mesh* (IPOM) tehniku, efikasna je i sigurna kao i otvorena operacija, ali sa nižom stopom recidiva. Neki hirurzi zagovaraju primenu primarnog zatvaranja fascije (*primary fascial closure* – PFC) sa postavljanjem intraperitonealne mreže, kako bi se smanjila stopa recidiva. Cilj rada bio je da se uporedi ishod lečenja kod bolesnika sa ventralnom hernijom za defekte ispod 4 cm, između dve laparoskopske tehnike: PFC tehnike i postavljanjem mrežice bez zatvaranja šava (IPOM tehnika). **Metode.** Istraživanjem je obuhvaćeno 50 bolesnika laparoskopski operisanih zbog ventralne kile u intervalu od 01. januara 2018. do 31. decembra 2020. godine. Polovina bolesnika bila je podvrgnuta samo postavljanju mrežice IPOM tehnikom (grupa bolesnika bez suture), dok je kod preostalih tome prethodilo zatvaranje kilnog prstena (grupa bolesnika sa suturom). Sve kile bile su medijalne a svi defekti ispod 4 cm. Ispitivane grupe bile su homogene prema polnoj i starosnoj strukturi. Praćene su komplikacije, komorbiditeti i postoperativni oporavak bolesnika. **Rezultati.** Najveći broj kila bile su primarne (76%), a najčešći komorbiditet bila je arterijska hipertenzija (28%). Jedan (2%) bolesnik imao je intraoperativno krvarenje a najčešća postoperativna komplikacija bio je bol kod 7 (14%) bolesnika. Posle trogodišnjeg praćenja, 10 (20%) bolesnika imalo je komplikacije – jednom bolesniku se vratila hernija, dok je 9 (18%) bolesnika preminulo. Nije bilo razlike u vrstama pojave kila, komorbiditetima i intraoperativnim komplikacijama.

Correspondence to: Bojan Jovanović, University Clinical Center Niš, Center for Minimally Invasive Surgery, Blrd "Dr. Zorana Đinđića" 48, 18 000 Niš, Serbia. E-mail: bokistet@gmail.com

Distribucija postoperativnih komplikacija značajno se razlikovala među grupama (p = 0,007). Bol je bio statistički značajno zastupljeniji kod bolesnika sa suturom. Tokom prva tri meseca posle operacije, značajno više bolesnika sa suturom imalo je hronični bol (χ^2 = 8,140; p = 0,004). **Zaključak.** Preporučujemo primenu PFC tehnike u odabranim slučajevima operacija ventralnih hernija, mada može dovesti do povećanja postoperativnog bola (koji se, srećom, lako tretira).

Ključne reči:
fascija; hernija; laparoskopija; postoperativni period; kvalitet života; hirurška mrežica; hirurgija, operativne procedure; šavovi, tehnike.

Introduction

Ventral incisional hernias can be operated on either through an open or laparoscopic approach. The incidence of incisional hernias is up to 30%, and they are one of the more frequent long-term complications after laparotomy [1].

The main issues after hernia surgery are recurrence and pain, and the technique used in the hernia repair procedure affects both the rate of recurrence as well as postoperative pain.

Laparoscopic ventral hernia surgery has progressed in terms of performance and safety of the procedure. It has been shown to be as effective and safe as open surgery, with a lower recurrence rate. Laparoscopy, especially the intraperitoneal onlay mesh (IPOM) technique, is a popular method used for ventral hernia surgery [2]. While laparoscopy has reduced the incidence of surgical site infection (SSI) and recurrence rates, some surgeons advocate laparoscopic primary fascial closure (PFC) with intraperitoneal mesh placement to reduce recurrence rates. Certain studies indicate that in patients undergoing laparoscopic ventral hernia repair, PFC, compared to mesh placement without defect closure, reduces the rate of hernia recurrence but increases postoperative pain [3, 4]. In general, fascial defects larger than 4 cm always require suturing. However, the choice of whether to suture the defect is up to the surgeon when the defects are under 4 cm in diameter. As the dilemma about whether to perform PFC in these cases persists in the surgical community, the aim of this article was to compare the pros and cons of suturing the abdominal wall fascial defect between the patients who underwent PFC and those who did not, ultimately aiming to reduce the recurrence rates, SSIs, and postoperative pain, while providing adequate cosmetic results for our patients.

Methods

The research was comprised of 50 patients who underwent ventral hernia repair with a small defect (< 4 cm) done laparoscopically from January 1, 2018, until December 31, 2020, in the Center for Minimally Invasive Surgery, University Clinical Center Niš, Serbia. The study was approved by the Ethics Committee of the University Clinical Center Niš (Decision No. 19486/5, from July 7, 2023).

The study included 25 (50%) male and 25 (50%) female patients, with a mean age of 54.84 ± 6.86 years. The patients included in the study had a midline ventral hernia (either umbilical or epigastric), classified as a primary ventral hernia, postoperative incisional hernia, or a recurrent one. The patients were divided into two groups according to the type of surgery: in 25 (50.0%) patients, the hernial opening was closed with a suture, followed by placement of prosthetic material (i.e., the IPOM-plus technique), and in the other 25 (50.0%), the hernial opening was not closed, and only a mesh was placed using the standard IPOM technique. In all patients, after carbon dioxide insufflation at 12 mmHg utilizing a Veress needle at Palmer's point, a 10 mm camera port was placed in the anterior axillary line on the right side in the projection of the umbilicus. Then, a 12 mm port was placed under direct vision in the medioclavicular line below the right costal margin. Finally, a 5 mm port is placed in the anterior axillary in the projection of the anterior superior iliac spine.

When there were adhesions to the anterior abdominal wall, they were divided with the UltraScision™ device. A polypropylene composite mesh was used in all patients, with absorbable tacks along with non-absorbable sutures used for mesh fixation. When done, intracorporeal laparoscopic interrupted sutures were used for PFC (in 50% of patients). During the period when the studied operations were performed, the hernia sacs were not removed in any patients. While performing the IPOM-plus technique, when closing the defect, the hernia sac was sutured along with the edges of the fascial defect in order to reduce the dead space. As for the IPOM technique, the dead space was reduced by postoperative compression on the skin using a packet of gauze. Since all hernias had a minor fascial defect, no surgical cosmetic treatment of excess skin was required.

The patient's comorbidities were monitored – the presence of diabetes, chronic obstructive pulmonary disease, renal insufficiency, hypertension, and smoking. Complications monitored were: intraoperative bleeding, postoperative seroma, hematoma, and pain. Patients were also monitored for the presence of mesh bulging; however, there were no cases of bulging during the study period, which is why it was excluded from the monitored complications. The perioperative data was collected from the patient's electronic medical records, while the postoperative complications and quality of life (QoL) data were obtained at the follow-up examination after three and six months, and then after one, two, and three years, when the patients answered questions about comfort and postoperative pain. The questions stem from a modified iteration of the EuraHS QoL questionnaire, designed for

evaluating patients' QoL. In this modified rendition, alterations were made to the scale of the answers, with the traditional 0 to 10 scale being replaced by a more concise scale, spanning from 0 to 5, prompting patients to provide their responses within this refined framework [5]. The groups were compared according to the type of operation and QoL [6].

Statistical calculations were performed using SPSS version 22. Of the basic descriptive statistical parameters, standard statistical methods were used for qualitative and quantitative assessment of the obtained results. The normality of the distribution was tested with the Kolmogorov-Smirnov test. Sample comparison was performed with the Student's *t*-test and the Mann-Whitney *U* test for cases of irregular data distribution. The Chi-square and Fisher test were used to test the statistical significance of absolute frequency differences between samples. A difference between samples was considered significant if $p < 0.05$.

Results

The basic characteristics of two compared groups of patients with ventral hernias are shown in Table 1. Only the distribution of postoperative complications differed significantly among the studied groups ($\chi^2 = 12.105$; $p = 0.007$). A total of 10 (20%) patients had complications. Seromas and hematomas were more common in patients who did not have sutures of the hernial defect but without statistical significance, while pain was statistically significantly more prevalent in patients with the sutures.

A comparison between the groups regarding the QoL is shown in Table 2. When asked about the pain at the hernia site at rest, it was determined that there was a significant difference in the answers in the examined groups ($\chi^2 = 7.053$; $p = 0.029$). Concerning the answers regarding pain at the site of the hernia during activity, a significant difference was found in the responses by the examined groups ($\chi^2 = 15.797$; $p = 0.003$). Patients in whom a suture was performed gave answers 3, 4, and 5 in a significantly higher number compared to patients without a suture. There was no significant difference in the answers to the questions about activity restriction and aesthetic discomfort (Table 2).

Table 3 shows the results of patient follow-up in the first three years. It was found that there was a significant difference between the studied groups during the first three months ($\chi^2 = 8.140$; $p = 0.004$), where patients with sutures had chronic pain in a significantly greater number. There were a total of 7 patients who died after three years (non-significant value) under the diagnosis of COVID-19 infection. In all subsequent periods, there was no significant difference between the groups.

Table 1

Comparison of patients with and without defect suturing

Parameter	With a suture (n = 25)	Without a suture (n = 25)	*p*-value
Gender			
female	14 (56.0)	11 (44.0)	
male	11 (44.0)	14 (56.0)	0.396[1]
Age, years	55.44 ± 7.69	54.24 ± 6.01	0.542[2]
Type of occurrence			
incisional	4 (16.0)	3 (12.0)	
primary	18 (72.0)	20 (80.0)	
recurrence	3 (12.0)	2 (8.0)	0.799[1]
Comorbidities			
without	5 (20.0)	6 (24.0)	
diabetes mellitus	5 (20.0)	4 (16.0)	
COPD	1 (4.0)	4 (16.0)	
renal disease	3 (12.0)	2 (8.0)	
hypertension	7 (28.0)	7(28.0)	
smoker	4 (16.0)	2 (8.0)	0.720[1]
Intraoperative complications			
no	25 (100.0)	24 (96.0)	
bleeding	0 (0.0)	1 (4.0)	1.000[3]
Postoperative complications			
no	18 (72.0)	20 (80.0)	
seroma vulneris	0 (0.0)	3 (12.0)	
hematoma	0 (0.0)	2 (8.0)	
pain	7 (28.0)	0 (0.0)	0.007[1]

COPD – chronic obstructive pulmonary disease. [1]Chi-square test. [2]Student's *t*-test. [3] Fisher test. All values are given as numbers (percentages) or mean ± standard deviation.

Table 2

Comparison of quality of life of patients with and without defect suturing

Questions	Response	With a suture (n = 25)	Without a suture (n = 25)	p^1-value
1. Do you feel pain at the site of hernia in rest?	1	10 (40.0)	15 (60.0)	
	2	9 (36.0)	10 (40.0)	
	3	6 (6.0)	0 (0.0)	0.029
2. Do you feel pain at the site of the hernia during activities?	1	8 (32.0)	15 (60.0)	
	2	5 (20.0)	10 (40.0)	
	3	2 (8.0)	0 (0.0)	
	4	5 (20.0)	0 (0.0)	
	5 (worst pain imaginable)	5 (20.0)	0 (0.0)	0.003
3. Do you experience restrictions of activities/daily activities?	2	5 (20.0)	8 (32.0)	
	3	0 (0.0)	3 (12.0)	
	4	6 (24.0)	6 (24.0)	
	5 (completely)	14 (56.0)	8 (32.0)	0.149
4. Do you experience esthetical discomfort concerning the shape of your abdomen?	1	17 (68.0)	17 (68.0)	
	2	8 (32.0)	8 (32.0)	1.000

[1]Chi-square test. All values are given as numbers (percentages).

Table 3

Follow-up of patients with and without defect suturing

Time	Complication	With a suture (n = 25)	Without a suture (n = 25)	p^1-value
Follow-up				
3 months	chronic pain	7 (28.0)	0 (0.0)	0.004
6 months	chronic pain	2 (8.0)	0 (0.0)	0.149
1 year	death	1 (4.0)	0 (0.0)	0.312
2 years	death	2 (8.0)	3 (12.0)	0.637
3 years	reccurence	3 (12.0)	6 (24.0)	
	death	0 (0.0)	1 (4.0)	0.301

[1]Chi-square test. All values are given as numbers (percentages).

Discussion

The laparoscopic intraperitoneal approach with IPOM was first described in 1993. At that time, little was known about the foreign body reaction of the IPOM-mesh, which covered the defect of the parietal peritoneum. This is becoming increasingly important, as the IPOM procedure with resection of the hernial sac and closure of the hernial opening (IPOM-plus) is increasingly being utilized [1].

Despite excellent results of laparoscopic repair of ventral hernias, numerous controversies are associated with the procedure – how to create the pneumoperitoneum, how to perform adhesiolysis, how to adequately deal with the hernial sac, the evolution, and complications related to a postoperative seroma, the type and size of the mesh, as well as the method of insertion and fixation of the mesh and postoperative pain [2, 3].

There are arguments for and against the utilization of the IPOM technique as a method of solving defects in ventral hernias. The surgeon makes only a few smaller incisions for placing laparoscopic ports (ranging from 5 mm to 10–12 mm in size), which reduces the risk of infection, bleeding, and other complications associated with open techniques. Due to the minimally invasive nature of the IPOM technique, patients often have a faster recovery. Smaller incisions mean less pain, less blood loss, and a shorter hospital stay. Furthermore, the IPOM technique involves strengthening the abdominal wall using special prosthetic implants, which provide firm support and reduce the risk of hernia recurrence after surgery [7].

Arguments against the IPOM technique are that it requires advanced surgical skills and specific equipment. The IPOM technique can be more expensive when compared to other conventional methods of hernia repair. This can be problematic for patients who do not have insurance or have limited financial means. Since the IPOM technique is relatively new, there is a lack of meta-analyses that would provide solid evidence of its long-term efficacy and safety. More research is needed to get more substantial verification of the long-term results of this technique [8].

The IPOM with the suturing of the hernial defect involves a suture or sutures on the hernial opening to close it

before placing the mesh. The surgeon joins the edges of the defect using sutures or other closure techniques. It was established that chronic pain might be related to non-absorbable suture material. Additionally, sutures used for closing the fascial defect pass through the excellently innervated peritoneum and also carry the risk of injuring vessels and nerves in the anterior abdominal wall, both of which may lead to increased postoperative pain [9, 10]. The pain was relieved with non-steroidal anti-inflammatory drugs or, in more severe cases, with local anesthetic injections. After the defect is closed, the mesh is placed over the defect with an additional 1.5 cm on each side of the defect and fixed in place with sutures, tackers, or other fixation methods. This technique aims to strengthen the abdominal wall and provide additional support to prevent the hernia from recurring.

IPOM without suturing the hernial defect is an approach in which the hernial opening is not sutured or closed before placing the mesh. Instead, the mesh is placed directly over the defect without any closure. This technique relies on the mesh itself to provide support and prevent hernia recurrence without relying on the closure of the defect [11, 12]. Furthermore, as there is dead space left, this can lead to seroma formation postoperatively. In our series, there were both more seromas and more recurrences in patients who underwent IPOM without PFC, although without statistical significance, which may be caused by the size of the samples. Other authors' findings demonstrate higher rates of seroma formation and recurrence following IPOM without PFC, although evidence is still inconsistent [13, 14]. Mesh bulging is a relatively common complication following IPOM ventral hernia repair. The fact there was no bulging in our series might be the consequence of a small number of patients included. There is conflicting data regarding differences between IPOM and IPOM-plus concerning mesh bulging – some authors' results suggest more bulging occurs following IPOM, while others indicate similar rates for both techniques, which necessitates further research [13, 15].

The choice between IPOM with suturing the defect or without depends on various factors such as the size and location of the hernia, the surgeon's expertise, the patient's condition, and other individual considerations [12].

Postoperative complications that occur after large opening hernia surgeries are most commonly seroma formation [16]. Late complications include chronic pain and mesh bulging. Seroma formation often compromises the patient's aesthetic appearance and causes discomfort, pain, and/or infection. The true incidence of seroma formation after IPOM is unknown because its presence is variable and depends on many factors [9]. In our series, seroma occurred in 12% of patients without a hernia defect suture, although this is a small series of patients, and the hernias were M1 (≤ 4 cm). In the comparison between the two study groups, patients with an unstitched hernia had statistical significance in the occurrence of seroma compared to patients in whom the hernia was closed with a suture [6]. The diagnosis of seroma in the literature is based on different diagnostic criteria of different authors. In a comparative study by Suwa et al. [9], the ratio of the occurrence of seroma in IPOM with and without sutures is 14% vs. 25%.

Following the recurrence results after IPOM, in our series, one (2%) recurrence occurred after three years. There was no statistical significance of recurrence in the laparoscopic IPOM group in relation to the open IPOM group [17]. In other studies, the percentage of recurrence was 16% in large M3 hernias; the most common cause was insufficient overlapping of the mesh over the hernial opening [10]. In our series, 18% died after three years with the diagnosis of COVID-19 infection.

Another issue with laparoscopic IPOM is chronic pain. It is defined as pain lasting more than three months. This complication occurs in 2–9.5% of cases of laparoscopic IPOM surgery [18]. The pain is related to the method of fixation of the mesh, especially with non-absorbable material, and in our series, it was observed in 14% of cases.

Conclusion

In summary, despite the ongoing conflicting evidence and the need for further investigation, the authors advocate for the use of PFC in selected cases of ventral hernia repair. This approach is suggested due to its potential to mitigate occurrences of seromas and reduce recurrence rates, albeit at the expense of heightened postoperative pain. Nonetheless, given the manageable nature of postoperative pain, we assert that the benefits outweigh the associated trade-offs.

REFERENCES

1. *Eickhoff RM, Kroh A, Eickhoff S, Heise D, Helmedag MJ, Tolba RH,* et al. A peritoneal defect covered by intraperitoneal mesh prosthesis effects an increased and distinctive foreign body reaction in a minipig model. J Biomater Appl 2021; 35(6): 732−9.
2. *LeBlanc K.* Proper mesh overlap is a key determinant in hernia recurrence following laparoscopic ventral and incisional hernia repair. Hernia 2016; 20(1): 85−99.
3. *Morales-Conde S.* Laparoscopic ventral hernia repair: advances and limitations. Semin Laparosc Surg 2004; 11(3): 191−200.
4. *Wennergren JE, Askenasy EP, Greenberg JA, Holihan J, Keith J, Liang MK,* et al. Laparoscopic ventral hernia repair with primary fascial closure versus bridged repair: a risk-adjusted comparative study. Surg Endosc 2016; 30(8): 3231−8.
5. *Muysoms F, Campanelli G, Champault GG, DeBeaux AC, Dietz UA, Jeekel J,* et al. EuraHS: the development of an international online platform for registration and outcome measurement of ventral abdominal wall hernia repair. Hernia 2012; 16(3): 239–50.
6. *Silecchia G, Campanile FC, Sanchez L, Ceccarelli G, Antinori A, Ansaloni L,* et al. Laparoscopic ventral/incisional hernia repair: updated Consensus Development Conference based guidelines [corrected]. Surg Endosc 2015; 29(9): 2463–84. Erratum in: Surg Endosc 2015; 29(9): 2485.

7. *Mikamori M, Nakahara Y, Iwamoto K, Hyuga S, Naito A, Ohtsuka M,* et al. Intraperitoneal-onlay-mesh repair with hernia defect closure via the hernial orifice approach: A case series of 49 patients. Int J Surg Open 2021; 36: 100418.
8. *Köckerling F, Lammers B, Weyhe D, Reinpold W, Zarras K, Adolf D,* et al. What is the outcome of the open IPOM versus sublay technique in the treatment of larger incisional hernias?: A propensity score-matched comparison of 9091 patients from the Herniamed Registry. Hernia 2021; 25(1): 23–31.
9. *Suwa K, Okamoto T, Yanaga K.* Is fascial defect closure with intraperitoneal onlay mesh superior to standard intraperitoneal onlay mesh for laparoscopic repair of large incisional hernia? Asian J Endosc Surg 2018; 11(4): 378–84.
10. *Giuffrida M, Rossini M, Pagliai L, Del Rio P, Cozzani F.* Laparoscopic Intraperitoneal Onlay Mesh (IPOM): Short- and Long-Term Results in a Single Center. Surgeries 2023; 4(1): 98−107.
11. *Megas IF, Benzing C, Winter A, Raakow J, Chopra S, Pratschke J,* et al. A propensity-score matched analysis of ventral-TAPP vs. laparoscopic IPOM for small and mid-sized ventral hernias. Comparison of perioperative data, surgical outcome and cost-effectiveness. Hernia 2022; 26(6): 1521−30.
12. *Van Hoef S, Tollens T.* Primary non-complicated midline ventral hernia: is laparoscopic IPOM still a reasonable approach? Hernia 2019; 23(5): 915–25.
13. *Basukala S, Tamang A, Rawal SB, Malla S, Bhusal U, Dhakal S,* et al. Comparison of outcomes of laparoscopic hernioplasty with and without fascial repair (IPOM-Plus vs IPOM) for ventral hernia: A retrospective cohort study. Ann Med Surg (Lond) 2022; 80: 104297.
14. *Tandon A, Pathak S, Lyons NJR, Nunes QM, Daniels IR, Smart NJ.* Meta-analysis of closure of the fascial defect during laparoscopic incisional and ventral hernia repair. Br J Surg 2016; 103(12): 1598–607.
15. *Lambrecht JR, Vaktskjold A, Trondsen E, Øyen OM, Reiertsen O.* Laparoscopic ventral hernia repair: outcomes in primary versus incisional hernias: no effect of defect closure. Hernia 2015; 19(3): 479–86.
16. *Helgstrand F, Rosenberg J, Kehlet H, Jorgensen LN, Bisgaard T.* Nationwide prospective study of outcomes after elective incisional hernia repair. J Am Coll Surg 2013; 216(2): 217–28.
17. *Lavanchy JL, Buff SE, Kohler A, Candinas D, Beldi G.* Long-term results of laparoscopic versus open intraperitoneal onlay mesh incisional hernia repair: a propensity score-matched analysis. Surg Endosc 2019; 33(1): 225–33.
18. *Liot E, Bréguet R, Piguet V, Ris F, Volonté F, Morel P.* Evaluation of port site hernias, chronic pain and recurrence rates after laparoscopic ventral hernia repair: A monocentric long-term study. Hernia 2017; 21(6): 917–23.

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Portal vein thrombosis in patients with liver cirrhosis

Tromboza vene porte kod bolesnika sa cirozom jetre

Željka Savić*†, Dimitrije Damjanov*†, Olgica Latinović Bošnjak†, Nebojša Janjić*†, Božidar Dejanović*†, Žarko Krnetić†, Vladimir Vračarić†

***University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; †University Clinical Center of Vojvodina, Clinic for Gastroenterology and Hepatology, Novi Sad, Serbia**

Abstract

Background/Aim. Portal vein thrombosis (PVT) in patients with liver cirrhosis (LC) has a prevalence of 0.6–26%. It is most commonly discovered incidentally as part of the evaluation of LC or in the context of acute decompensation of LC due to portal hypertension. The aim of the study was to determine the prevalence of PVT in patients with LC in relation to the severity of the disease and individual elements of portal hypertension. **Methods.** A total of 326 patients treated for LC decompensation were included in a retrospective study. Standard laboratory analyses, abdominal ultrasonography and/or computed tomography, and esophagogastroduodenoscopy were performed. **Results.** The diameter of the portal vein (PV) differed between patients without esophageal varices (12.2 mm) and those with large varices (13.6 mm), $p = 0.026$. PVT was identified in 6.1% of patients with LC. The patients were classified according to the Child-Pugh scoring system, which has the A, B, and C categories used to assess the severity of liver disease. PVT was present in 3.0% of patients in class C and 12.0% in class B, while none of the patients in class A had PVT ($p = 0.005$). PVT was present in 4.4% of patients with small varices and 16.7% with large varices ($p < 0.001$). There was no difference in the presence of PVT between the groups of patients with and without variceal bleeding nor between groups with different degrees of ascites. A fatal outcome occurred in 29.4% of patients, but there was no difference between patients with and without PVT. **Conclusion.** PVT is present in more advanced stages of LC and predominantly in patients with large esophageal varices. There was no higher prevalence of PVT observed with the occurrence of variceal bleeding or with the death outcome in patients with LC.

Key words:
esophageal and gastric varices; hypertension, portal; liver cirrhosis; portal vein; thrombosis.

Apstrakt

Uvod/Cilj. Tromboza vene porte (TVP) kod bolesnika sa cirozom jetre (CJ) ima prevalenciju od 0,6–26%. Najčešće se TVP otkriva slučajno, u sklopu evaluacije CJ, ili u sklopu akutne dekompenzacije CJ zbog portne hipertenzije. Cilj rada bio je da se ustanovi zastupljenost TVP kod bolesnika sa CJ u odnosu na težinu bolesti i pojedine elemente portne hipertenzije. **Metode.** Retrospektivnim istraživanjem obuhvaćeno je 326 bolesnika lečenih zbog dekompenzacije CJ. Bolesnicima su rađene standardne laboratorijske analize, ultrasonografija abdomena i/ili kompjuterizovana tomografija abdomena i ezofagogastroduodenoskopija. **Rezultati.** Dijametar vene porte (VP) razlikovao se kod bolesnika bez variksa jednjaka (12,2 mm) i onih koji su imali velike varikse (13,6 mm), $p = 0,026$. TVP je ustanovljena kod 6,1% bolesnika sa CJ. Bolesnici su klasifikovani u skladu sa *Child-Pugh* sistemom bodovanja, kojim se težina oboljenja jetre izražava kategorijama A, B i C. TVP je bila prisutna kod 3.0% bolesnika iz kategorije C, kod 12,0% bolesnika iz kategorije B, a nije bila prisutna ni kod jednog bolesnika iz kategorije A ($p = 0,005$). TVP je bila prisutna kod 4,4% bolesnika sa malim variksima i kod 16,7% bolesnika sa velikim variksima ($p < 0,001$). Nije bilo razlike u prisustvu TVP između grupa bolesnika sa i bez variksnog krvarenja, kao ni između grupa sa različitim stepenom ascita. Kod 29,4% bolesnika nastupio je smrtni ishod, ali nije bilo razlike između bolesnika sa i bez TVP. **Zaključak.** TVP je prisutna kod težih stadijuma CJ i pretežno kod bolesnika sa velikim variksima jednjaka. Nije ustanovljena veća prevalencija TVP povezana sa pojavom variksnog krvarenja ili smrtnim ishodom bolesnika sa CJ.

Ključne reči:
jednjak, variksi; hipertenzija, portalna; jetra, ciroza; v.portae; tromboza.

Correspondence to: Dimitrije Damjanov, University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21 137 Novi Sad, Serbia.
E-mail: dimitrije.damjanov@mf.uns.ac.rs

Introduction

Liver cirrhosis (LC) is a disease characterized by two stages – the compensated and decompensated stage. Clinical characteristics of decompensation include ascites, variceal bleeding (VB), and overt hepatic encephalopathy [1]. Portal hypertension (PH) is a clinical syndrome characterized by increased pressure in the portal vein (PV). The values of the hepatic venous pressure gradient > 10 mm indicate clinically significant PH. Esophageal varices (EVs) are present in 50–60% of patients with compensated and 85% with decompensated LC [2, 3].

Portal vein thrombosis (PVT) refers to thrombus formation within the PV or its intrahepatic branches, with or without extension to the superior mesenteric and splenic veins. The condition is commonly incidentally diagnosed during the evaluation of LC or, in the case of acute LC decompensation associated with PH. It is necessary to identify the initial site, number of affected blood vessels, and obstruction rate and determine whether the disease is acute or chronic [4–6]. PVT prevalence in patients with LC ranges between 0.6% and 26%, with a yearly incidence rate of 4.6–26% [7, 8]. Chronic PVT is associated with complications of PH, such as esophagogastric varices, with an increased risk of VB, splenomegaly, and hypersplenism [7, 9, 10].

Although the first description of PVT dates back to 1868 [11], there are still questions related to predisposing factors, primarily whether PVT in patients with LC is clinically significant or merely an epiphenomenon of advanced liver disease (LD) [12].

The pathogenesis of PVT is multifactorial and pathophysiologically related to the vertices of Virchow's triad (venous stasis, hypercoagulability, and endothelial dysfunction) [13]. In LC, reduced portal flow velocity (FV) occurs as a consequence of changes in porto-collateral circulation combined with increased PV diameter, which is commonly seen in patients with clinically significant PH. A decrease in portal FV to less than 15 cm/s measured by Doppler ultrasonography (US) is a significant predictive factor for the development of PVT [6, 14]. Endothelial dysfunction is commonly present in LC patients and is associated with procedures such as sclerotherapy, portosystemic shunt surgery, and splenectomy [15]. A rebalanced coagulation concept, a delicate balance between procoagulant and anticoagulant factors, characterizes LC. The existence of relative hypercoagulability in the PV and splanchnic circulation compared to systemic circulation has been established [16–18]. Hyperfunction and increased platelet aggregation potential, especially in portal circulation, can be explained by increased stimulation by the lipopolysaccharide derived from the leaky gut [19].

PVT can be regarded as the underlying cause and consequence of decompensated LC. The prognosis and therapy depend on the localization, extension and progression rate, the presence of relevant risk factors, and the stage of chronic LD [6]. US is the method of choice in the initial evaluation of the portal venous system because it has an accuracy of 88–98% in detecting PVT (sensitivity and specificity range from 80–100% in most studies). Various techniques are employed, including 2D grey-scale US, which displays thrombosis as isoechoic or hypoechoic material filling the vessel either involving a part of the lumen (partial thrombosis) or the entire lumen (complete thrombosis). Color Doppler US, spectral Doppler (pulsed wave US), and contrast-enhanced US improve the characterization of thrombosis, enabling confirmation of the absence of flow in complete PVT. Portal hemodynamics depends on whether the thrombosis is partial or complete [20]. In the evaluation of PVT, contrast-enhanced computed tomography (CT) or magnetic resonance imaging is also applied, especially their venous phase, to determine the extent of thrombus spread in the branches of the PV and to detect potential complications such as intestinal infarction [21].

Classification of PVT should be treatment-oriented and, *inter alia*, include extension of thrombus and grade of occlusion [22].

PVT is currently treated similarly to any other thrombosis with anticoagulant therapy, but the response to this treatment is poor. Thirty to sixty percent of patients with PVT do not achieve thrombus resolution, suggesting that the composition of the thrombus in the PV likely differs from that in the systemic circulation [4, 14, 23].

The objective of this study was to establish the occurrence of PVT in patients with LC related to cirrhosis severity and individual elements of PH.

Methods

A retrospective study encompassed 326 patients treated at the Clinic for Gastroenterology and Hepatology of the University Clinical Center of Vojvodina, Serbia for decompensated LC from January 1, 2020, to December 31, 2022. The research was approved by the Ethics Committee of the Clinical Center of Vojvodina (from July 28, 2023; No. 00-136).

Although PVT is common in patients with hepatocellular carcinoma, they were not included in the study because they represent a heterogeneous population with different disease behaviors [24]. Likewise, the study did not include patients with inflammation of abdominal organs (gallbladder and biliary tree, pancreas, intestine) and those with hematological disorders.

All patients underwent clinical examination and laboratory analysis. The analysis of blood parameters was performed using the hematology analyzer Sysmex XN 1000. Biochemical analysis – urea, creatinine, electrolytes, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, bilirubin, and albumin – were performed using an automated biochemical analyzer Abbott Alinity c, and with the original reagents supplied by Abbott. Hemostatic parameters were determined by the automated coagulation analyzer Sysmex CS – 5100 and original reagents. Patients were classified according to the Child-Pugh (C-P) scoring system, a modification of the original Child-Turcotte score. This scoring system is generally used to assess the severity of LD.

Based on the degree of expression of ascites and encephalopathy, as well as on the basis of serum values of bilirubin, albumin, and prothrombin time (1–3 points are assigned for each parameter), the C-P score of the patients was calculated. The patients were classified into classes A, B, or C. Patients in classes B and C, with higher C-P scores, have advanced LD with an increased risk of one-year mortality [25, 26]. The Model for End-Stage LD (MELD) score (range from 6–40), which has the ability to stratify patients with end-stage LD according to their three-month mortality, was calculated as well. The formula for calculating the MELD score includes serum values of bilirubin, creatinine, and international normalized ratio (INR):

MELD = 3.8 × $\log_e$ serum bilirubin (mg/dL) + 11.2 × $\log_e$INR + 9.6 × $\log_e$ serum creatinine (mg/dL) + 6.4 [27–29].

Samsung RS 85 US system was used for the evaluation of liver parenchyma homogeneity, liver margins, dimensions of the hepatic lobes (right, left, and caudate), bipolar spleen diameter, and the AP diameter of the main trunk of the PV. Doppler US was applied to assess PV FV (main PV and the left and right portal branches). The diameter of PV of 7–13 mm and PV FV of 20–40 cm/s were considered normal values. Multilayer CT on the GE Revolution Evo CT scanner enabled insight into the thrombus extension into the lienal and superior mesenteric vein. Esophagogastroduodenoscopy was performed by applying Fujinon EPH 4400. The presence and size of EVs were classified into three grades – no EVs, small EVs (less than 5 mm), and large EVs (over 5 mm).

The data obtained were analyzed using descriptive statistics, absolute numbers and percentages, and central tendency and dispersion measures. The χ^2 test was used to analyze the differences between category parameters. The differences between numerical values for parametric and non-parametric data were analyzed using one-way Analysis of Variance and the Kruskal-Wallis test, respectively, following up with a *post hoc* evaluation of the obtained results. In all applied tests, statistical hypotheses were tested at the level of statistical significance 95% ($p < 0.05$). All data were entered into the specifically created database of the Microsoft Excel package. Statistical analysis was performed using the SPSS v23 software program.

Results

Patient characteristics

The majority of patients (80%) had alcoholic LC, while one-fifth of patients had LC of other etiologies. The LC of other etiologies included the following: autoimmune, cholestatic, metabolic, cardiogenic, and cryptogenic. The average C-P score was 9.9, and the MELD score was 19.3. The average age of the patients was 59.0 years. The majority of the patients were male. The characteristics of patients are presented in Table 1.

Portal hypertension

Esophagogastroduodenoscopy was performed in 276/326 (84.7%) patients. EVs were not detected in 48/276 (17.4%) patients, whereas small and large EVs were identified in 163/276 (59.1%) and 65/276 (23.6%) patients, respectively.

Of 228 patients with EVs, VB was endoscopically confirmed in 59 (25.9%) patients. In the group with established varicosity, there were no differences in bleeding incidence associated with the LC etiology [27% vs. 20.9%, $\chi^2 = 0.676$, degree of freedom (df) = 1, $p = 0.411$].

The average PV diameter was 12.8 ± 2.5 mm (range 9–25 mm). Portal venous FV was measured in 24/296 (8.1%) patients, and it was 14.7 ± 6.3 cm/s (range 2–28 cm/s). PV

Table 1

Demographic and laboratory characteristics of patients

Characteristics	Values
Males	233 (71.5)
Age, years	59.0 ± 10.8
Liver cirrhosis etiology	
alcohol	260 (79.8)
autoimmune	14 (4.3)
cholestatic	19 (5.8)
metabolic	4 (1.2)
cardiogenic	7 (2.1)
cryptogenic	22 (6.7)
Child-Pugh score	9.9 ± 2.2
MELD score	19.3 ± 7.7
Albumin (g/L) (RR 34–52)	27.3 ± 5.3
Prothrombin time (INR) (RR 0.83–1.30)	1.5 ± 0.5
Bilirubin total (μmol/L) (RR 3.0–21.0)	54.2 (92.2) †
Creatinine (μmol/L) (RR 49–115)	90.0 (67.0) †
Sodium (mmol/L) (RR 135–150)	136.5 ± 5.8
Platelets (×10^9/L) (RR 140–400)	127.5 (104.8) †

MELD – Model for End-Stage Liver Disease; RR – reference range; INR – international normalized ratio. Categorical variables are presented as numbers (percentage) and continuous variables as mean ± standard deviation or median with interquartile range where indicated with †.

diameter in patients without EVs was 12.2 ± 1.9 mm, with small EVs, the diameter was 12.9 ± 2.4 mm, and in those with large EVs, it was 13.6 ± 3.5 mm, $p = 0.035$. *Post hoc* analysis revealed significant differences in the PV diameter between patients without EVs and those with large EVs ($p = 0.026$). The results are presented in Figure 1.

We established the differences in the spleen diameter between patients with large EVs (15.0 ± 3.3 cm) and those without EVs (13.2 ± 3.1 cm) or with small EVs (13.6 ± 2.3 cm); $p = 0.003$ and $p = 0.004$, respectively. The results are displayed in Figure 2.

The average platelet count in patients without EVs was 168.5 ± 107.4 $\times 10^9$/L, compared to 144.9 ± 83.5 $\times 10^9$/L in patients with small EVs and 136.0 ± 83.7 $\times 10^9$/L in those with large EVs. The difference was not statistically significant ($p = 0.216$).

Of the 296/326 patients who underwent abdominal inspection by visualization methods as a part of the standard evaluation of LC, 198 (66.9%) manifested with large ascites volume, and 40 (13.5%) had moderate-volume ascites. In contrast, ascites was not detected in 58 (19.6%) patients.

Portal vein thrombosis

The standard evaluation included abdominal US, CT, and, in some cases, magnetic resonance imaging examination in 90.8% of patients. Abdominal examination revealed PVT in 18/298 (6.1%) patients, by using the abdominal Doppler US in 7/18 (38.9%) by using Doppler US and in 11/18 (61.1%) patients by using CT. Occlusive PVT was diagnosed in 6/18 (33.3%) and non-occlusive in 12/18 (66.7%) patients. Of the 18 patients with PVT, single-branch and main-trunk thrombosis were established in three and four patients, respectively. Three patients had a main trunk and single-branch thrombosis, while eight manifested with the main trunk TVP and both right and left hepatic branches (Figure 3). Thrombus extension into the superior mesenteric and splenic vein was observed in 1/18 (5.6%) patient.

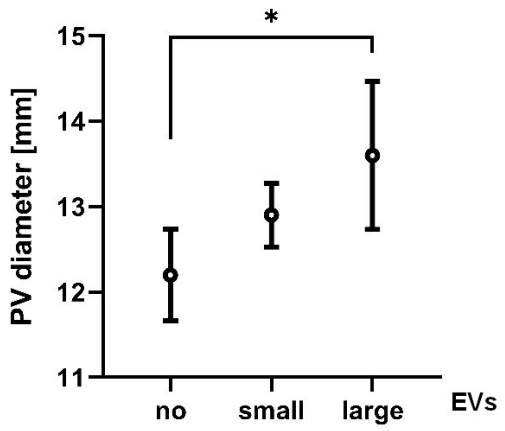

Fig. 1 – The average portal vein (PV) diameter related to the presence and grade of esophageal varices (EVs). Results are presented as the mean value with 95% confidence interval. *$p < 0.05$.

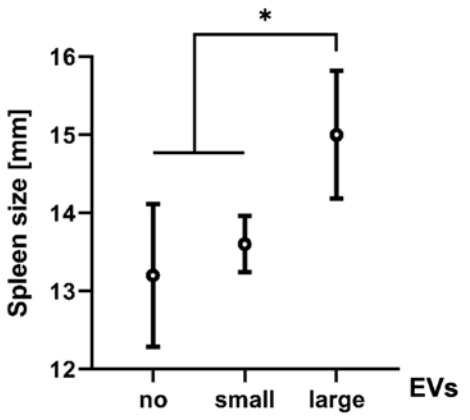

Fig. 2 – The average spleen size related to the presence and grade of esophageal varices (EVs). Results are presented as the mean value with 95% confidence interval. *$p < 0.05$.

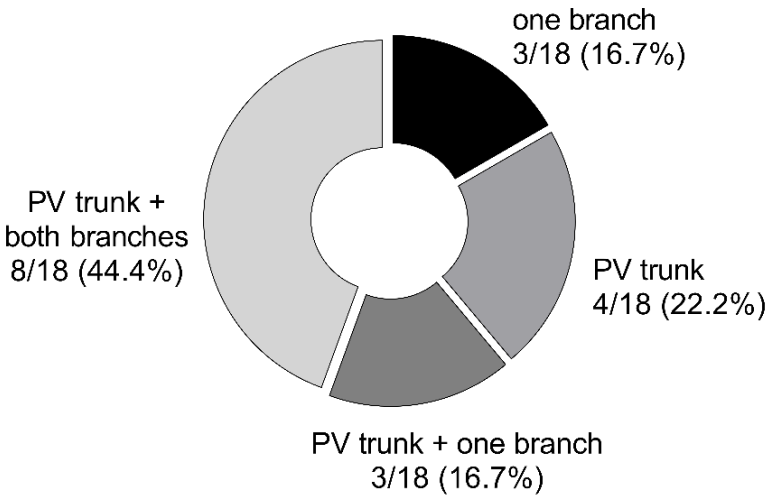

Fig. 3 – The distribution of thrombus extension into the portal vein (PV) and its hepatic branches.

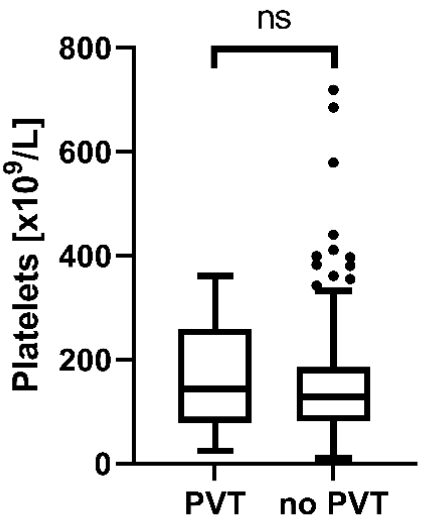

Fig. 4 – Median platelet values related to the presence of portal vein thrombosis (PVT).
Results are presented as the mean value with 95% confidence interval; ns – non-significant.

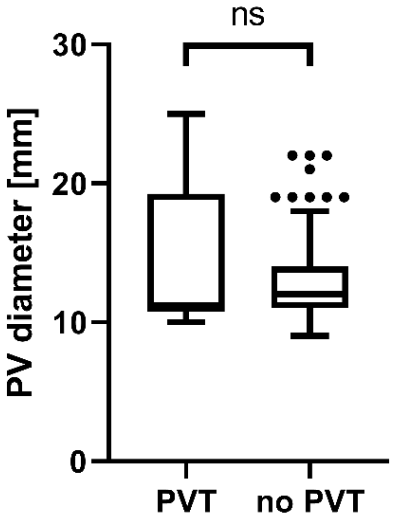

Fig. 5 – The average portal vein (PV) diameter related to the presence and grade of PV thrombosis (PVT).
Results are presented as the mean value with 95% confidence interval; ns – non-significant.

PVT was diagnosed in 12/236 (5.1%) patients with alcoholic LC and 6/60 (10.0%) of those with other LC types. The difference was not statistically significant (χ^2 = 2.02, df = 1, p = 0.155).

In 296 patients who underwent abdominal inspection by visualization methods, thrombosis was established in 5/168 (3.0%) patients with C-P class C and 13/108 (12.0%) patients with C-P class B, whereas none (0/20) of the C-P class A patients had thrombosis (χ^2 = 10.7, df = 2, p = 0.005).

Mann-Whitney *U* test revealed statistically significantly lower PT INR (1.3 vs. 1.56, p = 0.007) and total bilirubin (50.5 μmol/L vs. 104.1 μmol/L, p = 0.009) values in the group with PVT vs. group without PVT, respectively, whereas albumin values did not differ (p = 0.619).

Median platelet values did not differ between the PVT and no PVT group (143.5 ×10^9/L vs. 128.0 ×10^9/L, respectively; p = 0.45) (Figure 4).

PV diameter did not differ significantly between PVT and no PVT group (median 11 mm vs. 12 mm, respectively; p = 0.77) (Figure 5).

We established the differences in PVT prevalence among our patient population depending on the EV grade (χ^2 = 14.0, df = 2, p < 0.001). PVT was established in 2/45 (4.4%) patients without EVs, 4/150 (2.7%) patients with small EVs, and 9/54 (16.7%) patients with large EVs.

There were no differences in PVT prevalence between the groups with or without VB (χ^2 = 0.151, df = 1, p = 0.698) or groups with different ascites grades (χ^2 = 3.19, df = 2, p = 0.203).

Lethal outcomes during the hospitalization period occurred in 96/326 (29.4%) patients, and there were no differences in the mortality rate of patients with or without PVT (16.7% vs. 29.2%, χ^2 = 1.31, df = 1, p = 0.252).

Discussion

Patient characteristics and portal vein thrombosis

This investigation included a retrospective analysis of 326 patients treated for LC to establish the incidence of PVT and its potential association with LD characteristics and individual elements of PH. The majority of patients were males and had LC of alcoholic etiology. PVT was confirmed in 6.1% of patients. PVT was present in 3.0% of patients with C-P class C and 12.0% with C-P class B. However, none of the patients with C-P class A had PVT. There were no differences between PVT prevalence in patients with alcoholic LC and those with LC of other etiologies. The investigation did not include the analysis of beta-blocker administration or previous endoscopic treatment of EVs. Yerdel et al. [30] established that male gender, treatment for PH, C-P class C, and alcoholic LD were associated with PVT. According to the PVT etiology, some studies reported alcoholic and post-viral cirrhosis as the most common causes of PVT; however, other investigations did not establish any association between LC etiology and PVT [31].

According to the data from the literature, the PVT prevalence in LC and PH ranges from 0.6% to 15.8% and increases with cirrhosis grade. It is most commonly classified as C-P class B or C. In compensated LC, the incidence rate is below 1%, corresponding with our investigation [30, 32]. The results of an Italian multicenter prospective study involving 753 patients with chronic LD found a prevalence of PVT in 17% of patients. Cirrhotic patients with PVT were older, but no difference in the etiology of LC was observed. Cirrhotic patients with PVT exhibited a more advanced and decompensated disease, and the presence of ascites and encephalopathy was more frequently observed [33]. Dong et al. [34] did not confirm the

importance of the C-P score for developing PVT. That can be due to the fact that most of the participants were C-P class A, with average C-P scores of 6.6 and 5.8 in the PVT and non-PVT groups, respectively. Conversely, most patients in our study were C-P class B or C.

In our study, the survival rate was 70.6%. The mortality rate was not associated with the presence or absence of PVT. Likewise, Dong et al. [34] did not establish any influence of PVT on the survival rate in patients with LC. Luca et al. [35] reported spontaneous improvement of partial PVT in 45% of patients with LC, as well as the lack of association between partial PVT progression and clinical outcome of the disease, consistent with the severity of the LC. A prospective study by Nery et al. [36] established an association between PVT development and severity of LD at baseline; however, it does not follow a recent progression of LD. There is no evidence that the development of PVT is responsible for the further progression of LD. Borjas-Almaguer et al. [37] reported that the presence of PVT itself does not lead to a worse prognosis of LC. It could be just an epiphenomenon and not a marker of advanced cirrhosis. The authors indicated the MELD score as the most reliable predictor for clinical outcome. Stine et al. [38] conducted a meta-analysis, establishing an increased risk for ascites and mortality in PVT patients; however, the authors emphasized that the data were insufficient to determine the effects of other decompensation markers, such as VB or hepatic encephalopathy. After conducting a systematic review of the literature, Qi et al. [39] concluded that heterogeneity in data reporting did not allow conclusions to be drawn about PVT consequences on LC outcomes. Our study did not reveal any differences in the prevalence of PVT between the patients with or without VB or groups with different ascites grades.

Most authors agree that PVT in LC patients has a minor influence on the course of LD, except in those with PVT after liver transplantation, which is associated with increased graft failure, morbidity, and mortality rates [40–43]. Our study revealed the presence of occlusive and non-occlusive PVT in 33.3% and 66.7% of patients, respectively. Non-occlusive PVT most commonly has an asymptomatic course with a spontaneous recanalization in about 70% of cases, which may be attributed to the improvement of liver function [31, 44].

Portal vein characteristics and EVs, spleen diameter, and platelet count

EVs are a characteristic of PH. EVs can be classified as small or large, with or without red color signs [45]. In our study, EVs were diagnosed in 82.6% of patients. Singh et al. [46] investigated the correlation between EVs and PV diameter, reporting an EV prevalence of 78%. Endoscopic examination confirmed the VB in 25.9% of our patients with LC, which corresponds with the data from the literature, reporting prevalence rates ranging from 25 to 40% [47]. PVT was more common in patients with large EVs compared to those with small EVs and those without EVs. Our investigation did not reveal any association between the presence of PVT and VB, which is contrary to the results of D'Amico et al. [48]. Our study established the differences in the PV diameter between patients without EVs and those with large EVs. Rani et al. [49] have compared the PV diameter with the occurrence of EVs in patients with LC and PH. The authors reported a PV diameter of 11.1 ± 0.8 mm in patients without EVs and 13.1 ± 2.1 mm in those with EVs ($p < 0.001$), which corresponds with the results of our investigation. We established the differences in spleen diameters between patients without EVs or small EVs and those with large EVs. This result is consistent with the report of Rani et al. [49], who reported spleen diameters of 14.0 ± 1.1 cm and 15.2 ± 1.4 cm ($p < 0.01$) in patients without and with EVs, respectively.

Similar results were reported by Gyawali and Acharya [50], who concluded that US measurement of the PV and spleen diameter is recommendable as a non-invasive predictor of esophagogastric varices in LC patients.

In our study, there was no statistically significant difference in the number of platelets among patients in relation to the presence of EVs. Conversely, Rani et al. [49] reported the platelet counts in patients without EVs and those with EVs of 158.6 ± 31.9 $\times 10^9$/L and 114.6 ± 54.0 $\times 10^9$/L, respectively, $p < 0.001$. Bhattarai et al. [51] detected sensitivities of 92.7% and 94.5%, while specificities for the presence of EVs were 90% and 75%, and the cut-off values for PV diameter and spleen size were 12.3 mm and 13.9 cm, respectively. The platelet count cut-off point < 144 $\times 10^9$/L had 87.9% sensitivity. High specificity has also been established for serum albumin at a cut-off point of 25.5 g/L. The authors concluded that these parameters can be recommended as non-invasive predictors for gastro-esophageal varices in LC patients. The study of Mandal et al. [52] established a direct correlation between EV rate and PV diameter and spleen size ($r = 0.707$ and $r = 0.467$, respectively). In higher-grade EVs, the average PV diameter was 14.4 ± 0.9 mm, and the spleen diameter was 15.4 ± 2.1 cm. Schepis et al. [53] reported an association between PV diameter of 13 mm and higher-grade EVs, which corresponds with our investigation. Our results correspond with the study of Uppalapati and Lokesh [54] who reported that a PV diameter at a cut-off value above 13 mm had a strong significant relationship ($p < 0.01$) with the presence of EVs (sensitivity of 100%, specificity of 90%, and positive predictive value of 95.2%). Singh et al. [55] reported an association between the grade of EVs and increased spleen diameter (the mean spleen size of the patients with grade I EVs was 12.1 ± 0.7 cm, grade II EVs was 14.3 ± 0.9 cm, grade III EVs was 16.4 ± 1.1 cm, and with grade IV EVs was 19.1 ± 1.2 cm), as well as the increased PV diameter (grade I, II, III, and IV where the corresponding EVs were 13.2 ± 0.6 mm, 14.6 ± 0.6 mm, 16.4 ± 0.7 mm, and 19.0 ± 1.0 mm, respectively). These results are consistent with the findings in our study. Dong et al. [34] published a study confirming a positive association between PV diameter and PVT in patients with LC. The

average PV diameter in patients with PVT was 14.0 ± 3.0 mm and 10.8 ± 1.1 mm in non-PVT patients. The authors reported that the PV diameter cut-off value with predictive capacity for PVT development was > 12.5 mm and suggested that B-type US should be considered a potential initial diagnostic method. The authors also identified the portal flow, platelet count, and D-dimer as potential risk factors for PVT development. PV diameter proved to be the most predictive factor for developing PVT (odds ratio: 3.96; area under the receiver operating characteristic curve = 0.88, $p < 0.01$).

Unlike Dong et al. [34], other authors found a lower platelet count in patients with PVT compared to patients without PVT [56, 57]. In our study, no statistically significant difference was found in platelet count between patients who had and those who did not have PVT. Such a result was also obtained by Maruyama et al. [58]. Platelet count and function are altered *in vivo* in patients with chronic LD. A complex approach is essential to quantify the real-time platelet function to monitor the unstable counterbalancing between hyperaggregable and hypoaggregable states [59]. In the conducted research, no significant difference was found in the diameter of the PV between patients who had and those who did not have PVT. However, an established difference in the diameter of the PV between patients with large varices and patients without varices could have prognostic significance in assessing the course and degree of severity of LC. Bhattarai et al. [51] found a significantly larger PV diameter in the group of patients with varices compared to patients without varices, as well as a significant association between the Child-Pugh class and the presence of varices. They also reported that the risk for VB in patients of C-P class C was 1.43 times higher than in class B.

Limitations of our study

This study has some limitations. First of all, the data are retrospective. Secondly, PVT was not categorized into acute or chronic forms. Chronic PVT is characterized by the development of venous collaterals known as portal cavernoma. However, PH and potential pre-existing collaterals associated with LC often cause difficulties differentiating between acute and chronic PVT. Moreover, the analysis of therapeutic modalities before and after establishing the diagnosis of PVT was not performed. Finally, the investigation did not include the analysis of beta-blocker administration or previous endoscopic treatment of EVs (this was not the objective of this investigation). Therefore, further prospective studies, including the abovementioned data, are necessary.

Conclusion

Among patients with LC, PVT was diagnosed in the severe stages of the disease (C-P classes B and C). However, there were no differences in the mortality rates in patients with and without PVT. There is a significant difference in the PV diameter between patients without EVs and those with large EVs. PVT is more frequent in patients with large EVs than in those with small EVs and no EVs, even though the existing PVT was not associated with the presence or absence of VB. Esophagogastroduodenoscopy is required in patients with PVT. On the other hand, Doppler US and/or abdominal CT are indicated in patients with large EVs and those of C-P classes B and C. In patients with LC, it is always necessary to consider thrombosis, not only hemorrhagic conditions.

REFERENCES

1. *De Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C; Baveno VII Faculty*. Baveno VII – Renewing consensus in portal hypertension. J Hepatol 2022; 76(4): 959–74. Erratum in: J Hepatol 2022.
2. *Garcia-Tsao G, Bosch J*. Management of varices and variceal hemorrhage in cirrhosis. N Engl J Med 2010; 362(9): 823–32. Erratum in: N Engl J Med 2011; 364(5): 490.
3. *Diaz-Soto MP, Garcia-Tsao G*. Management of varices and variceal hemorrhage in liver cirrhosis: a recent update. Therap Adv Gastroenterol 2022; 15: 17562848221101712.
4. *Caiano LM, Riva N, Carrier M, Gatt A, Ageno W*. Treatment of portal vein thrombosis: an updated narrative review. Minerva Med 2022; 112(6): 713–25.
5. *Ma J, Yan Z, Luo J, Liu Q, Wang J, Qiu S*. Rational classification of portal vein thrombosis and its clinical significance. PLoS One 2014; 9(11): e112501.
6. *Von Köckritz L, De Gottardi A, Trebicka J, Praktiknjo M*. Portal vein thrombosis in patients with cirrhosis. Gastroenterol Rep (Oxf) 2017; 5(2): 148–56.
7. *Tsochatzis EA, Senzolo M, Germani G, Gatt A, Burroughs AK*. Systematic review: portal vein thrombosis in cirrhosis. Aliment Pharmacol Ther 2010; 31(3): 366–74.
8. *Turon F, Driever EG, Baiges A, Cerda E, García-Criado Á, Gilabert R*, et al. Predicting portal thrombosis in cirrhosis: A prospective study of clinical, ultrasonographic and hemostatic factors. J Hepatol 2021; 75(6): 1367–76.
9. *European Association for the Study of the Liver*. EASL Clinical Practice Guidelines: Vascular diseases of the liver. J Hepatol 2016; 64(1): 179–202.
10. *De Franchis R; Baveno VI Faculty*. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatol 2015; 63(3): 743–52.
11. *Balfour GW, Stewart TG*. Case of enlarged spleen complicated with ascites, both depending upon varicose dilatation and thrombosis of the portal vein. Edinb Med J 1869; 14(7): 589–98.
12. *Faccia M, Ainora ME, Ponziani FR, Riccardi L, Garcovich M, Gasbarrini A*, et al. Portal vein thrombosis in cirrhosis: Why a well-known complication is still matter of debate. World J Gastroenterol 2019; 25(31): 4437–51.
13. *Anton A, Campreciós G, Pérez-Campuzano V, Orts L, García-Pagán JC, Hernández-Gea V*. The pathophysiology of portal vein thrombosis in cirrhosis: getting deeper into Virchow's triad. J Clin Med 2022; 11(3): 800.
14. *Turon F, Hernández-Gea V, García-Pagán JC*. Portal vein thrombosis: yes or no on anticoagulation therapy. Curr Opin Organ Transplant 2018; 23(2): 250–6.

15. *Odriozola A, Puente Á, Cuadrado A, Rivas C, Anton Á, González FJ,* et al. Portal vein thrombosis in the setting of cirrhosis: a comprehensive review. J Clin Med 2022; 11(21): 6435.
16. *Lisman T, Porte RJ*. Rebalanced hemostasis in patients with liver disease: evidence and clinical consequences. Blood 2010; 116(6): 878–85.
17. *Delahousse B, Labat-Debelleix V, Decalonne L, d'Alteroche L, Perarnau JM, Gruel Y*. Comparative study of coagulation and thrombin generation in the portal and jugular plasma of patients with cirrhosis. Thromb Haemost 2010; 104(4): 741–9.
18. *Praktiknjo M, Trebicka J, Carnevale R, Pastori D, Queck A, Ettorre E,* et al. Von Willebrand and factor VIII portosystemic circulation gradient in cirrhosis: implications for portal vein thrombosis. Clin Transl Gastroenterol 2020; 11(2): e00123.
19. *Zanetto A, Campello E, Senzolo M, Simioni P.* Assessment of whole blood platelet aggregation in patients with cirrhosis: challenges and opportunities. Platelets 2023; 34(1): 2178823.
20. *Berzigotti A, García-Criado A, Darnell A, García-Pagán JC*. Imaging in clinical decision-making for portal vein thrombosis. Nat Rev Gastroenterol Hepatol 2014; 11(5): 308–16.
21. *DeLeve LD, Valla DC, Garcia-Tsao G; American Association for the Study Liver Diseases*. Vascular disorders of the liver. Hepatology 2009; 49(5): 1729–64.
22. *Senzolo M, Garcia-Tsao G, García-Pagán JC*. Current knowledge and management of portal vein thrombosis in cirrhosis. J Hepatol 2021; 75(2): 442–53.
23. D*elgado MG, Seijo S, Yepes I, Achécar L, Catalina MV, García-Criado Á,* et al. Efficacy and safety of anticoagulation on patients with cirrhosis and portal vein thrombosis. Clin Gastroenterol Hepatol 2012; 10(7): 776–83.
24. *Khan AR, Wei X, Xu X*. Portal vein tumor thrombosis and hepatocellular carcinoma – the changing tides. J Hepatocell Carcinoma 2021; 8: 1089–115.
25. *Child CG, Turcotte JG*. Surgery and portal hypertension. Major Probl Clin Surg 1964; 1: 1–85.
26. *Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R*. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60(8): 646–9.
27. *Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL,* et al. A model to predict survival in patients with end-stage liver disease. Hepatology 2001; 33(2): 464–70.
28. *Ruf A, Dirchwolf M, Freeman RB*. From Child-Pugh to MELD score and beyond: Taking a walk down memory lane. Ann Hepatol 2022; 27(1): 100535.
29. *Gülcicegi DE, Goeser T, Kasper P*. Prognostic assessment of liver cirrhosis and its complications: current concepts and future perspectives. Front Med (Lausanne) 2023; 10: 1268102.
30. *Yerdel MA, Gunson B, Mirza D, Karayalçin K, Olliff S, Buckels J,* et al. Portal vein thrombosis in adults undergoing liver transplantation. Transplantation 2000; 69(9): 1873–81.
31. *Mantaka A, Augoustaki A, Kouroumalis EA, Samonakis DN*. Portal vein thrombosis in cirrhosis: diagnosis, natural history, and therapeutic challenges. Ann Gastroenterol 2018; 31(3): 315–29.
32. *Amitrano L, Guardascione MA, Brancaccio V, Margaglione M, Manguso F, Iannaccone L,* et al. Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis. J Hepatol 2004; 40(5): 736–41.
33. *Violi F, Corazza GR, Caldwell SH, Perticone F, Gatta A, Angelico M,* et al. Portal vein thrombosis relevance on liver cirrhosis: Italian Venous Thrombotic Events Registry. Intern Emerg Med 2016; 11(8): 1059–66.
34. *Dong G, Huang XQ, Zhu YL, Ding H, Li F, Chen SY*. Increased portal vein diameter is predictive of portal vein thrombosis development in patients with liver cirrhosis. Ann Transl Med 2021; 9(4): 289.
35. *Luca A, Caruso S, Milazzo M, Marrone G, Mamone G, Crinò F,* et al. Natural course of extrahepatic nonmalignant partial portal vein thrombosis in patients with cirrhosis. Radiology 2012; 265(1): 124–32.
36. *Nery F, Chevret S, Condat B, de Raucourt E, Boudaoud L, Rautou PE,* et al. Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: Results of a longitudinal study. Hepatology 2015; 61(2): 660–7.
37. *Borjas-Almaguer OD, Cortez-Hernández CA, González-Moreno EI, Bosques-Padilla FJ, González-González JA, Garza AA,* et al. Portal vein thrombosis in patients with cirrhosis: just a common finding or a predictor of poor outcome? Ann Hepatol 2017; 15(6): 902–6.
38. *Stine JG, Shah PM, Cornella SL, Rudnick SR, Ghabril MS, Stukenborg GJ,* et al. Portal vein thrombosis, mortality and hepatic decompensation in patients with cirrhosis: A meta-analysis. World J Hepatol 2015; 7(27): 2774–8.
39. *Qi X, Dai J, Yang M, Ren W, Jia J, Guo X*. Association between portal vein thrombosis and survival in non-liver-transplant patients with liver cirrhosis: a systematic review of the literature. Gastroenterol Res Pract 2015; 2015: 480842.
40. *Ghabril M, Agarwal S, Lacerda M, Chalasani N, Kwo P, Tector AJ*. Portal vein thrombosis is a risk factor for poor early outcomes after liver transplantation: Analysis of risk factors and outcomes for portal vein thrombosis in waitlisted patients. Transplantation 2016; 100(1): 126–33.
41. *Englesbe MJ, Schaubel DE, Cai S, Guidinger MK, Merion RM*. Portal vein thrombosis and liver transplant survival benefit. Liver Transpl 2010; 16(8): 999–1005.
42. *Zanetto A, Rodriguez-Kastro KI, Germani G, Ferrarese A, Cillo U, Burra P,* et al. Mortality in liver transplant recipients with portal vein thrombosis - an updated meta-analysis. Transpl Int 2018; 31(12): 1318–29.
43. *Xian J, Tang Y, Shao H, Wang X, Zhang M, Xing T*. Effect of portal vein thrombosis on the prognosis of patients with cirrhosis without a liver transplant: A systematic review and meta-analysis. Medicine (Baltimore) 2021; 100(16): e25439.
44. *Qi X, Yang Z, Fan D*. Spontaneous resolution of portal vein thrombosis in cirrhosis: Where do we stand, and where will we go? Saudi J Gastroenterol 2014; 20(5): 265-6.
45. *Abby Philips C, Sahney A*. Oesophageal and gastric varices: historical aspects, classification and grading: everything in one place. Gastroenterol Rep (Oxf) 2016; 4(3): 186–95.
46. *Singh H, Sharma S, Singh G, Kaur D*. Study of the correlation of oesophageal varices with portal vein diameter and ratio of platelet count to splenic diameter and their comparative evaluation in liver cirrhosis. Int J Adv Med 2021; 8(9): 1405–11.
47. *Lisman T, Hernandez-Gea V, Magnusson M, Roberts L, Stanworth S, Thachil J,* et al. The concept of rebalanced hemostasis in patients with liver disease: Communication from the ISTH SSC working group on hemostatic management of patients with liver disease. J Thromb Haemost 2021; 19(4): 1116–22.
48. *D'Amico G, De Franchis R; Cooperative Study Group*. Upper digestive bleeding in cirrhosis. Post-therapeutic outcome and prognostic indicators. Hepatology 2003; 38(3): 599–612.
49. *Rani S, Sudarsi B, Siddeswari R, Manohar S*. Correlation of portal vein size with esophageal varices severity in patients with cirrhosis of liver with portal hypertension. Int J Sci Res Publ 2015; 5(1): 1–5.
50. *Gyawali M, Acharya RR*. Role of ultrasonography in predicting gastro-oesophageal varices in patients with liver cirrhosis. J Coll Med Sci-Nepal 2021; 17(1): 10–5.
51. *Bhattarai S, Dewan KR, Shrestha G, Patowary BS*. Non-invasive predictors of gastro-oesophageal varices. J Nepal Med Assoc 2017; 56(207): 298–303.
52. *Mandal L, Mandal SK, Bandyopadhyay D, Datta S*. Correlation of portal vein diameter and splenic size with gastro-oesophageal

varices in cirrhosis of liver. J Indian Acad Clin Med 2011; 12(4): 266–70.
53. *Schepis F, Cammà C, Niceforo D, Magnano A, Pallio S, Cinquegrani M,* et al. Which patients with cirrhosis should undergo endoscopic screening for esophageal varices detection? Hepatology 2001; 33(2): 333–8.
54. *Uppalapati S, Lokesh S*. Correlation of portal vein diameter with the presence of oesophageal varices in chronic liver disease: a prospective study. Int J Adv Med 2018; 5(4): 859–64.
55. *Singh I, Sehgal R, Singh H, Chuchra P, Neki NS*. Correlation of portal vein diameter, splenomegaly and thrombocytopenia with gastro-espohageal varices in cirrhotic patients. Int J Curr Res Biol Med 2017; 4(12): 38–44.
56. *Abdel-Razik A, Mousa N, Elhelaly R, Tawfik A*. De-novo portal vein thrombosis in liver cirrhosis: risk factors and correlation with the Model for End-stage Liver Disease scoring system. Eur J Gastroenterol Hepatol 2015; 27(5): 585–92.
57. *Quan X, Ye X, Qian S, Wei B, Tong H, Wang Z,* et al. Portal vein thrombosis associates with high platelet-fibrin clot strength and platelet activation in decompensated cirrhosis: A retrospective study. Dig Liver Dis 2023; 55(5): 629–36.
58. *Maruyama H, Okugawa H, Takahashi M, Yokosuka O*. De novo portal vein thrombosis in virus-related cirrhosis: predictive factors and long-term outcomes. Am J Gastroenterol 2013; 108(4): 568–74.
59. *Chen SH, Tsai SC, Lu HC*. Platelets as a gauge of liver disease kinetics? Int J Mol Sci 2022; 23(19): 11460.

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Comparative evaluation of titanium-prepared platelet-rich fibrin with and without herbal extract: a histological study

Poređenje titanijumom-pripremljenog fibrina obogaćenog trombocitima sa i bez biljnog ekstrakta: histološka studija

Shiva Shankar Gummaluri*†, Kaarthikeyan Gurumoorthy‡§, Trinath Kishore Damera*†, Anusha Boddeda*†, Tejaswi Kodem*†, [1]Sivaram Lekkala*

***GITAM Dental College and Hospital, †Department of Periodontology and Implantology, Andhra Pradesh, India; ‡Saveetha University, §Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Department of Periodontology, India**

[1]Bachelor of Dental Surgery (BDS) undergraduate student

Abstract

Background/Aim. Injecting herbal extract into platelet concentrates is one of the newer treatment protocols, which enables platelet concentrates to act as sustained drug delivery (DD) systems. Histological analysis of titanium-prepared platelet-rich fibrin (T-PRF) injected with herbal extract, could help assess the appearance (pattern) and structural changes of T-PRF. The aim of the study was to evaluate the appearance of the fibrin network, cellularity, and fibrin border area of T-PRF alone and T-PRF injected with herbal extract. **Methods.** A total of 40 histological slides were prepared from 10 mL of blood from each patient, 20 with T-PRF alone and 20 with T-PRF+herbal extract. The slides were divided into a group consisting of T-PRF injected with neem gel (test group) and a group consisting of T-PRF alone (control group). The preparation protocol was made according to Bankroft's manual adapted for light microscopy. **Results.** Regarding the fibrin network features (dense vs. loose), no statistical significance was found among the studied groups ($p = 0.172$). A statistically significant difference was shown in the packeting ($p = 0.018$) and layered ($p = 0.028$) patterns of the fibrin network, and there was no statistically significant difference in the scattered ($p = 0.749$) pattern among the examined groups. Cellularity and cell pattern values were not statistically significantly different for both groups ($p = 1.00$, $p = 0.3111$, respectively). Moreover, the values determined for red blood cells, white blood cells, and platelets were not statistically significantly different ($p = 0.147$), as well as for the fibrin border area between cells and meshwork ($p = 0.206$). **Conclusion.** The obtained results could be useful for the development of a new treatment strategy in dentistry, by utilizing T-PRF with incorporated herbal extracts or antibiotics, as a local sustained DD system.

Key words:
drug delivery systems; histological techniques; periodontitis; plants; platelet-rich plasma.

Apstrakt

Uvod/Cilj. Ubrizgavanje biljnog ekstrakta u koncentrat trombocita jedan je od novijih protokola lečenja, koji omogućava da koncentrat trombocita deluje kao sistem za kontinuiranu isporuku lekova. Analiza histoloških preseka titanijumom-pripremljenog fibrina obogaćenog trombocitima (*titanium-prepared platelet-rich fibrin* – T-PRF) u koji je ubrizgan biljni ekstrakt, mogla bi biti korisna za procenu izgleda (obrasca) i promena strukture T-PRF. Cilj rada bio je da se proceni izgled fibrinske mreže, celularnost i granično područje fibrina samog T-PRF i T-PRF u koji je ubrizgan biljni ekstrakt. **Metode.** Od 10 mL krvi svakog pacijenta pripremljeno je ukupno 40 histoloških pločica, 20 samo sa T-PRF i 20 sa T-PRF i dodatkom biljnog ekstrakta. Preparati su podeljeni na grupu T-PRF u koji je ubrizgan *neem* gel (testirana grupa) i grupu samog T-PRF (kontrolna grupa). Protokol pripreme napravljen je prema Bankroftovom priručniku prilagodjenom za svetlosnu mikroskopiju. **Rezultati.** Nije bilo statističke značajnosti među ispitivanim grupama ($p = 0{,}172$) u pogledu izgleda fibrinske mreže (gusto vs. rastresito). Pokazana je statistički značajna razlika u obrascima fibrinske mreže „pakovanje" ($p = 0{,}018$) i „slojevitost" ($p = 0{,}028$), a nije bilo statistički značajne razlike u obrascu „rasipanje" ($p = 0.749$) među ispitivanim grupama. Vrednosti za parametre distribucija

Correspondence to: Kaarthikeyan Gurumoorthy, Department of Periodontology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India. E-mail: drkarthik79@yahoo.co.in

ćelija i celularnost nisu bile statistički značajno različite za obe grupe (p = 1.00, p = 0.3111, redom). Takođe, ni vrednosti koje su utvrđene za crvena krvna zrnca, bela krvna zrnca i trombocite nisu bile statistički značajno različite (p = 0,147), kao ni za granično područje fibrina između ćelija i mreže (p = 0,206). **Zaključak.** Dobijeni rezultati bi mogli biti korisni za razvoj nove strategije lečenja u stomatologiji, korišćenjem T-PRF sa inkorporiranim biljnim ekstraktima ili antibioticima, kao sistema za lokalnu kontinuiranu isporuku lekova.

Ključne reči:
lekovi, sistemi za isporuku; histološke tehnike; periodontitis; biljke; plazma bogata trombocitima.

Introduction

Periodontitis is a painless disease that is difficult to treat because of its multifactorial origin [1]. Various treatment strategies, including some non-surgical treatments such as scaling and root planing and local drug delivery (DD) systems, have been used [2]. Surgical treatments such as open flap debridement, guided tissue regeneration, and guided bone regeneration have been utilized. Various biomaterials, which were reported to achieve good results, like bone grafts, collagen membranes, and platelet concentrates (PCs), were also used for controlling postoperative recession and restoring lost periodontal tissues [3]. PCs are considered a boon to the medical field because of their autologous nature, sustained holding, and release of growth factors (GFs) at surgical sites [4]. Initially, leukocyte platelet-rich fibrin (L-PRF) was introduced by Choukroun et al. [5]. However, because of its shorter resorption time and possible silica cross-contamination, titanium-prepared platelet-rich fibrin (T-PRF) was introduced by Tunali et al. [6], where medical grade titanium tubes (Grade IV) were used for the preparation of PCs.

T-PRF had better fibrin meshwork, a thicker membrane, and better cellular entrapment. It demonstrates better hemocompatibility, and the titanium passivates itself into the titanium dioxide layer to activate platelets [7]. In the studies by Chatterjee et al. [8] and Yajamanya et al. [9], it was stated that T-PRF had a better fibrin meshwork, border, and thicker membrane pattern than L-PRF. As stated in the study by Bhattacharya et al. [10], both T-PRF and L-PRF shared a similar pattern, but T-PRF had thicker fibrin meshwork with a thicker structure in the mid-membrane region. Histological sectioning of human specimens was always a difficult task because gaining ethical permissions and patient acceptance was troublesome.

Ercan et al. [11] used doxycycline hyclate gel that was incorporated in T-PRF clot and compared it with collagen sponge incorporated with doxycycline hyclate gel and concluded that T-PRF+ doxycycline (Doxy) – (T-PRF+ Doxy) group had longer release than collagen sponge group during their kinetic studies. Furthermore, their microbiological analysis also depicted the best outcome with the T-PRF+Doxy group regarding the zone of inhibition, thus depicting the newer treatment modality of DD at the required periodontal site [11]. Various antibiotics have been tried, such as lincomycin, minocycline, and clindamycin, and neem gels were used as local DD systems in non-surgical and surgical treatments, and good concentration levels of the drug in the pocket were achieved [12].

Histological analysis is one of the major types of assessment for diagnosing a clinical case scenario by a possible clinical correlation. However, it is not possible to perform this in every situation. Histological evaluation of membrane clots using light microscopy (LM) would be an indirect evaluation of cells and fibrin structure, which would hold the GFs, which in turn help in the formation of lost tissue cells.

Considering that a small number of studies has been done on this topic, the present study aimed to evaluate the features of the fibrin network (including distribution pattern and arrangement), cellular entrapment (cellularity), and the border area between fibrin meshwork and cellularity in T-PRF with and without injecting herbal extract using LM.

Methods

Sample size

With 80% power, an effect size of 0.25, and an alpha value error of 5%, a sample of 20 slides *per* group was sufficient to conduct the study. A total of 40 samples were considered for the study. The entire calculation was performed on G* Power software 3.0.

Study design

The present study was a histological study using LM. A total of 20 healthy volunteers were recruited after getting informed consent from the outpatient Department. The study was conducted by the Department of Periodontology and Implantology, GITAM Dental College and Hospital, Visakhapatnam. The study was performed according to the 1975 Helsinki Declaration, modified in 2013. Prior to the conduction of the study, ethical approval was obtained from the GITAM Dental College and Hospital Ethics Committee (Nº 6908606s33523, from July 28, 2023). There were no problems at the patient blood drawing sites.

The test group consisted of T-PRF injected with neem gel, and the control group consisted of T-PRF alone.

The study was performed based on the below inclusion and exclusion criteria.

Inclusion and exclusion criteria

Patients above 18 years of age, with a Plaque Index < 20%, with healthy periodontium, and patients with a range of platelets between 150,000 to 400,000/mm^3 were included in the study. Patients who were systemically ill, who were not interested in participating, lactating and pregnant women,

who underwent periodontal treatment in the last six months, and who were on medications that affect the status of periodontium were excluded from the study.

Procedure

T-PRF clots were prepared based on modified Tunali et al. [6], Bhattacharya et al. [13], and Mitra et al. [14] criteria. For the test group, the T-PRF clot was loaded with commercially prepared neem gel just immediately after clot retrieval from titanium test tubes, while for the control group, T-PRF alone in the clot form was used. With no further delay, these clots of both groups were immediately placed in 10% formalin solution (for fixing up to 24 hrs) and transferred to the Department of Oral Pathology and Oral Microbiology for slide preparation and LM analysis.

Neem gel preparation

Neem gel was commercially formulated from the PerioBiologics™ lab. The neem gel was prepared in the following manner. Aqueous liquid extract of *Azadirachta indica* was prepared by 20 g of neem leaves in 1 L of water. This was reduced to 100 g of neem extract. To obtain a minimum inhibitory concentration at 25 µg/mL, 20 g of Carbopol® 934P was added in 1,000 mL of water and soaked for 24 hrs to prepare the base gel. All the prepared ingredients were mixed, and 0.02% calcium chloride and 0.9% sodium chloride were added to prepare the neem gel [15]. These gels were loaded in µ syringe needles with the quantity of 10 U *per* syringe, which creates a concentration of 8 g of neem *per* syringe.

T-PRF clots preparation

Ten mL of blood was drawn from the antecubital vein and immediately transferred to medical-grade sterile titanium tubes, divided into 5 mL per tube. Figure 1 shows the titanium tubes, retrieval of T-PRF clots, and T-PRF injected with neem gel. Then, the tubes filled with blood were subjected to centrifugation using the modified Tunali et al. [6, 16] protocol [3,500 revolutions *per* minute (rpm) for 15 min]. Further, the upper layer of platelet-poor plasma was discarded, the middle layer, which was the fibrin clot with a buffy coat, was retrieved, and the bottom layer of red blood cell (RBC) layers was allowed to settle down at the bottom of the tube. The middle layer of T-PRF clots was retrieved, and those clots were placed on a sterile kidney tray.

Sample preparation for histological analysis

The considered samples were placed in a 10% formalin solution and fixed for a period of 24 hrs as a process of preventing the autolysis and maintenance of the intact structure. These clots were then retrieved and subjected to the process of desiccation in various concentrations of alcohol (25%, 50%, 75%, and 100%). Further clearing and paraffinization were continued, which takes up to 16 hrs. Later on, these clots were taken into blocks and made into thin sections using a microtome and transferred to a slide (Figure 2). In the

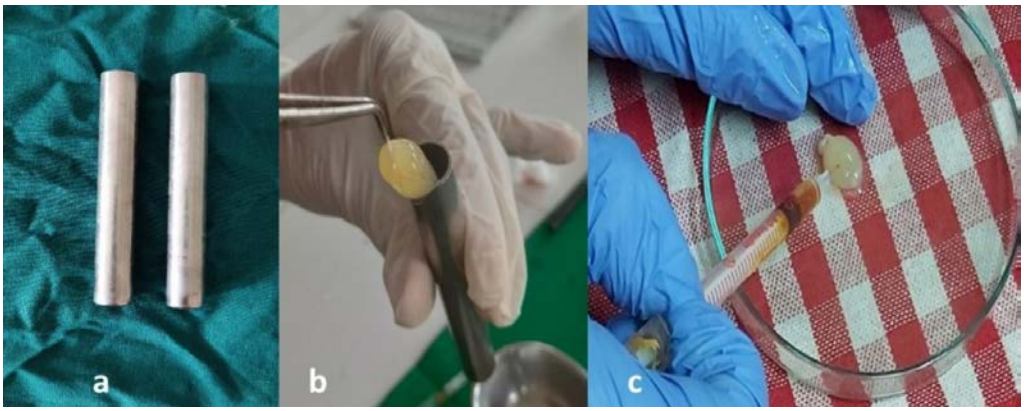

Fig. 1 – a) Titanium tubes; b) Retrieval of T-PRF clots; c) T-PRF injected with neem gel.
T-PRF – titanium-prepared platelet-rich fibrin.

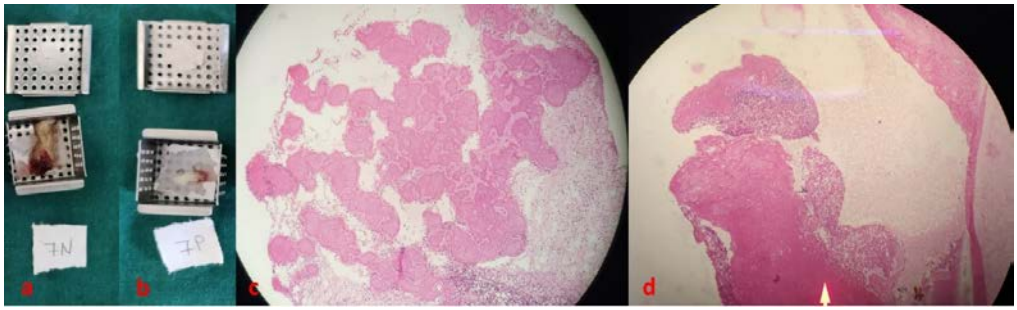

Fig. 2 – a, b) Paraffin block preparation of T-PRF incorporated with neem gel and plain T-PRF; Histological section of: c) plain T-PRF and d) T-PRF incorporated with neem gel (at 10× magnification, HE staining).
T-PRF – titanium-prepared platelet-rich fibrin; HE – hematoxylin eosin.

next step, deparaffinization was done by heating the slide at 55 ˚C and kept in xylene to remove any paraffin particles. Hence, as a part of the study, 40 slides (20 for each of the two groups) with proper coding and numbers were subjected to LM analysis. The entire process of histological analysis was performed according to Bancroft's manual [17] and observed under a penta head microscope.

Parameters assessed from the prepared slides

Based on the modified Tunali et al. [6] criteria, all the T-PRF slides, both with and without neem gel, were analyzed. In addition, it was noted whether the fibrin network pattern was loose or dense; the pattern was recorded as packeting, layered, or scattered. Concerning cellularity, presence and absence were scored. If present, the score of 0 was given when the presence of cellularity was < 25%; a score of 1 was given when there was 26–50% of cellularity; a score of 2 was recorded when there was 51–75% of cellularity; a score of 3 was given for > 75% of cellularity. Furthermore, the presence and absence of RBCs were assessed, including whether the border area between fibrin meshwork and cellularity was thick or thin.

The slides were examined by an experienced oral pathologist.

Statistical analysis

Entire data was noted in a Microsoft Excel spreadsheet and subjected to statistical analysis. Data analysis was done using Statistical Package for Social Sciences (SPSS) version 22, IBM Pvt Ltd, Chicago, Illinois, USA. Study data were presented as percentages of the frequency distribution for the following: fibrin network pattern; cell distribution; presence or absence of cellularity; presence of red and white blood cells (WBCs), platelets, and fibrin border area between cellularity and fibrin network. For these parameters, Fisher's exact test was used. The score was also noted and an unpaired *t*-test was used to demonstrate the cellularity significance. The value of $p < 0.05$ was considered statistically significant.

Results

In the present study regarding the fibrin network features, plain T-PRF showed a 60% dense pattern and 40% loose pattern, whereas, in the case of the T-PRF neem gel group, it was vice versa. No significant statistical difference was shown between the groups ($p = 0.172$). In the case of pattern, T-PRF had 50% of packeting pattern, 10% showed layered, and 40% had scattered pattern, while neem incorporated T-PRF showed 15% of packeting pattern, 40% showed layered, and 45% had scattered pattern. Their comparisons were significant for the packeting ($p = 0.018$) and layered ($p = 0.028$) patterns and non-significant for the scattered ($p = 0.749$) pattern. Regarding cell distribution patterns, both groups (T-PRF plain and neem gel incorporated T-PRF) reported non-significant differences ($p = 1.000$), i.e., 30% of narrow and 70% of wide patterns were detected. In the case of cellularity, 95% of plain T-PRF showed the presence of cells, whereas 100% of neem-incorporated T-PRF showed the presence of cellularity, which was not statistically significantly different ($p = 0.311$). Although the values were not statistically significant, 35 to 50% of both groups showed a range of cellularity greater than 50%, i.e., a score range of 2 and 3. Regarding RBCs, WBCs, and platelets, both groups showed 90 to 100% of presence without a statistically significant difference between the groups ($p = 0.147$) (Table 1).

Table 1

Frequency distribution percentage comparison of fibrin network, their pattern, cell distribution, cellularity cum scoring, presence of red blood cells, white blood cells, platelets, and the fibrin border area between cells, and fibrin meshwork of plain T-PRF and neem gel incorporated T-PRF

Parameters	T-PRF plain	T-PRF neem gel	*p*-value
Fibrin network			
dense	12 (60)	8 (40)	0.172
loose	8 (40)	12 (60)	
Pattern			
packeting	10 (50)	3 (15)	0.018*
layered	2 (10)	8 (40)	0.028*
scattered	8 (40)	9 (45)	0.749
Cell distribution			
narrow	6 (30)	6 (30)	1.000
wide	14 (70)	14 (70)	
Cellularity			
absent	1 (5)	0 (0)	0.311
present	19 (95)	20 (100)	
Red blood cells			
absent	2 (10)	0 (0)	0.147
present	18 (90)	20 (100)	
Platelets and white blood cells			
absent	2 (10)	0 (0)	0.147
present	18 (90)	20 (100)	

T-PRF – titanium-prepared platelet-rich fibrin; *statistically significant.
All values are given as numbers (percentages).

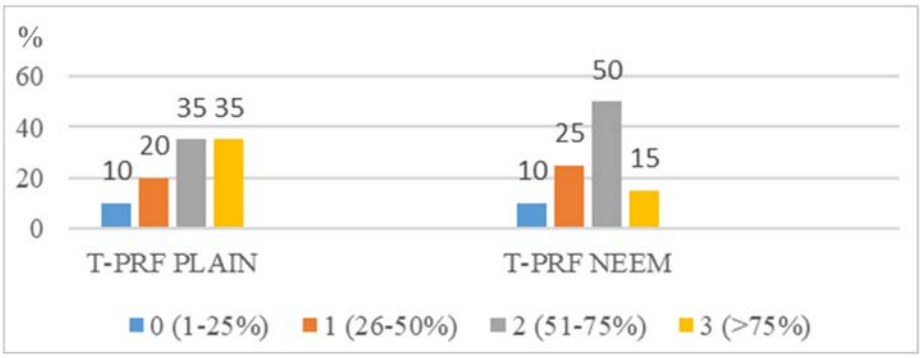

Fig. 3 – Score range criteria for plain T-PRF and T-PRF neem groups (p = 0.535). T-PRF – titanium-platelet rich fibrin.

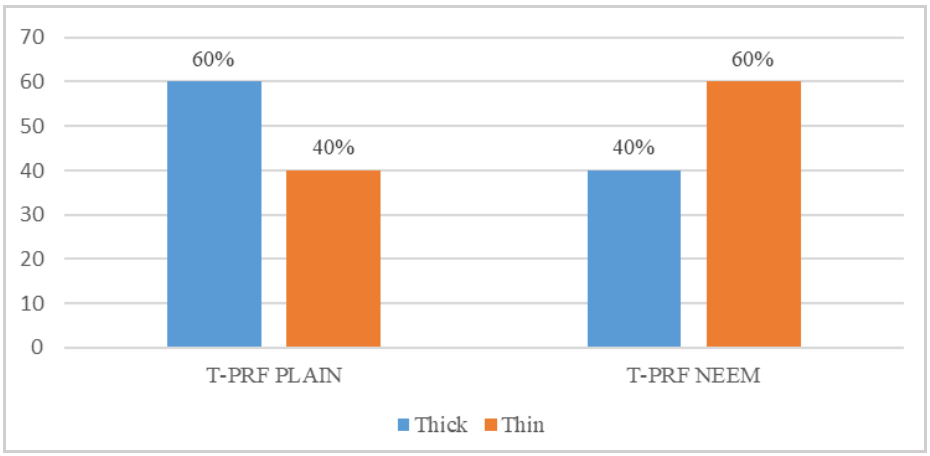

Fig. 4 – Graphical representation of the border area between fibrin meshwork and cellularity (p = 0.206). T-PRF – titanium-prepared platelet rich fibrin.

When comparing the scores regarding the percentage of cellularity, there was no statistically significant difference between the tested groups (p = 0.535). The score range criteria for both groups, plain T-PRF and T-PRF neem, as well as values, are presented in Figure 3.

When it comes to the border area between the fibrin network and cellularity, plain T-PRF had a 60% thick border area, whereas neem incorporated T-PRF showed a 40% thick border area, but the values shown were not statistically significantly different between the groups (p = 0.206) (Figure 4).

Discussion

Autologous PCs always helped in the promotion of soft and hard tissue healing [18]. Initially, L-PRF was prepared in silica tubes or silica-coated glass test tubes. Silica within these tubes activates the platelets in the blood (particularly alpha and beta granules) and fibrin clots form as a result. The shorter resorption time of 7–11 days and possible silica contamination lead the researchers to search for better material [19]. Due to this quest, the researchers were attracted to titanium metal, which led to the introduction of T-PRF by Tunali et al. [6]. Animal studies on the rabbit model revealed that T-PRF had thicker fibrin meshwork and uniform distribution of cells over the fibrin [16]. Most clinical studies [7, 14, 20] checked for post-operative bone fill, decreased probing pocket depth, and gain in clinical attachment level from baseline to 6 or 9 months based on their respective criteria. Histological sectioning and surgical re-entry always raise a situation of ethical concerns. Hence, histological analysis of PCs will be an indirect way to assess and predict the possibility of periodontal soft and hard tissue healing.

Local DD of antibiotics like amoxicillin, metronidazole, tetracycline, clindamycin, etc., is one of the alternative ways of treating the periodontal disease non-surgically. Even some herbal extracts like neem, aloe vera, etc., have been tried as local DD systems and mouthwashes, and good results have been reported [21]. However, sustained drug release was one of the recent advancements. The drug was delivered at the surgical or inflamed sites that would control the post-operative infection and improve clinical parameters with constant timely drug release [22]. Most delivery systems were laboratory-prepared, such as carbo-polymers, collagen membranes, gel foams, etc. Although these have reported good results, because of their raised sensitivity reaction concerns and the cost of the material, researchers were made to search for better autologous biomaterial [23]. Because of this, in the study by Ercan et al. [11], T-PRF was used as a

sustained drug carrier system where doxycycline hyclate antibiotic liquid was incorporated. The results of their study suggested that T-PRF can be a good biomaterial that is able to withstand the drug because of its thicker fibrin meshwork and better GF holding capacity. The present study was an attempt to check whether injecting the neem gel into T-PRF led to any changes in the fibrin characteristics, cellularity, and intactness of fibrin meshwork and to compare results with plain T-PRF using LM.

Regarding the fibrin network, there was a numerically denser fibrin network for plain T-PRF than for neem gel-incorporated T-PRF, and it was statistically non-significant. Therefore, there was not much difference in both groups overall. Present plain T-PRF results were in accordance with previous studies done by Tunali et al. [6] and Chatterjee et al. [8], where a denser fibrin network in T-PRF was reported. The results of these studies were in contrast with the presented neem gel incorporated T-PRF. These variations of loose fibrin network in the test group might be due to the incorporation of neem gel into the fibrin clot, which lead to spaces in between the fibrin structure. Regarding the pattern, 50% of plain T-PRF had a packeting pattern, and the T-PRF neem gel group had a scattered pattern. This is contrary to the study done by Bhattacharya et al. [10], where a mostly scattered pattern was reported. Present study results were also in accordance with Tunali et al. [6] and Chatterjee et al. [8], where a packeting pattern and thicker fibrin network were reported. This might be due to the greater activation of platelets by the passivated titanium dioxide layer within titanium tubes, which led to intact fibrin mesh.

Concerning cell distribution (26–75% of score range), RBCs, WBCs, and platelets, there was a non-significant, wider distribution pattern. RBC, WBC, and platelet presence was uniform, and this is in accordance with the study results by Bhattacharya et al. [10], Tunali et al. [6], and Chatterjee et al. [8], where a wider distribution of cellularity and various types of cells was reported. This greater cellular entrapment might be due to the modified Tunali et al. [16] centrifugation protocol (35,00 rpm at 15 min) utilized in the study, which could help incorporate RBCs, WBCs, and platelets.

In the present study, plain T-PRF had a thicker fibrin border area than the neem gel-incorporated T-PRF. This is contrary to the study done by Bhattacharya et al. [10] regarding the plain T-PRF group, where a thicker fibrin border area was reported for L-PRF compared to T-PRF. Their results favored neem gel incorporated T-PRF. In the study by Tunali et al. [6], plain T-PRF had a thicker fibrin border area, while neem gel incorporated T-PRF had a thinner fibrin border area. This variation in neem gel incorporated T-PRF might be due to the injected gel having disrupted the fibrin structure leading to much scattered and layered patterns with thin fibrin border areas. It can be thus indirectly understood that neem gel was incorporated into the T-PRF clot which might be washed out during the process of histological slide preparation and depicted as spaces within membrane meshwork. Apart from this, the inherent property of expansion of T-PRF clot might also have influenced the reporting of gaps in the T-PRF incorporation of neem gel.

In the present study, there was the presence of cells, i.e., RBC, WBC, and platelets, with a wider area of cell distribution and 51–75% cell percentage over the slide area in both, T-PRF alone and neem gel incorporated T-PRF. This is in accordance with the study by Ercan et al. [11], where doxycycline hyclate was incorporated into T-PRF, and a greater withhold capacity of the drug by T-PRF was reported. This might be due to the inbuilt capacity of T-PRF and it will make the addition of drugs into T-PRF a treatment protocol and give hope for a sustained DD system.

The incorporation of antibiotics into blood prior to centrifugation is always a concern for the centrifugation process, clotting mechanism, and fibrin structure quality. Studies done by Marzaman et al. [24] and Pillai et al. [25] used antibiotics and painkillers with PRF and concluded that there was no loss of structure, good anti-microbial efficacy was maintained, and post-operative pain after the third molar extraction was reduced. The present study utilized a similar pattern of incorporation of antibiotics after the process of centrifugation. In the present study, it was reported that there were gaps in the T-PRF incorporated with neem gel clots. However, there was no disturbance regarding the wider cell distribution, cell pattern, or presence of cells and layered pattern of fibrin meshwork. This might be due to the incorporation of 0.5 mL of gel into the clot. This was even supported by Polak et al. [26], where it was reported that PRF clots, incorporated with antibiotics beyond 0.5 mL, lost their structural integrity. Hence, 0.5 mL is the maximum amount of drug that can be incorporated into the clots.

A smaller sample size might be considered a limitation of the present study. Histological analysis of membrane clots may predict the healing to some extent, but further scanning electron microscopy, drug kinetics with spectrophotometry, GF release assessment for platelet viability, and animal or human clinical trial might help identify the efficacy of this treatment strategy of T-PRF incorporated with neem gel.

Thus, this might open a gateway for a newer treatment protocol of sustained DD system, utilizing T-PRF as a vehicle incorporated with herbal extracts or antibiotics in the form of gels or liquids.

Conclusion

Although neem gel incorporated T-PRF showed a thin fibrin network with scattered or layered pattern, wider distribution of cells (RBCs, WBCs, and platelets), and a 26–75% score range extended over the clot, it was not much significantly different from T-PRF plain which depicted thicker fibrin network, packeting pattern, and wider cellular distribution. Hence this may help deliver a newer treatment strategy to the dental fraternity utilizing T-PRF as a sustained DD system by incorporating herbal extracts or any antibiotics making it a part of the local DD system.

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R E F E R E N C E S

1. *Könönen E, Gursoy M, Gursoy UK.* Periodontitis: a multifaceted disease of tooth-supporting tissues. J Clin Med 2019; 8(8): 1135.
2. *Amato M, Santonocito S, Polizzi A, Tartaglia GM, Ronsivalle V, Viglianisi G,* et al. Local Delivery and Controlled Release Drugs Systems: A New Approach for the Clinical Treatment of Periodontitis Therapy. Pharmaceutics 2023; 15(4): 1312.
3. *Alqahtani AM.* Guided Tissue and Bone Regeneration Membranes: A Review of Biomaterials and Techniques for Periodontal Treatments. Polymers (Basel) 2023; 15(16): 3355.
4. *Giannotti L, Di Chiara Stanca B, Spedicato F, Nitti P, Damiano F, Demitri C,* et al. Progress in Regenerative Medicine: Exploring Autologous Platelet Concentrates and Their Clinical Applications. Genes (Basel) 2023; 14(9): 1669.
5. *Choukroun J, Adda F, Schoeffer C, Vervelle A.* PRF: an opportunity in perio-implantology. Implantodontie 2000; 42: 55−62.
6. *Tunali M, Özdemir H, Küçükodacı Z, Akman S, Fıratlı E.* In vivo evaluation of titanium-prepared platelet-rich fibrin (T-PRF): a new platelet concentrate. Br J Oral Maxillofac Surg 2013; 51(5): 438−43.
7. *Gummaluri SS, Bhattacharya HS, Astekar M, Cheruvu S.* Evaluation of titanium-prepared platelet-rich fibrin and leucocyte platelet-rich fibrin in the treatment of intra-bony defects: A randomized clinical trial. J Dent Res Dent Clin Dent Prospects 2020; 14(2): 83−91.
8. *Chatterjee A, Debnath K, Ali MM, Babu C, Gowda PL.* Comparative histologic evaluation of titanium platelet-rich fibrin and platelet-rich fibrin in hypertensive and smoker participants: A cell cytology study. J Indian Soc Periodontol 2017; 21(3): 195−200.
9. *Yajamanya SR, Chatterjee A, Babu CN, Karunanithi D.* Fibrin network pattern changes of platelet-rich fibrin in young versus old age group of individuals: A cell block cytology study. J Indian Soc Periodontol 2016; 20(2): 151−6.
10. *Bhattacharya HS, Gummaluri SS, Astekar M, Sapra G, Shiva Manjunath RG.* Comparative histological evaluation of L-PRF with T-PRF: A scanning electron microscopic study. J Exp Ther Oncol 2022; 13(3): 207−13.
11. *Ercan E, Suner SS, Silan C, Yilmaz S, Siddikoglu D, Sahiner N,* et al. Titanium platelet–rich fibrin (T-PRF) as high-capacity doxycycline delivery system. Clin Oral Investig 2022; 26(8): 5429−38.
12. *Sholapurkar A, Sharma D, Glass B, Miller C, Nimmo A, Jennings E.* Professionally delivered local antimicrobials in the treatment of patients with periodontitis—A narrative review. Dent J (Basel) 2020; 9(1): 2.
13. *Bhattacharya HS, Gummaluri SS, Astekar M, Gummaluri RK.* Novel method of determining the periodontal regenerative capacity of T-PRF and L-PRF: An immunohistochemical study. Dent Med Probl 2020; 57(2): 137−44.
14. *Mitra DK, Potdar PN, Prithyani SS, Rodrigues SV, Shetty GP, Talati MA.* Comparative study using autologous platelet-rich fibrin and titanium prepared platelet-rich fibrin in the treatment of infrabony defects: An in vitro and in vivo study. J Indian Soc Periodontol 2019; 23(6): 554−61.
15. *Lwin HY, Linn ZZ, Oo AK, Hla TT.* The Efficacy of Locally Delivered Azadirachta indica (Neem) Extract Gel in Chronic Periodontitis. J Dent Sci UDMY 2020; 1(1): 18–25.
16. *Tunali M, Özdemir H, Küçükodacı Z, Ezirganlı S, Barış E, Akman S,* et al. A novel platelet concentrate for guided bone regeneration: Titanium Prepared Platelet-Rich Fibrin (T-PRF). Gulhane Med J 2015; 57(2): 102−6.
17. *Bancroft JD, Cook H, Turner D.* Manual of Histological Techniques and Their Diagnostic Application. 4th ed. Philadelphia: Elsevier; 1996.
18. *Mijiritsky E, Assaf HD, Kolerman R, Mangani L, Ivanova V, Zlatev S.* Autologous Platelet Concentrates (APCs) for Hard Tissue Regeneration in Oral Implantology, Sinus Floor Elevation, Peri-Implantitis, Socket Preservation, and Medication-Related Osteonecrosis of the Jaw (MRONJ): A Literature Review. Biology (Basel) 2022; 11(9): 1254.
19. *Miron RJ, Kawase T, Dham A, Zhang Y, Fujioka-Kobayashi M, Sculean A.* A technical note on contamination from PRF tubes containing silica and silicone. BMC Oral Health 2021; 21(1): 135.
20. *Chatterjee A, Pradeep AR, Garg V, Yajamanya S, Ali MM, Priya VS.* Treatment of periodontal intrabony defects using autologous platelet-rich fibrin and titanium platelet-rich fibrin: a randomized, clinical, comparative study. J Investig Clin Dent 2017; 8(3): e12231.
21. *Da Rocha HA, Silva CF, Santiago FL, Martins LG, Dias PC, De Magalhães D.* Local drug delivery systems in the treatment of periodontitis: a literature review. J Int Acad Periodontol 2015; 17(3): 82−90.
22. *Liang J, Peng X, Zhou X, Zou J, Cheng L.* Emerging applications of drug delivery systems in oral infectious diseases prevention and treatment. Molecules 2020; 25(3): 516.
23. *Binlateh T, Thammanichanon P, Rittipakorn P, Thinsathid N, Jitprasertwong P.* Collagen-based biomaterials in periodontal regeneration: Current applications and future perspectives of plant-based collagen. Biomimetics (Basel) 2022; 7(2): 34.
24. *Marzaman ANF, Roska TP, Sartini S, Utami RN, Sulistiawati S, Enggi CK,* et al. Recent Advances in Pharmaceutical Approaches of Antimicrobial Agents for Selective Delivery in Various Administration Routes. Antibiotics (Basel) 2023; 12(5): 822.
25. *Pillai AK, Thomas S, Seth S, Jain N, Chobey A.* Platelet Rich Fibrin (PRF) Gel as Efficient Vehicle for Local Drug Delivery in Minor Oral Surgical Defects. J SVOA Dent 2021; 2: 185−91.
26. *Polak D, Clemer-Shamai N, Shapira L.* Incorporating antibiotics into platelet-rich fibrin: a novel antibiotics slow-release biological device. J Clin Periodontol 2019; 46(2): 241−7.

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Acoustic evoked potentials characteristics in patients with vertebral artery hypoplasia and posterior circulation stroke

Karakteristike akustičnih evociranih potencijala kod bolesnika sa hipoplazijom vertebralne arterije i moždanim udarom u vertebrobazilarnom slivu

Biljana Živadinović*†, Radomir Damjanović*, Stefan Todorović*, Jelena Živadinović‡, Jelena Stamenović*†, Mila Bojanović†§, Aleksandar Bojanović†‖, Ivana Ćirić Mladenović‖, Hristina Jovanović**

University Clinical Center of Niš, *Clinic for Neurology, ‡Clinic for Anesthesiology, §Clinic for Otorhinolaryngology, ‖Clinic for Radiology, Niš, Serbia; †University of Niš, Faculty of Medicine, **Department of Pharmacology with Toxicology, Niš, Serbia; ‖Special Hospital for Rehabilitation, Prolom Banja, Serbia

Abstract

Background/Aim. Acoustic evoked potentials (AEPs) represent an electrophysiological method used in diagnosing pathological changes of the brain stem (BSt), the acoustic nerve (its peripheral and central part), in patients in coma, in the confirmation of cerebral death, etc. The response includes seven negative waves which are generated in the structures of the BSt vascularized by the arteries of the posterior circulation. However, in everyday practice, due to their constancy, the first five waves are followed. The vertebral artery hypoplasia (VAH) is assumed to affect the AEPs finding. The current definition of VAH includes the criterion that the diameter of the blood vessel is ≤ 2 mm and that the ratio of the diameter of the left and right vertebral artery is ≥ 1 : 1.7. VAH is found in 5.3% of cases of the total population and its presence increases the risk of posterior circulation stroke (PCS). The aim of this study was to show a higher frequency of pathological findings of AEPs in patients with VAH and PCS and demonstrate the characteristics of AEP in that group of patients. **Methods.** This prospective study included 163 patients diagnosed with PCS over a period of two years. Computed tomography (CT) and magnetic resonance (MR) imaging (MRI) established the diagnosis of PCS. Suspicion of VAH was found by Color Doppler ultrasonography and confirmed by CT and MR angiography. All patients underwent AEPs testing. Wave amplitudes and interwave latencies (IWL) were monitored. **Results.** There was no statistically significant difference between gender ($\chi^2 = 1.823$; $p = 0.176$) and age in relation to VAH ($p = 0.815$). A statistically significant greater number of patients with multiple PCS had a positive VAH finding (VAH group, 42.3%) compared to those without VAH (noVAH group, 26.6%) ($\chi^2=4.278$; $p=0.038$). A statistically significant greater number of pathological AEPs was found in the group of patients with PCS and VAH ($\chi^2 = 4.899$; $p = 0.026$). A statistically significant IWL change accompanied by low amplitude waves in the VAH group has been determined ($\chi^2 = 4.465$; $p = 0.034$). **Conclusion.** The distribution of VAH is not gender- or age-related. The frequency of pathological AEPs findings (presence of associated changes in wave amplitudes and prolonged IWL) is statistically significantly higher in patients with VAH and PCS.

Key words:
brain stem infarctions; electrophysiology; evoked potentials, auditory, brain stem; vertebrobasilar insufficiency.

Apstrakt

Uvod/Cilj. Akustični evocirani potencijali (AEP) predstavljaju elektrofiziološku metodu koja se koristi u dijagnostici patoloških promena moždanog stabla, akustičnog nerva (njegovog perifernog i centralnog dela), kod bolesnika u komi, kod potvrđivanja moždane smrti itd. Odgovor uključuje sedam negativnih talasa koji se generišu u strukturama moždanog stabla vaskularizovanih arterijama zadnjeg sliva. Ipak, u svakodnevnoj praksi se zbog svoje konstantnosti prati prvih pet talasa. Pretpostavlja se da hipoplazija vertebrlne arterije (HVA) utiče na nalaz AEP. Važeća definicija HVA obuhvata kriterijum da je dijametar krvnog suda ≤ 2 mm i da je odnos dijametara leve i desne vertebralne arterije ≥ 1 : 1,7. HVA se sreće u 5,3% slučajeva ukupne populacije i njeno prisustvo povećava rizik od infarkta mozga u zadnjem slivu (*posterior circulation stroke* – PCS). Cilj istraživanja bio je da ukaže na veću učestalost patoloških nalaza AEP kod bolesnika sa HVA i PCS i da pokaže karakteristike AEP u toj grupi bolesnika. **Metode.**

Correspondence to: Radomir Damjanović, University Clinical Center of Niš, Clinic for Neurology, Bulevar dr. Zorana Đinđića 48, 18 000 Niš, Serbia. E-mail: rdamjanovic@gmail.com

Sprovedena je prospektivna studija koja je obuhvatila 163 bolesnika sa PCS, u periodu od dve godine. Metodama kompjuterizovane tomografije (KT) i magnetne rezonance (MR) ustanovljena je dijagnoza PCS. Sumnja na postojanje HVA nakon *Color Doppler* ultrasonografije potvrđivana je KT i MR angiografijom. Svim bolesnicima urađeno je AEP ispitivanje. Praćene su promene talasnih amplituda i intertalasne latence (ITL). **Rezultati.** Nije postojala statistički značajna razlika između polova ($\chi^2 = 1,823$; $p = 0,176$) i godina starosti bolesnika u odnosu na prisustvo HVA ($p = 0,815$). Statistički značajno veći broj bolesnika sa višestrukim PCS imalo je pozitivan nalaz HVA (grupa HVA – 42,3%) u odnosu na bolesnike koji nisu imali HVA (grupa bezHVA – 26,6%) ($\chi^2 = 4,278$; $p = 0,038$). Statistički značajno veći broj patoloških AEP nalaza ustanovljen je kod bolesnika u grupi koja je imala PCS i HVA ($\chi^2 = 4,899$; $p = 0,026$). Utvrđeno je postojanje statistički značajne promene ITL praćene talasima niske amplitude u grupi HVA ($\chi^2 = 4,465$; $p = 0,034$). **Zaključak.** Distribucija HVA ne zavisi od pola ni starosti. Statistički značajno je veća učestalost patološkog AEP nalaza (prisustvo udruženih promena amplituda talasa i produženih ITL) kod bolesnika sa HVA i PCS.

Ključne reči:
moždano stablo, infarkti; elektrofiziologija; evocirani potencijali moždanog stabla, auditorni; vertebrobazilarna insuficijencija.

Introduction

Acoustic (auditory) evoked potentials (AEPs) represent an electrophysiological method used in diagnosing pathological changes of the brain stem (BSt), in addition to numerous other indications (neurodegenerative, demyelinating diseases of the nervous system, tumors in the BSt area, damage to the acoustic pathway, determination of brain death, etc.). The stimulation of the acoustic nerve is done by a specific type of sound signal (alternant click), and responses generated along the auditory pathway from the cochlea to the cortical center responsible for hearing are monitored. Both central and peripheral parts are thus observed because the response to the stimulus includes seven negative waves within 10 ms after the stimulation, with different amplitudes and latencies, but because of their constancy and reproducibility, the first five are used. Numerous studies have shown that wave I of AEPs is generated in the cochlear nerve portion, wave II in the cochlear nuclei portion, and wave III in the *medulla oblongata* area (superior olive and projections to the lateral *lemniscus*, as well as the medial nucleus of the trapezoid body). Wave IV is generated in the *pons* region (lateral *lemniscus*), and wave V is generated in the superior *pons* or inferior *colliculus*. Wave VI originates in the midbrain; wave VII is believed to be a response of auditory radiations and the primary motor auditory cortex. The first five responses are considered clinically important since waves VI and VII are only variably present and often cannot be replicated [1]. Each wave has a specific place of origin, and they are all generated in the structures of the BSt, which are vascularized by the arteries of the posterior circulation (acoustic nerve, acoustic nuclei, *medulla oblongata*, lower and upper *pons*, *mesencephalon*) [2, 3]. The method is, therefore, significant as an additional diagnostic tool in diagnosing vascular lesions of the BSt and localizing the lesion. Its significance comes from the logical assumption that damage to the wave 'generator' region or 'chronic vascularization insufficiency' of the region from which the waves originate leads to changes in morphology and other characteristics of the waves that can be monitored and measured [4, 5]. The AEPs method is applicable in cerebrovascular disorders because the point of wave generation is in the regions vascularized by the vertebral artery (VA) and other arteries of the posterior circulation, even though it has long been used only in diagnosing cerebellopontine angle tumors, demyelinating diseases, comatose patients, and brain death [6–9]. It is highly significant that they change very little under the influence of anesthetics and barbiturates [1].

Posterior circulation stroke (PCS) is a common indication for the application of the AEPs method. Although its diagnosis is primarily clinical and radiological, electrophysiology can also give useful information about lesion location, and it can even show predictive potential in estimating clinical outcomes [10].

Alternating syndromes located in the area of the *medulla oblongata* and *pons*, as well as those in the *mesencephalon*, also cause changes in AEPs. The dominant changes occur in the wave form and the amplitudes of waves III, IV, and V. Amplitude variation is noticed in waves I and V. Pathological findings show changes in interwave latency (IWL) of waves III–V [11–13].

In addition to already familiar risk factors in the development of PCS (age, hypertension, diabetes mellitus, male gender, heart diseases, heart rhythm diseases, etc.), VA hypoplasia (VAH) is also becoming one of the risk factors in PCS [14, 15]. The VA is the first lateral branch of the subclavian artery. In rare cases, it can originate directly from the aortic arch. Considering the path of the VA, four topographic points are described: V1 – prevertebral (*pars prevertebralis)*; V2 – cervical or transverse part (*pars cervicalis*); V3 – the part on the final arch of the atlas (*pars atlantica*); V4 – the part in the posterior cranial cavity or intracranial part (*pars intracranialis*) [16]. The basilar artery (BA) is formed by joining the left and right VA at the height of the lower edge of the cerebral bridge (*pons*). At the same time, the common asymmetry of the VA has been observed in the normal population, with a wider lumen of the left VA in 50% of cases and of the right VA in 25% of cases, while the same diameter of both blood vessels has been found in a quarter of the population [14]. The symptoms of 'vascular insufficiency' in posterior circulation, despite asymmetry in the size of the blood vessels, have not been observed in 75% of the population, whereas it has been shown that people with ischemic changes in VA circulation have a significantly higher percentage of VAH. There is no absolute agreement on the definition of

the VAH. The current definition includes the diameter of the blood vessel at 2 mm or less (literature rarely shows ≤ 3 mm) and the ratio of the diameter of left and right VA ≥ 1 : 1.7 [17]. The results of two separate studies show a greater frequency of VAH on the right side than on the left [18]. Another group of authors describes a greater extent of right-sided VAH (6.2%) than the left-sided one (4.6%) [19]. It is more frequent in patients who have migraines with aura (28%) and patients with vestibular neuronitis (65%) [14]. It is often associated with stenosis and hypoplasia of the BA, which increases the risk of PCS [17]. Furthermore, there can be compensatory enlargement of the diameter of the contralateral blood vessel up to 5 mm. In addition to measuring the diameter of the blood vessel, ultrasound examination can be used to monitor hemodynamic parameters of the VA: reduced blood flow (in the VAH group, it was 81.6 ± 1.5 mL/min, and in the noVAH group, it was 123.2 ± 13.5 mL/min), decreased peak systolic velocity (less than 40 cm/sec), and increased resistance index (RI > 0.75) [14, 17].

The aim of this study was to show a higher frequency of pathological AEPs findings in patients with VAH and PCS and to find out specificities of AEPs findings in patients with both conditions.

Methods

The research was conducted as a prospective study, and it included 163 patients diagnosed with PCS, hospitalized, and treated for a period of two years at our department. The research was approved by the Ethics Committee of the Faculty of Medicine, University of Niš (No. 01-2625-6, from April 8, 2014).

All patients were admitted with symptoms of PCS. A computerized tomography (CT) scan established the diagnosis of PCS. In cases where CT was not sufficiently precise or the lesions in the BSt area were small, magnetic resonance (MR) imaging (MRI) of the brain was done to diagnose PCS. A neurological examination was done on admission and during the patient's stay, and it was scored according to the National Institutes of Health Stroke Scale modified for posterior circulation [12]. Standard risk factors for stroke were monitored (hypertension, hyperlipidemia, diabetes mellitus, atrial fibrillation, previous myocardial infarction, heart disorders, malignancies, hematological diseases, etc.). Neck vessel Color Doppler ultrasonography (CDU) was done for all patients. CDU was performed at the Clinic for Neurology, University Clinical Center of Niš, Serbia using the Esaote MyLab 70 apparatus. A linear probe (4–11 MHz) with a pulse repetition frequency of 1–1.8 kHz was applied. Patients suspected of VAH, besides CT or MRI, also underwent CT angiography (CTA) and MR angiography (MRA), which determined the presence or absence of VAH. CTA and MRA examination of extra- and intracranial arteries was done at the Clinic for Radiology, University Clinical Center of Niš, using MSCT General Electronic Healthcare BrightSpeed (Fairfield, Connecticut, United States) with 2.5 mm cross-section thickness and magnetic resonance Avanto Siemens with a magnetic field strength of 1.5 T and 3 mm cross-section thickness. CTA is a contrast method for which an iodine contrast material, Ultravist, was used. MRA included the noncontrast Time of Flight method. All patients in the tested group underwent the AEPs assessment method. The AEPs test was done at the Electrophysiology Cabinet of the Clinic for Neurology in a room with appropriate sound and electromagnetic isolation. Following a specific preparation for recording, with the aim of allowing the patients to relax in order to avoid artifacts caused by swallowing, blinking, and movement, and after the necessary skin preparation (removal of impurities from the surface), superficial silver disc electrodes were taped on the skin. The active electrodes were on the mastoid processes (A1, A2), the referential electrode was on the vertex (Cz), and the ground electrode was on the forehead. After determining the hearing threshold, a click intensity 70 dB stronger than the threshold was added; one ear was stimulated with it, while the other ear was "masked" with a sound of 30 dB (due to the crossing of the acoustic path at the level of the BSt and the conduction of the sound through the skull). Stimulation was performed with 2,048 stimuli, with a frequency of 5 stimuli per second, a time base of 10 ms and a filter range of 100 Hz (low cut) and 3 kHz (high cut). The duration of the click was 0.1 ms. The total number of 5/sec stimuli was 2,048 (Sapphire, Medelec); of the 10/sec stimuli, it was 2,000 (Nihon Kohden Neuropack M1). The degree of amplitude sensitivity was 0.5 μV. Our devices have a limit of 105 dB, so we worked with a volume of 100 dB for all patients whose hearing threshold was above 35 dB. The patients were divided into two groups, depending on the presence or absence of hypoplasia of the VA (VAH group and noVAH group), and all of them had PCS. A pathological finding of AEPs was considered if a decrease in amplitude by more than 50% and a prolongation of IWL occurred, the normal values of which are determined by the standard values of our laboratory.

Statistical data analysis

Statistical calculations were done using the SPSS program, version 20. Out of basic descriptive statistical parameters, standard statistical methods for qualitative and quantitative analysis of the obtained results were used: absolute numbers, relative numbers (%), arithmetic mean (%), and standard deviation. The Kolmogorov-Smirnov test was used to test the normality of the distribution. Comparing arithmetic means between two samples was performed by a *t*-test, while a nonparametric Mann-Whitney *U* test was used in cases where data did not follow a normal distribution. Statistically significant differences between absolute frequencies were tested by the Chi-square (χ^2) test. A statistical hypothesis was tested at a significance level risk of $\alpha = 0.05$, meaning that a *p*-value < 0.05 was considered statistically significant.

Results

The distribution of patients according to gender in relation to VAH findings is shown in Table 1. There is no statistical significance between males and females in relation to

the VAH finding (χ^2 = 1.823; p = 0.176). Both genders have an equal distribution in both groups of patients (Table 1).

The age groups show homogeneity in both study groups, indicating no statistically significant difference in age structure between VAH and noVAH patients (p = 0.815) (Table 2).

The distribution of infarct localization depends on the presence or absence of VAH. VAH distribution in relation to infarct localization is shown in Table 3. It has been determined that a positive VAH finding was significantly less present in patients with *cerebellum* infarction (VAH 13.0% vs. noVAH 25.5%) (χ^2 = 3.843; p = 0.048) in comparison to all other localizations. A statistically significantly greater number of patients with infarction at multiple locations have a positive VAH finding (VAH 42.3% vs. noVAH 26.6%) (χ^2 = 4.27; p = 0.038). No significant difference was observed between other infarct localizations.

Results show that a statistically significantly greater number of AEPs pathological findings were found in the group of patients with PCS and VAH (χ^2 = 4.899; p = 0.026) (Table 4).

There is a statistically significant IWL change accompanied by low amplitude waves in the VAH group (χ^2 = 4.465; p = 0.034) (Table 5).

Table 1

Gender distribution of patients in relation to vertebral artery hypoplasia (VAH) finding

Gender	VAH	noVAH	*p**
Male	40 (60.9)	50 (53.1)	
Female	29 (39.1)	44 (46.9)	0.176
Total	69 (100.0)	94 (100.0)	

Results are shown as numbers (percentages). *Chi-square test.

Table 2

Age distribution of patients in relation to vertebral artery hypoplasia (VAH) finding

Age (years)	VAH	noVAH	*p**
31–40	2 (2.8)	2 (2.1)	
41–50	4 (5.7)	3 (3.2)	
51–60	12 (17.5)	17 (18.1)	
61–70	21 (30.4)	29 (30.9)	
71–80	18 (26.1)	21 (22.3)	
> 78	12 (17.5)	22 (23.4)	0.815
Total	69 (100.0)	94 (100.0)	

Results are shown as numbers (percentages). *Fisher test.

Table 3

Distribution of vertebral artery hypoplasia (VAH) in relation to infarct localization

Infarct localization	VAH	noVAH	*p**
Medulla oblongata	4 (5.7)	11 (11.7)	0.197
Pons	17 (24.6)	18 (19.2)	0.399
Cerebellum	9 (13.0)	24 (25.5)	0.048
Mesencephalon	6 (8.7)	10 (10.6)	0.680
Occipital lobe	4 (5.7)	6 (6.4)	0.877
Multiple localizations	29 (42.3)	25 (26.6)	0.038
Total	69 (100.0)	94 (100.0)	

Results are shown as numbers (percentages). *Chi-square test.

Table 4

Distribution of acoustic evoked potentials (AEPs) findings in patients with posterior circulation stroke in relation to vertebral artery hypoplasia (VAH) presence

Parameter	AEPs finding		*p**
	normal	pathological	
noVAH	24 (25.5)	70 (74.5)	
VAH	8 (11.6)	61 (88.4)	0.026
Total	32 (19.6)	131 (80.4)	

Results are shown as numbers (percentages). *Chi-square test.

Table 5

Distribution of the type of pathological acoustic evoked potentials wave in patients with posterior circulation stroke in relation to vertebral artery hypoplasia (VAH) presence

Parameter	VAH	noVAH	*p**
Low amplitude waves	33 (54.1)	25 (35.7)	0.034
IWL change	10 (16.4)	15 (21.4)	0.464
IWL + low amplitude waves	18 (29.5)	30 (42.9)	0.113
Total	61 (14.0)	70 (100.0)	

IWL – interwave latency. Results are shown as numbers (percentages). *Chi-square test.

Discussion

Anatomic variations regarding the origin, course, and termination of the VA are numerous [18]. Studies dealing with VAH show left-sided dominance in 50–75% of the population. The right side is dominant in 25% of the population, and the same diameter of both VAs is found in about 25% of the population [20]. The reason for left-sided dominance is probably in the origin and "potency" of the site of origin (in 6% of the population, it originates directly from the arch of the aorta and separates without a brachiocephalic trunk). Since VAH is a congenital vessel abnormality, its frequency rate is not age-related. VAH was first described in the 19th century. It is an uncommon embryonic variation of the posterior circulation. The frequency of VAH has been reported to range from 2–6% in normal, healthy individuals on angiography imaging and autopsies [14]. Some authors report its frequency at 5–10% [20], while others report a percentage of 2.6% [21]. The VAH definition states that the VA diameter is ≤ 2 mm. Some authors have accepted the values of 2.2 mm or 2.5 mm in their studies, as well as the ratio between left and right VA of 1 : 1.7. Besides vessel diameter measurements, there are changes in hemodynamic parameters measured by blood vessel ultrasound: reduced flow volume (in the group VAH, it was 81.6 ± 16.5 mL/min, while in the group no-VAH, it was 123.2 ± 13.5 mL/min), peak systolic velocity decrease below 40 cm/sec, and increase in RI (> 0.75) [14, 22]. The fact that 75% of individuals with VA asymmetry have no signs of posterior circulation insufficiency is indicative of multiple-factor significance: collateral circulation, the Circle of Willis, and compensatory mechanisms of brain circulation for blood redistribution depending on the short- and long-term needs of certain brain parts [23]. Left VA dominance is associated with left hemisphere dominance and the dominance of right-handed people in the world's population, but the data about this is scarce. A group of authors introduced the term "VA dominance" [24], with a greater number of PCS in the territory curvature on the side of the non-dominant VA. The incidence of PCS is significantly higher in the group with VA dominance. A greater number of posterior inferior cerebellar artery infarctions were described on the non-dominant VA side, and a greater number of pontine infarctions were described on the side of dominant VA, explained by the traction of BA branches (*rami pontis*) [25]. For a long time, many authors have pointed out the importance of VAH presence in PCS associated with other risk factors for the onset of brain stroke (BS) [19]. In recent years, it has been proven that VAH is an independent risk factor for PCS onset [26]. Our prospective study enrolled 163 hospitalized patients with the diagnosis of PCS. Out of the total number, 69 (42.33%) patients had VAH, and 94 (57.67%) did not have VAH. The prevalence of gender is not seen in any of the groups. Gaigalaite et al. [27] found VA to be wider in males and VAH more common in females than in males (33% vs. 23.5%). Since VAH is a congenital vessel abnormality, its frequency rate is not age-related. Considering congenital variations of VAH, we expected an approximately equal distribution of PCS in VAH age groups. In relation to age, there is a greater number of patients aged 60 to 70 in both groups, which can be attributed to the presence of associated risk factors for PCS onset. Baran et al. [2] described BS sites in PCS in a group of 227 PCS patients and found an incidence of 10.1% at the *mesencephalon*, *pons* infarct in 62%, and *medulla* infarct in 30.5% of patients. Posterior cerebral artery territory infarction was noted in 26% and multiple site infarcts in 9% of patients. As for etiological factors, large artery atherosclerosis was found in > 50% of patients and cardioembolism in 15–30%, depending on the infarct site, mostly at the *mesencephalon* (26%). Small vessel disease of the posterior inferior cerebellar artery was the cause of BS in 6.5% of patients. Rare causes of stroke (vasculitis disorders, arterial dissection, malignancies) were found in 6.7% of patients. Despite diagnostic examinations, in 7.5% of patients, no cause for stroke could be found. In a study by Lin et al. [26] in patients with BSt infarcts, the incidence of *pons* infarcts was found in 85.4% of patients, 10% had *medulla* infarcts, and 1.5% had *mesencephalon* infarcts. Only 3% of patients had infarcts in other localizations. The etiological factors considered were the same as in the previous study. Considering the vascularization of posterior circulation structures, in cases of VAH, a greater incidence of infarction would be expected in the medullary region and lower *pons* (VA vascularization and posterior inferior cerebellar artery) in comparison to BSt parts vascularized by superior cerebellar artery, anterior cerebellar artery, BA (vascularization of *pons*, *cerebellum*, *mesencephalon*, and occipital lobe), or an equal distribution of ischemia in multiple locations or the simultaneous presence of multiple ischemic changes regarding the chronic inadequate circulation of a hypoplastic vessel. In our group of patients without VAH, the greatest number of patients experiencing a PCS (25.5%) with determined statistical significance was in the group with *cerebellum* localization (influence of other

risk factors for BS onset). A similar number of patients (26.6%) was noted in PCS at multiple localizations. In the group with VAH, a slightly greater number of patients had pontine infarction without statistically significant difference in comparison to the group without VAH, while the greatest number of patients showed the expected statistical significance of having PCS at multiple localizations in the posterior circulation region. The number of other PCS localizations in the VAH group is similar. Infarct localization significantly affects AEP wave morphology. It is explained by the fact that each wave of the AEPs is generated by a specific structure of BSt that is vascularized by the posterior circulation, so each vascular damage and other types of damage to these structures (multiple sclerosis, cerebellopontine angle tumor, chemotherapy in malignancies, ischemia, or compression during neurosurgical interventions) results in alternations of the wave morphology of AEPs [1]. In the studies that followed up AEPs in lacunar infarctions of the BSt, a group of authors found altered AEPs in BA dolichoectasia due to BSt ischemia and compression [4]. Thorwirth et al. [28] detected the absence of wave III in pontine lesions. Drake et al. [29] showed absolute latency elongation of waves in patients with posterior circulation transient ischemic attack (TIA). They detected the absence of wave III in pontine lesions. Earlier studies describe AEPs changes in patients with TIA. In this group of patients, improvements in clinical presentation correlate with the regression of findings. In the case of recurrent persistent TIA over a longer period of time (chronic posterior circulation deficiency), wave morphology changes occur; all of them are dominant but exhibit poor shape and often low amplitudes [11]. In Budd-Chiari syndrome, the authors describe the elongation of IWL I–V [30]. Wang et al. [12] describe the elongation of latencies in waves III and V in patients with PCS. In patients who experienced PCS during stent implantation, elongation of IWL I–V was registered [31]. Yoshikatsu et al. [32] reported pathological AEPs findings in 56% of patients in the group with PCS. Pathological findings were mostly associated with the pontine tegmentum and cerebellar peduncle lesions. Normal AEPs findings showed lesions dominantly in the *medulla oblongata*, pontine base, cerebral peduncles, and *cerebellum* hemispheres. Waves III, IV, and V are not seen in the group with tegmentum lesions of the upper to mid *pons*. There was no difference in findings between the group with *medulla oblongata* lesions and the midbrain lesions, implying that they do not generate AEPs, as the authors hypothesized. In our group of patients, there is a statistically significantly greater number of AEPs pathological findings in patients with VAH and PCS. By following changes in the amplitudes of AEPs waves and changes in the IWL of waves I–III, III–V, and I–V in this group of patients, decreased amplitudes associated with prolonged IWL are statistically significant in comparison to the group with noVAH patients.

Conclusion

In our group of patients with PCS, there is a statistically significantly higher rate of VAH. The distribution of VAH is not gender-related. The incidence of PCS was highest in the group of patients aged 60 to 70 in both groups of patients. Our study revealed a higher frequency of pathological AEPs findings in patients with VAH and PCS. Pathological AEPs findings showed the presence of associated changes in wave amplitudes and prolonged IWL in a statistically significant number of patients with VAH.

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Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Berger JR, Blum AS*. Brainstem Auditory Evoked Potentials. In: *Blum AS, Rutkove SB*, editors. The Clinical Neurophysiology Primer. New Jersey: Humana Press; 2007. pp. 475–84.
2. *Baran G, Gultekin TO, Baran O, Deniz C, Katar S, Yildiz GB,* et al. Association between etiology and lesion site in ischaemic brainstem infarcts: a retrospective observational study. Neuropsychiatr Dis Treat 2018; 14: 757–66.
3. *Thai-Van H, Cozma S, Boutitie F, Disant F, Truy E, Collet L*. The pattern of auditory brainstem response wave V maturation in cochlear-implanted children. Clin Neurophysiol 2007; 118(3): 676–89.
4. *Passero S, Nuti D*. Auditory and vestibular system findings in patients with vertebrobasilar dolichoectasia. Acta Neurol Scand 1996; 93(1): 50–5.
5. *Henry-Le Bras F, Fischer C, Nighoghossian N, Salord F, Trouillas P, Mauguière F*. Early and middle latency auditory evoked potentials in vertebrobasilar strokes. Neurophysiol Clin 1994; 24(6): 399–412. (French)
6. *De Biase S, Gigli GL, Lorenzut S, Bianconi C, Sfreddo P, Rossato G,* et al. The importance of polysomnography in the evaluation of prolonged disorders of consciousness: sleep recordings more adequately correlate than stimulus-related evoked potentials with patients' clinical status. Sleep Med 2014; 15(4): 393–400.
7. *Rogowski M, Michalska BI*. The importance of brain stem evoked potentials in the diagnosis of neurosurgical patients. Neurol Neurochir Pol 2001; 35(4): 667–79. (Polish)
8. *Iakupov EZ, Kuznetsova EA*. Evoked potentials in patients with secondary headaches. Zh Nevrol Psikhiatr Im S S Korsakova 2010; 110(1): 73–7. (Russian)
9. *Jiang ZD, Zhou Y, Yin R, Wilkinson AR*. Amplitude reduction in brainstem auditory response in term infants under neonatal intensive care. Clin Neurophysiol 2013; 124(7): 1470–6.
10. *Živadinovic B, Stamenović J, Ljubisavljevic S*. The comparative analyses of the auditory evoked potentials and color Doppler sonography findings in patients diagnosed with vertebrobasilar insufficiency. Neurol Res 2014; 36(11): 939–44.
11. *Živadinović B, Đurić S, Jolić M, Stamenović J*. Diagnostic importance of auditory brainstem potentials of patients with vertebrobasilar insufficiency. Mak Med Pregl 2004; 58(supp1.61): 55.

12. *Wang H, Zhou H, Guo Y, Wang H*. Value of high-frequency stimulation ABR in the diagnosis and treatment of posterior circulation ischemia. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2012; 26(16): 724–6.
13. *Stone JL, Fino J, Patel K, Calderon-Arnulphi M, Suss N, Hughes JR*. Modified brain stem auditory evoked potentials in patients with intracranial mass lesions. Clin EEG Neurosci 2012; 43(4): 291–302.
14. *Chuang YM, Chan L, Wu HM, Lee SP, Chu YT*. The clinical relevance of vertebral artery hypoplasia. Acta Neurol Taiwan 2012; 21(1): 1–7.
15. *Katsanos A, Kosmidou M, Kyritsis AP, Giannopoulos S*. Is vertebral artery hypoplasia a predisposing factor for posterior circulation cerebral ischemic events? A comprehensive review. Eur Neurol 2013; 70(1–2): 78–83.
16. *Antić S*. Vascularization of the central nervous system. In: *Pavlović S, Stefanović N, Vučetić R, Antić S, Čukuranović R, Arsić S*, editors. Anatomy of the central nervous system and senses. Niš: Sven; 2006. p. 48–157. (Serbian)
17. *Mitsumura H, Miyagawa S, Komatsu T, Hirai T, Kono Y, Iguchi Y*. Relationship between Vertebral Artery Hypoplasia and Posterior Circulation Ischemia. J Stroke Cerebrovascular Dis 2016; 25(2): 266–9.
18. *Iqbal S*. Vertebrobasilar variants and their basic clinical implications. Int J Med Res Health Sci 2013; 2(4): 799–808.
19. *Szárazová AS, Bartles E, Turčáni P*. Vertebral artery hypoplasia and the posterior circulation stroke. Perspect Med 2012; 1(1–12): 198–202.
20. *Vilimas A, Barkauskas E, Vilionskis A, Rudzinskaitë J, Morkûnaitë R*. Vertebral artery hypoplasia: importance for stroke development, the role of posterior communicating artery, possibility for surgical and conservative treatment. Acta medica Lituanica 2003; 10(2): 110–4.
21. *Demarin V, Škarić-Jurić T, Lovrencić-Huzjan A, Puretić MB, Vuković V*. Vertebral artery hypoplasia-sex-specific frequencies in 36 parent-offspring pairs. Coll Antropol 2001; 25(2): 501–9.
22. *Chen YY, Chao AC, Hsu HY, Chung CP, Hu HH*. Vertebral artery hypoplasia is associated with a decrease in net vertebral flow volume. Ultrasound Med Biol 2010; 36(1): 38–43.
23. *Hendrikse J, van Raamt AF, van der Graaf Y, Mali WP, van der Grond J*. Distribution of cerebral blood flow in the circle of Willis. Radiology 2005; 235(1): 184–9.
24. *Zhu W, Wang YF, Dong XF, Feng HX, Zhao HQ, Liu CF*. Study on the correlation of vertebral artery dominance, basilar artery curvature and posterior circulation infarction. Acta Neurol Belg 2016; 116(3): 287–93.
25. *Hong JM, Chung CS, Bang OY, Yong SW, Joo IS, Huh K*. Vertebral artery dominance contributes to basilar artery curvature and peri-vertebrobasilar junctional infarcts. J Neurol Neurosurg Psychiatry 2009; 80(10): 1087–92.
26. *Lin Y, Zhang L, Bao J, Zhang B, Li H, Chen S,* et al. Risk factors and etiological subtype analysis of brainstem infarctions. J Neurol Sci 2014; 338(1–2): 118–21.
27. *Gaigalaite V, Vilimas A, Ozeraitiene V, Dementaviciene J, Janilionis R, Kalibatiene D,* et al. Association between vertebral artery hypoplasia and posterior circulation stroke. BMC Neurol 2016; 16: 118.
28. *Thorwirth V, Volles E, Lossi C, Grunwald F*. Auditory evoked brain stem potentials, visual pattern evoked and somatosensory evoked potentials in transient ischemic attacks (TIA). Schweiz Arch Neurol Neurochir Psychiatr 1983; 132(1): 41–54. (German)
29. *Drake ME Jr, Pakalnis A, Padamadan H, Hietter SA*. Auditory evoked potentials in vertebrobasilar transient ischemic attacks. Clin Electroencephalogr 1990; 21(2): 96–100
30. *Moncho D, Poca MA, Minoves T, Ferré A, Rahnama K, Sahuquillo J*. Brainstem auditory evoked potentials and somatosensory evoked potentials in Chiari malformation. Rev Neurol 2013; 56(12): 623–34. (Spanish)
31. *Pandey P, Kansara A, Thirumala P, Tamkus AA, Xavier AR*. Neurophysiological monitoring with brainstem evoked potentials can be a valuable tool for patients undergoing vertebrobasilar stenting and angioplasty-initial experience. J Clin Neurophysiol 2013; 30(1): 55–8.
32. *Yoshikatsu S, Masahiro S, Makoto H, Syuichi M*. Topographical Relationships Between the Brainstem Auditory and Somatosensory Evoked Potentials and the Location of Lesions in Posterior Fossa Stroke. Neurol Med Chir 2003; 43(6): 282–92.

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CASE REPORT
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Atraumatic rupture of the spleen caused by *Plasmodium falciparum*, challenge in UN peacekeeping operations – a case report

Atraumatska ruptura slezine prouzrokovana *Plasmodium falciparum*-om, izazov u mirovnim operacijama UN

Saša Micković*, Goran Milojković†, Jugoslav Stojiljković†, Nemanja Borovčanin‡§, Sanja Jović†, Saša Kovačević†

Military Medical Academy, *Clinic for Vascular and Endovascular Surgery, ‡Institute of Transfusiology and Hemobiology, Belgrade, Serbia; †Military Hospital Niš, Niš, Serbia; §University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Introduction. Unlike traumatic rupture of the spleen, which is the most common consequence of blunt abdominal trauma and is well documented in the literature, and unlike the atraumatic rupture of the spleen, which is less common but also well described, spontaneous rupture of the spleen in a patient with malaria is a rare complication, with a potentially fatal outcome. **Case report.** A soldier aged 46 years, was hospitalized, with the clinical picture of abdominal colic and diarrhea, previously treated for a primary attack of acute malaria. During hospitalization, deterioration of health condition occurred, with the development of a clinical picture of an acute abdomen due to spontaneous rupture of the spleen which was successfully resolved by splenectomy. **Conclusion.** Splenectomy is the method of choice for patients with unstable clinical presentation due to atraumatic rupture of the spleen.

Key words:
central african republic; malaria; splenectomy; splenic rupture; united nations.

Apstrakt

Uvod. Za razliku od traumatske rupture slezine, koja je najčešća posledica tupe traume abdomena i dobro je dokumentovana u literaturi, i za razliku od atraumatske rupture slezine, koja je ređa, ali takođe dobro opisana, spontana ruptura slezine kod bolesnika sa malarijom je retka komplikacija, sa potencijalno fatalnim ishodom. **Prikaz bolesnika.** Vojnik star 46 godina, prethodno lečen od primarnog napada akutne malarije, hospitalizovan je zbog kliničke slike trbušne kolike i dijareje. Tokom hospitalizacije došlo je do pogoršanja zdravstvenog stanja sa razvojem kliničke slike akutnog abdomena usled spontane rupture slezine koja je uspešno rešena splenektomijom. **Zaključak.** Splenektomija je metoda izbora kod bolesnika sa nestabilnom kliničkom slikom usled atraumatske rupture slezine.

Ključne reči:
centralna afrička republika; malarija; splenektomija; slezina, ruptura; ujedinjene nacije.

Introduction

During the six-month rotation of the Serbian Armed Forces (SAF) contingent in peacekeeping operations – Multidimensional Stabilization Mission in the Central African Republic (MINUSCA), Bangui, Central African Republic (CAR), in the Level 2 hospital, a total of 1,002 patients were tested for suspected malaria, mainly repeated or recurrent infections. Malaria was confirmed in 375 patients, with 98% of cases caused by *Plasmodium (P.) falciparum*, and they were treated according to the protocol.

The SAF started the engagement in the CAR in the United Nations (UN) mission MINUSCA on September 20, 2014. This mission had the most numerous engagement of the SAF after the UN mission in Lebanon. Since 2020, the Serbian Level 2 military hospital of the UN mission in CAR has reached full autonomy and self-sufficiency, which means that all equipment and medical staff in the hospital were secured by the Republic of Serbia. The mission consisted of 72 members of the SAF, who provided the first and second lines of health care, emergency resuscitation/stabilization, emergency operations, limb and life salvage operations, basic

Correspondence to: Saša Micković, Military Medical Academy, Clinic for Vascular and Endovascular Surgery, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: mc.micko@gmail.com

dental care, and the evacuation of the injured to the next level of medical care.

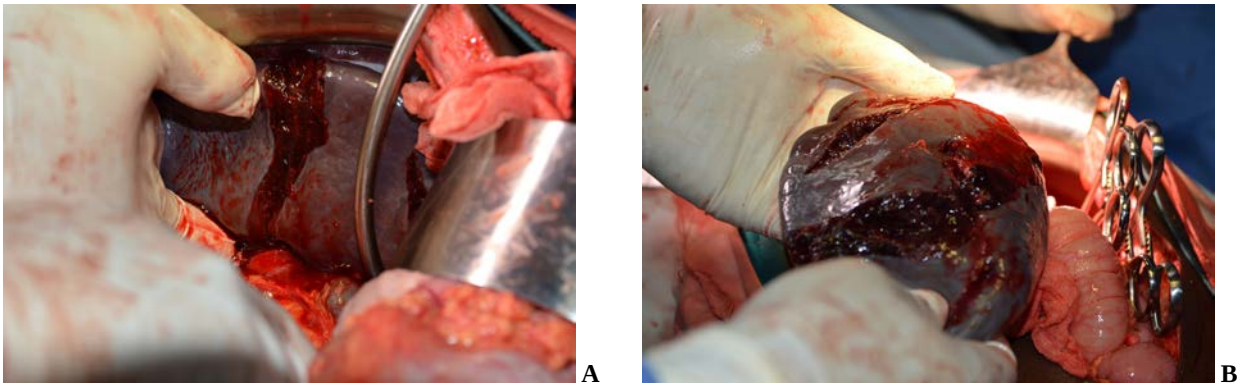
According to a World Health Organization report [1], the African region takes the first place in the world in the number of malaria cases and deaths, with 93% of all global cases of malaria, while 15 countries in sub-Saharan Africa have 80% of all global malaria cases. In 2016, a total of 91 countries reported 216 million cases of malaria, with an annual global mortality of 450,000 cases. Children under five years of age are the most vulnerable group, and there were 67% (272,000) of deaths from malaria in 2018 in this group. The resistance of malaria parasites to antimalarial medications is growing, making malaria one of the deadliest diseases. Abdominal pain, fever, hypotension, and particularly an enlarged spleen are the classic symptoms that accompany infection with malaria, and splenomegaly is practically a cardinal sign.

The incidence of spontaneous splenic rupture in the literature is 0.1–0.5%, and the pathological one is the most common, while a rupture in the absence of any agent is known as idiopathic spontaneous rupture [2].

There is no published data in the available literature on treated members of UN mission areas who suffered from malaria accompanied by complications such as pathological atraumatic spontaneous rupture (ASR) of the spleen. The aim of this paper was to present one potential fatal complication of splenic rupture that occurred during the first attack of *P. falciparum* infection in a member of a UN peacekeeping mission, which was resolved by splenectomy due to the clinical instability of the patient with hemorrhagic shock.

Case report

A 46-year-old soldier, a member of the contingent of the Police Force of Mauritania, was admitted to the Serbian Level 2 military hospital due to prolonged vomiting, diarrhea, and occasional abdominal pain. Two days before the hospitalization, due to a positive malaria rapid diagnostic test for *P. falciparum* and elevated body temperature, an outpatient doctor in the patient's home contingent administered him an antimalarial treatment, artemether ampoules 80 mg/24 h with antibiotic treatment, amoxicillin 500 mg/8 h, to which the patient reacted favorably. As a consequence, his condition improved partially, but the symptoms continued. On admission, the patient was conscious, afebrile, and spontaneously mobile. On physical examination, it was found that he had mild epigastric pain, and his spleen was enlarged by a length of three fingers below the left rib cage. His trauma history was negative. There was a history of multiple malaria attacks in the endemic area from which he came, the suburban area of the capital city, Nouakchott, Mauritania. His complete blood count and biochemical values were in the normal range. During the hospitalization, he vomited twice and had frequent stools with previous constipation; substitution rehydration therapy was administered. During the second day of hospitalization, intensive condition deterioration occurred, accompanied by severe abdominal pain with a drop in blood pressure and tachycardia. Urgent laboratory test showed a blood count deterioration with the following values: hemoglobin 76 g/L [reference range (RR) 115.0–165.0 g/L], red blood cell count 2.52×10^9/mL (RR $3.80–5.80 \times 10^9$/mL), hematocrit 22.9% (RR 37–47%). After repeated tests, the values were as follows: hemoglobin 65 g/L, red blood cell count 2.13×10^9/mL, hematocrit 19.1%. Due to a technical failure of an ultrasonic device, a diagnostic abdominal paracentesis was performed, which was positive for the presence of blood. A midline laparotomy was urgently performed, and intraoperatively, an enlarged spleen, transverse lacerations on both surfaces, and a rupture of the splenorenal ligament, accompanied by active hemorrhage, were found (Figure 1). A large amount of blood was in the abdomen (2,000 mL). A splenectomy was performed, and the abdomen was dried, drained, and closed. During the operation, the patient received a transfusion of three whole blood units (660 mL). On the first postoperative day, another unit of blood was administered. A control ultrasound of the abdomen on the sixth postoperative day did not detect the presence of any collection in the abdomen. A rapid diagnostic test for malaria was performed, and the smear was negative. Due to high levels of platelets in the postoperative period during hospitalization, a therapy with low-molecular-weight heparin Lovenox® 4,000 UI anti-Xa/0.4 mL s.c./12 h, Aspirin® tablets 75 mg up to 1 × 1 daily was administered. In addition, due to a high leukocyte count, an antibiotic therapy with ceftriaxone 2.0 g/12 h, vancomycin 1 g/12 h, and fluconazole tbl. 50 mg/24 h was applied. The further postoperative course was significantly improved. A histopathological specimen analysis was not performed due to the impossibility of sending the same, that is, the non-existence of a medical facility that would perform it in the capital city of CAR, Bangui. The



A **B**

Fig. 1 (A and B) – Transverse lacerations on both surfaces of the spleen.

patient was detained until complete recovery and departure to his country on the eleventh postoperative day. Because of the nature of the patient's medical condition, according to UN policy, Chapter 5, Annex A, Medical Support Manual 2015, the patient was not medically fit to stay on the mission. The patient was repatriated on the medical grounds. The patient was unable to continue his therapy in Bangui, CAR, for technical reasons, i.e., because of vaccine deficiency. Therefore, he was advised to continue with antibiotics and receive some vaccines (Pneumococcal, *Haemophilus influenzae*, and Meningococcal vaccine, in case of non-updated insight into the vaccination card) as an integral part of patient protocols after splenectomy, as quickly as possible after arriving to his home country [3].

Discussion

Malaria is the most common infectious disease in the CAR and originates from the *Plasmodium* family with its five species: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, while *Falciparum monospecies* is predominant [4]. At the same time, malaria is the number one killer in the CAR and the leading cause of death for children under five years of age [1].

In the population living in endemic areas, multiple malaria attacks result in the gradual enlargement of the spleen, contributing to a low probability of rupture, while it is more frequent in non-immunized people [5]. In 1958, Orloff and Peskin [6] proposed four criteria for defining a true spontaneous rupture of the spleen, which had to be met. The four criteria are the following: the absence of trauma, the absence of any disease that affects the spleen directly or indirectly, the absence of perisplenic adhesions, and the normal microscopic and macroscopic appearance of the spleen. In 1874, Atkinson [7] first described idiopathic spontaneous rupture of the spleen.

A large number of systematic papers describe only approximately the etiological cause of ASR of the spleen. ASR is an entity that is not precisely defined. In addition, there are numerous unknowns in the literature, particularly the characteristics of patients, incidence, and etiology, and there is no clear guide and management. The terms are often confusing, unclear, ambiguous, and even contradictory: spontaneous, idiopathic, atraumatic, occult, pathological, etc. The most common term is spontaneous rupture. The most extensive, comprehensive analysis of available papers published so far in the literature was systematized and analyzed by Renzulli et al. [8]. In a systematic review of 845 patients, with a total of 632 publications, the unknowns regarding ASR, excluding iatrogenic and traumatic ruptures of the spleen, were clearly defined, including ruptures of a vascular nature, such as thrombosis, or aneurysms of the splenic vein or artery. To clarify the nomenclature, the same authors propose the classification of splenic rupture into atraumatic idiopathic without etiological causes and traumatic pathological rupture of an already altered spleen that can arise spontaneously without trigger factors or with a minimal trigger (sneezing, coughing, vomiting, straining during defecation, and other muscular efforts), wherein the first one is present in 7% of patients and the second one in 93%. According to aetiological factors, ASRs are classified into six large aetiological groups (neoplastic, infectious, inflammatory – non-infectious, of a genetic origin, caused by medications and treatments, mechanical causes) and subdivided into 18 minor groups, stating that 93% of ASR patients had pathohistological changes in the spleen. As such, they should be classified as atraumatic pathological spontaneous rupture of the spleen. The knowledge of etiology is crucial for making decisions on the type of treatment, and splenomegaly is the common denominator in 55% of all ASR cases. The same study reports an overall mortality rate in ASR of 12.2%, which is related to malignant diseases and age over 40 years [8].

The average weight of a normal spleen is 156 (± 87) g in men and 140 (± 78) g in women, while the spleen size measured ultrasonographically is smaller than 110 × 70 × 50 mm [9]. In contrast to a normal spleen, the spleen is palpable in 50–80% of the cases in endemic areas with intense transmission of parasites, which is correlated with the immune response, the genetic predisposition, and the level of antibodies, while the enlarged spleen is practically present in 80% of patients with acute rupture [10].

A change in the spleen structure during malaria most frequently results in an asymptomatic enlargement of the spleen, i.e., splenomegaly, which acts by combining all three mechanisms and may lead to complications, the occurrence of a subcapsular hematoma and spontaneous rupture. In addition, we rarely find a detailed pathohistological substrate of a removed spleen in the literature. Therefore, Machado Siqueira et al. [11], in their representation of splenic rupture caused by a non-treated *P. vivax* infection provide a detailed description of a microscopic analysis with an expansion of the white pulp and hypercellularity in the red pulp with an intensive plasmablastic proliferation in subcapsular and perivascular compartments, and a large number of intact infected reticulocytes. Newly created secondary lymphoid follicles were also present. The histological feature resembles a B cell lymphoma and can be excluded with the polymerase chain reaction-PCR method.

A very extensive Vietnamese study [12], conducted in patients who died from complications of *P. falciparum* malaria, found loss of B cells from the marginal zone, as well as a significant change in the structure, in the form of an architectural reorganization of the spleen.

The mechanism of the occurrence of atraumatic spontaneous pathological rupture

One of the roles of the spleen as a complex organ, apart from the immunological sense, is selective blood filtration, the removal of red blood cells whose lifespan has passed, as well as the removal of various micro-organisms and red blood cells infected with *Plasmodium* parasites. Multiple malaria attacks by the endemic strain of *P. vivax* in the surroundings of the capital city, Nouakchott, the city of origin of the described case, caused an asymptomatic enlargement of the spleen. Such remodeling resulted in changes in the

structure of the spleen, such as a hematoma, focal necrosis, and thinning of the capsule. A new malaria attack by another species, in this case, *P. falciparum*, led to a critical increase, so a small trigger factor caused a rupture.

The clinical presentation of ASR patients is accompanied by local and systemic signs: the local presentation is a result of peritoneal irritation, while the systemic presentation is a result of hemorrhage. Therefore, its manifestations are, most commonly, hypotension, tachycardia, a decreased heart rate, oliguria, and, of course, a fever as a result of the infection itself. An early diagnosis is crucial. Moreover, the use of abdominal ultrasound is sufficient in evaluating the presence of blood through abdominal imaging windows and making decisions on surgical or non-surgical management [13]. A definite diagnosis of malaria infection is made after analyzing a peripheral blood smear and a thick blood smear. It is an integral part of a precise diagnosis and successful treatment.

Looking back through history, mortality from splenic rupture without surgery and therapy was almost 100%, which was described by Hershey and Lubitz [14] in their paper as early as 1948, compared to 12% after splenectomy, wherein a third of patients can be treated with non-surgical management, which includes exclusively resting in bed for 1–3 weeks and resuscitation with transfusion of blood and blood products if needed. Similar experiences with spontaneous atraumatic pathological rupture of the spleen caused by infection with *Plasmodium* parasites are also published by other authors in their articles as a quite rare but possible complication of individual cases. They applied an aggressive surgical approach – splenectomy, recommending surgical treatment in case of an emergency or as a result of worsening of the general condition in case of failure of conservative treatment [15–17].

Conclusion

Spontaneous splenic rupture caused by malaria is a potentially lethal complication when there is no adequate choice of treatment. The surgical approach is the method of choice for unstable patients with atraumatic spontaneous rupture.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *World Health Organization*. World malaria report 2019 [Internet]. 2019 [accessed on 2024 March 13]. Available from: https://www.who.int/publications/i/item/9789241565721
2. *Ehy A, Harbach L, Bhangu A.* Atraumatic splenic rupture: a 6-year case series. Eur J Emerg Med 2011; 18(2): 124–6.
3. *Jones AD, Khan M, Cheshire J, Bowley D.* Postsplenectomy Prophylaxis: A Persistent Failure to Meet Standard? Open Forum Infect Dis 2016; 3(4): ofw197.
4. *Nzoumbou-Boko R, Yambiyo BM, Ngoagouni C, Vickos U, Manirakiza A, Nakouné E.* Falciparum Malaria in Febrile Patients at Sentinel Sites for Influenza Surveillance in the Central African Republic from 2015 to 2018. Interdiscip Perspect Infect Dis 2020; 2020: 3938541.
5. *Jiménez BC, Navarro M, Huerga H, López-Vélez R.* Spontaneous splenic rupture due to Plasmodium vivax in a traveler: case report and review. J Travel Med 2007; 14(3): 188–91.
6. *Orloff MJ, Peskin GW.* Spontaneous rupture of the normal spleen, a surgical enigma. Int Abstr Surg 1958; 106(1): 1–11.
7. *Atkinson E.* Death from idiopathic rupture of spleen. Br Med J 1874; 2: 403–4.
8. *Renzulli P, Hostettler A, Schoepfer AM, Gloor B, Candinas D.* Systematic review of atraumatic splenic rupture. Br J Surg 2009; 96(10):1114–21.
9. *Frank K, Linhart P, Kortsik C, Wohlenberg H.* Sonographic determination of spleen size: normal dimensions in adults with a healthy spleen. Ultraschall Med 1986; 7(3): 134–7. (German)
10. *Neva FA, Sheagren JN, Shulman NR, Canfield CJ.* Malaria: host-defense mechanisms and complications. Ann Intern Med 1970; 73(2): 295–306.
11. *Machado Siqueira A, Lopes Magalhães BM, Cardoso Melo G, Ferrer M, Castillo P, Martin-Jaular L,* et al. Spleen rupture in a case of untreated Plasmodium vivax infection. PLoS Negl Trop Dis 2012; 6(12): e1934.
12. *Urban BC, Hien TT, Day NP, Phu NH, Roberts R, Pongponratn E,* et al. Fatal Plasmodium falciparum malaria causes specific patterns of splenic architectural disorganization. Infect Immun 2005; 73(4): 1986–94.
13. *Osman MF, Elkhidir IM, Rogers SO Jr, Williams M.* Non-operative management of malarial splenic rupture: The Khartoum experience and an international review. Int J Surg 2012; 10(9): 410–4.
14. *Hershey FB, Lubitz JM.* Spontaneous rupture of the malarial spleen: case report and analysis of 64 reported cases. Ann Surg 1948; 127(1): 40–57.
15. *Kassam N, Michael S, Hameed K, Ali A, Surani S.* Case Report: Sudden Splenic Rupture in a Plasmodium falciparum-Infected Patient. Int J Gen Med 2020; 13: 595–8.
16. *Weinberg Y, Feldman A, Jakobson DJ, Mishal J.* Spontaneous Pathologic Splenic Rupture in a Patient with Plasmodium falciparum Infection, First Case Reported in Israel. Infect Dis Rep 2020; 12(3): 121–6.
17. *Saad E, Elsamani E, Abdelrahman W.* Spontaneous Splenic Rupture Complicating Severe P. falciparum Infection: A Case Report and Literature Review. Case Rep Infect Dis 2019; 2019: 2781647.

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

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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

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

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Primeri referenci:

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

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

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

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

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.

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