YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

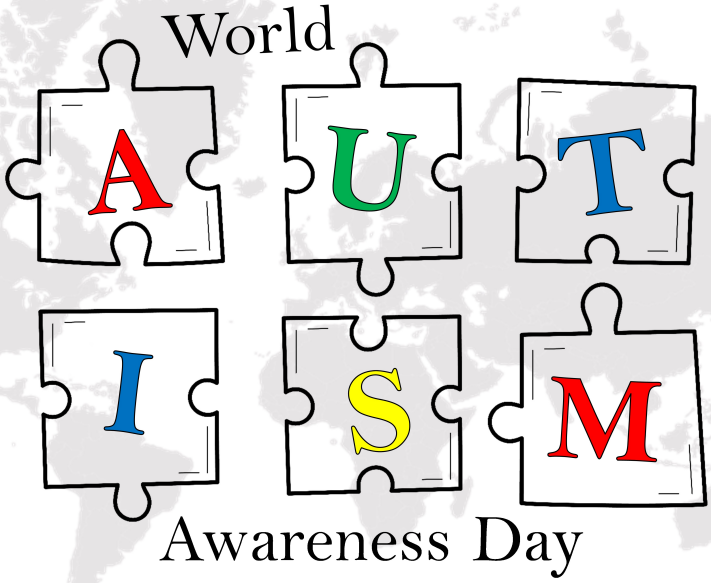
Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2025; April Vol. 82 (No. 4): pp. 195–258.

Vojnosanitetski Pregled 2025 April Vol. 82 (No. 4): pp. 195–258.



"Advancing Neurodiversity and the UN Sustainable Development Goals"

Vol. 82, No. 4, 2025

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of Vojno-sanitetski glasnik which was published between 1930 and 1941

PUBLISHER
Ministry of Defence of the Republic of Serbia, University of Defence, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

Editorial Office: University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia. E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Serbian Citation Index (SCIndex), DOAJ. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

Vol. 82, br. 4, 2025

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine
Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Ministarstvo odbrane Republike Srbije, Univerzitet odbrane, Beograd, Srbija

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

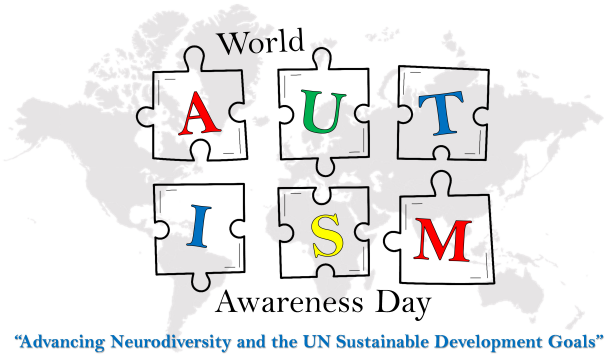
Adresa redakcije: Univerzitet odbrane, Medicinski fakultet Vojnomedicinske akademije, Centar za medicinske naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija. Informacije o pretplati (tel.): +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu" indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Srpski citatni indeks (SCIndeks), DOAJ. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ



Autism Spectrum Disorder (ASD) is a heterogeneous group of neurodevelopmental disorders usually diagnosed in childhood or early adulthood. These disorders are characterized by the presence of disabling impairments in social communication and interactions, along with restricted, repetitive behaviors, insistence on immutability, and unusual sensory sensitivities. The complex etiology of ASD includes numerous risk factors (genetic, neurobiological, environmental, sociocultural), and it has not been fully explored. The global prevalence of ASD has increased fourfold over the last three decades. Since the General Assembly of the United Nations (UN) declared April 2 as World Autism Awareness Day (WAAD) in 2007, the UN has been working on supporting people with autism to achieve their maximum potential in accordance with their health condition. The theme of this year's WAAD, "Advancing Neurodiversity and the UN Sustainable Development Goals", emphasizes the importance of practical solutions that improve the lives of autistic people and encourage their integration into society.

Poremećaji iz spektra autizma (PSA) su heterogena grupa neuroloških razvojnih poremećaja koji se obično dijagnostikuju u detinjstvu ili ranoj mladosti. Ove poremećaje karakteriše prisustvo onesposobljavajućih smetnji u socijalnoj komunikaciji i interakcijama, zajedno sa ograničenim, repetitivnim ponašanjima, insistiranjem na nepromenljivosti i neuobičajenim senzornim osetljivostima. Kompleksna etiologija PSA uključuje mnogobrojne faktore rizika (genetske, neurobiološke, ekološke, sociokulturne) i nije do kraja istražena. Globalna prevalencija PSA četvorostruko je porasla tokom poslednje tri decenije. Od kada je Generalna skupština Ujedinjenih nacija (UN) 2007. godine proglasila 2. april za Svetski dan svesti o autizmu (SDSA), UN rade na davanju podrške obolelima od autizma da u skladu sa njihovim zdravstvenim stanjem ostvare maksimalni potencijal. Tema ovogodišnjeg SDSA, „Napredovanje neurodiverziteta i ciljevi održivog razvoja UN", naglašava značaj praktičnih rešenja koja poboljšavaju život autističnih osoba i podstiču njihovu integraciju u društvo.

ORIGINAL ARTICLES

UDC: 616-002.2:616.1]:616.379-008.64
DOI: https://doi.org/10.2298/VSP241003015M

The association of IL-1β, IL-1α, IL-6, and E-selectin with the diastolic dysfunction in patients with type 2 diabetes mellitus and preserved ejection fraction

Povezanost IL-1β, IL-1α, IL-6 i E-selektina sa dijastolnom disfunkcijom kod obolelih od dijabetesa melitusa tipa 2 sa očuvanom ejekcionom frakcijom

Dejan M. Marinković*, Tamara Dragović*†, Predrag Djurić†‡, Jelena Rakočević§, Dragana Malović*, Saša Kiković*, Ivan Stanojević†‖, Bratislav Dejanović ¶, Petar Ristić*†, Zoran Hajduković†

Military Medical Academy, *Clinic for Endocrinology, ‡Clinic for Cardiology, ‖Institute for Medical Research, ¶Institute for Medical Biochemistry, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; §University of Belgrade, Faculty of Medicine, Institute for Histology and Embryology "Aleksandar Đ. Kostić", Belgrade, Serbia

Abstract

Background/Aim. The importance of chronic inflammation, endothelial dysfunction, certain cytokines, and selectins in the development of type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVDs) is increasingly evident and supported by evidence. However, the role of chronic inflammation in the development of diastolic dysfunction (DD) in the early stages of cardiomyopathy in T2DM patients is insufficiently studied. The aim of this study was to examine the possible association of interleukin (IL)-1β, IL-1α, IL-6, and E-selectin with DD in T2DM patients with still preserved ejection fraction (EF). **Methods.** The research included a total of 74 subjects divided into two groups: a group with proven T2DM, i.e., diabetes group (DG) (n = 45), and a healthy control group (HCG) (n = 29). Echocardiographic parameters of DD and serum levels of IL-1β, IL-1α, IL-6 and E-selectin were compared between the two groups, and the correlation of echocardiographic parameters of DD and serum biomarkers was examined in both groups. **Results.** Subjects with T2DM had significantly different values of DD parameters compared to HCG but also higher values of IL-6 (19 pg/mL vs. 12 pg/mL, $p = 0.002$), E-selectin (2,036 pg/mL vs. 1,522 pg/mL, $p < 0.001$), and IL-1α (46 pg/mL vs. 37 pg/mL, $p = 0.003$). The majority of subjects who met the echocardiographic criteria of DD were from DG. In subjects with proven DD, significantly higher values of IL-6 (20.5 pg/mL vs. 16 pg/mL, $p = 0.003$) and IL-1β (15.0 pg/mL vs. 11.4 pg/mL, $p = 0.036$) were verified compared to subjects without DD. **Conclusion.** The results of our study indicate the presence of a connection between chronic inflammation and echocardiographic parameters with the onset of DD in the phases of preserved cardiac EF in patients with T2DM.

Key words:
biomarkers; cardiomyopathies; cardiovascular diseases; cytokines; diabetes mellitus, type 2; echocardiography; heart failure.

Apstrakt

Uvod/Cilj. Značaj hronične inflamacije, endotelne disfunkcije, određenih citokina i selektina u razvoju dijabetesa melitusa tipa 2 (DMT2) i kardiovaskularnih bolesti (KVB) je sve očigledniji i potkrepljeniji dokazima. Međutim, uloga hronične inflamacije u razvoju dijastolne disfunkcije (DD) u ranim fazama kardiomiopatije kod obolelih od DMT2 je nedovoljno proučena. Cilj rada bio je da se ispita moguća povezanost interleukina (IL)-1β, IL-1α, IL-6 i E-selektina sa DD kod obolelih od DMT2 kod kojih je ejekciona frakcija (EF) srca još uvek očuvana. **Metode.** Istraživanjem je obuhvaćeno ukupno 74 ispitanika podeljenih u dve grupe: grupu sa dokazanim DMT2, odnosno dijabetes grupu (DG) (n = 45), i kontrolnu grupu (KG) (n = 29) zdravih ispitanika. Upoređivani su ehokardiografski parametri DD i nivoi IL-1β, IL-1α, IL-6 i E-selektina u serumu između dve grupe i ispitana je korelacija ehokardiografskih parametara DD i serumskih biomarkera u obe grupe. **Rezultati.** Kod ispitanika obolelih od DMT2 utvrđene su statistički značajno različite vrednosti parametara DD u poređenju sa KG, ali i više vrednosti IL-6

Correspondence to: Dejan M. Marinković, Military Medical Academy, Clinic for Endocrinology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: dejmarinkovic@gmail.com

(19 pg/mL vs. 12 pg/mL, $p = 0{,}002$), E-selektina (2 036 pg/mL vs. 1 522 pg/mL, $p < 0{,}001$) i IL-1α (46 pg/mL vs. 37 pg/mL, $p = 0{,}003$). Većina ispitanika koji su ispunjavali ehokardiografske kriterijume DD bila je iz DG. Kod ispitanika sa dokazanom DD utvrđene su statistički značajno više vrednosti IL-6 (20,5 pg/mL vs. 16 pg/mL, $p = 0{,}003$) i IL-1β (15,0 pg/mL vs. 11,4 pg/mL, $p = 0.036$) u odnosu na ispitanike bez DD. **Zaključak.** Rezultati našeg istraživanja ukazuju da postoji povezanost hronične upale i ehokardiografskih parametara sa nastankom DD u fazama očuvane srčane EF kod obolelih od DMT2.

Ključne reči:
biomarkeri; kardiomiopatije; kardiovaskularne bolesti; citokini; dijabetes melitus, tip 2; ehokardiografija; srce, insuficijencija.

Introduction

Type 2 diabetes mellitus (T2DM) and numerous cardiovascular diseases (CVDs) majorly contribute to the total morbidity, mortality, the number of hospitalizations, and overall medical costs in healthcare systems worldwide [1]. CVDs are one of the primary causes of death in patients with T2DM [1, 2]. T2DM is a major risk factor for the development of atherosclerosis and CVDs, including coronary artery disease and heart failure (HF) [1–3]. CVDs, stroke, and peripheral vascular disease are the main macrovascular complications of T2DM [4]. The mutual relationship and overall significance of T2DM and CVDs give high priority to better understanding their pathophysiology and correlation. Results of previous epidemiological, genetic, preclinical, and clinical studies pointed to the connection of aberrant inflammatory processes to both the progression from prediabetes to diabetes and the onset of late cardiovascular complications [1–8]. There is growing evidence of the involvement of chronic inflammation and certain pro-inflammatory cytokines in the development of HF in patients with T2DM [9, 10]. T2DM may significantly impact the heart, resulting in the clinical presentation of coronary artery disease or diabetic cardiomyopathy (DCM) [11]. Although different, they may progress to the same condition, clinical HF [12]. At the beginning of a typical metabolic but still specific cardiac muscle dysfunction, known as DCM, there is an early yet very long asymptomatic period designated as the subclinical period of the disease [13, 14]. During that initial phase, structural changes occur gradually leading to its remodeling and consequent diastolic dysfunction (DD) [15]. DD is viewed as an impaired left ventricular (LV) relaxation, with increased LV stiffness at its advanced stages and elevated filling pressures at more advanced ones [16]. Possible further deterioration of DD results in a decrease in relaxation and extensibility of the myocardium, which then leads to an increase in chamber filling pressure even with the smallest increase in volume. Finally, as the disease progresses, signs of LV systolic dysfunction develop, as well as first clinical manifestations. Those are a direct consequence of HF with preserved ejection fraction (EF) – HFpEF. If the disease continues to progress, the following conditions develop: HF with moderately reduced EF (HFmrEF), then HF with reduced EF (HFrEF), dilated LV of the heart, shortened ejection period, and increased ventricular filling pressure [17]. Gradually, pulmonary congestion starts manifesting more strongly as well as the development of a typical clinical picture of the final stage of HF [18].

The importance of pro-inflammatory cytokines, chronic inflammation, and endothelial dysfunction in the development of DD in the early stages of cardiomyopathy in patients with T2DM is not nearly as sufficiently studied. The potential correlation between inflammation and DD is of great significance for the early selection of T2DM patients for the echocardiographic assessment. An echocardiographic exam done during that period allows us to gather important information about LV diastolic function in the absence of valvular, ischemic, or uncontrolled hypertensive disease. Early diagnosis of DCM and intensive therapy in the primary asymptomatic stages of the disease is every clinician's imperative.

The aim of this study was to examine and identify the association of interleukin (IL)-1α, IL-1β, IL-6, and E-selectin with the LV DD in patients with T2DM.

Methods

Study population and design

Our study included a total of 74 participants, categorized into two groups: patients with T2DM, already known or newly diagnosed, i.e., diabetes group (DG) (n = 45), and age- and sex-matched healthy control group (HCG) (n = 29). The participants were selected during their regular visits or as a part of the outpatient clinic systematic examination of the Department of Endocrinology of the Military Medical Academy (MMA), Belgrade, Serbia. All participants were over 18 years of age. Patients' demographic and clinical data were gathered through patient interviews, medical records, blood test results, and echocardiographic examination. None of the participants had valvular or ischemic heart disease or uncontrolled hypertension. In inconsistent situations, the diagnosis of T2DM was defined using a two-hour oral glucose tolerance test according to official recommendations [19]. Body mass index (BMI) was calculated as body weight (in kilograms) divided by the square of height (in meters). Systolic blood pressure (BP) > 140 mmHg and diastolic BP > 90 mmHg, treated or not, was considered uncontrolled arterial hypertension. Exclusion criteria were as follows: diagnosed prediabetes, T1DM, ischemic cardiomyopathy, prior myocardial infarction, atrial fibrillation or other severe arrhythmias, LV EF < 50%, existing chronic kidney disease [estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2], acute inflammation, malignant or systemic autoimmune diseases, pregnancy,

people older than 70 years. Serum levels of biochemical parameters and inflammatory cytokines were analyzed from the morning venous blood sample: C-reactive protein (CRP) [reference range (RR): 0.0–4.0 mg/L], fibrinogen (RR: 2.1–4.0 g/L), D-dimer [RR: < 0.50 mg/L fibrinogen equivalent units (FEU)], fasting plasma glucose (FPG) (RR: 4.1–5.9 mmol/L), glycosylated hemoglobin (HbA1c) (RR: < 6.0 %), serum triglyceride (TG) (RR: < 1.7 mmol/L), total cholesterol (TC) (RR: < 5.2 mmol/L), low-density lipoprotein (LDL) (RR: < 3.5 mmol/L), high-density lipoprotein (HDL) (RR: > 1.3 mmol/L), IL-1β, IL-1α, IL-6, and E-selectin.

Echocardiography was performed in all included participants, with obtained values of LV EF, lateral wall diameter (LWD), posterior wall diameter (PWD), interventricular septum diameter (IVSD), LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), and left atrial volume (LAV). We used diagnostic criteria for DD assessment in patients with preserved LV EF mainly based on six parameters: tricuspid regurgitation peak velocity (TRpV), E wave, E/A ratio [the mitral inflow pattern is obtained with the peak of passive filling (E wave) and the peak of active filling (A wave)], septal e′, average E/e′, and left atrial volume index (LAVI). If the patients fulfilled more than two positive criteria (peak E velocity > 50 cm/s with adequate mitral E/A ratio, LAVI > 34 mL/m^2, TRpV > 2.8 m/s, average E/e′ > 14, septal e′ velocity < 7 cm/s), DD was diagnosed. For a more accurate DD assessment, we also measured isovolumic relaxation time (IVRT), a time delay between aortic valve closure and mitral valve opening, and E wave deceleration time (DT). The combination of E/A > 1, enlarged left atrial, IVRT > 100 ms, DT > 200 ms, and abnormal LV intrinsic relaxation (reduced e′) strongly suggests DD [20].

The study was approved by the Ethics Committee of the Faculty of Medicine of the MMA (No. 462-1, from February 02, 2023), and every patient provided a signed consent form.

Data collection

Patient's medical history, anthropometric measurements [waist circumference (WC), body height and weight, BMI], physical examination (BP, heart rate), and blood sampling (after a minimum of 15 minutes of rest) were done at the Department of Endocrinology of MMA. Standard laboratory analyses were measured on the same day at the Institute of Medical Biochemistry, MMA. The cytokine concentrations were measured at the Institute for Medical Research, MMA. A peripheral blood sample was submitted to the Institute for Medical Research MMA immunology laboratory within one hour of sampling, where serum was separated and stored at -70° C until analysis. All collected serum samples were analyzed in the same act. The cytokine concentrations (IL-1α, IL-1β, IL-6, and E-selectin) were measured in the patient's sera using a Premixed Multiplex Kit-Human Custom 10 Plex (N. Orange Grove Ave., Pomona, CA 91767, USA) and by bead-based multiplex immunoassays, performed according to the manufacturer's instructions (flow cytometer Beckman Coulter Navios EX). Detection kits were produced by AimPlex Biosciences, Inc. Echocardiography for the diagnosis of DD was performed at the Clinic for Cardiology, MMA, on a General Electric Vivid 7 PRO Ultrasound System machine.

Statistical analysis

The differences in demographic, clinical characteristics, and laboratory analyses between patients with diabetes and the control group were compared using the Chi-square test for categorical variables, the *t*-test for continuous variables with normal distribution, and the Mann-Whitney *U* test for non-normally distributed variables. Association between variables was tested using Pearson's or Spearman's correlation, where appropriate, according to the normality distribution. Statistical analyses were performed using IBM SPSS Statistics version 25 for Windows (IBM Corporation, Armonk, NY, USA). The level of statistical significance was set at $p < 0.05$.

Results

Basic clinical and biochemical parameters

The average age of the 74 study participants was 50.1 ± 10.5 years, with 60.8% of the study population being male. The mean BMI in the study cohort was 28.5 ± 5.6 kg/m^2. The mean WC was 100.8 ± 18.3 cm. Clinical characteristics of all the included participants were presented within the two given groups, DG and HCG. Patients in DG had significantly higher levels of BMI, WC, systolic BP, diastolic BP, FPG, HbA1c, and serum TG than the HCG patients. There were no significant differences between the two groups for heart rate, TC, HDL, LDL, CRP, fibrinogen, and D-dimer (Table 1).

Cytokines

Median IL-6 levels in DG subjects were significantly higher compared to HCG subjects (19 pg/mL vs. 12 pg/mL, $p = 0.002$), including E-selectin levels (2,036 pg/mL vs. 1,522 pg/mL, $p < 0.001$) and median IL-1α levels (46 pg/mL vs. 37 pg/mL, $p = 0.003$). Comparing serum levels of IL-1β between the groups showed no statistical significance (Table 2).

Echocardiographic parameters

No significant differences in LV EF mean value were shown between the two subject groups. Patients with T2DM had significantly higher LVESD (median: 3.3 mm vs. 3.0 mm, $p = 0.041$), wall thickness (IVSD – median 1.1 cm vs. 0.9 cm, $p < 0.001$; LWD – median: 1.0 cm vs. 0.9 cm, $p < 0.001$; PWD – median: 1.0 cm vs. 0.9 cm, $p < 0.001$), LAV (median: 36 mL vs. 31 mL, $p = 0.004$), A wave (median: 0.77 m/s vs. 0.65 m/s, $p < 0.001$), as well as septal a' (median: 0.10 m/s vs. 0.09 m/s, $p = 0.001$) compared to HCG (Table 3).

Table 1

Baseline subject characteristics of study participants

Parameters	HCG (n = 29)	DG (n = 45)	*p*-value
Body mass index (kg/m^2)	26.5 ± 4.8	29.8 ± 5.8	**0.014**
Waist circumference (cm)	93 (78.6–105.7)	108.5 (96.2–117)	**0.01**
Systolic BP (mmHg)	120 (110–130)	130 (120–140)	**< 0.001**
Diastolic BP (mmHg)	80 (70–85)	85 (80–85)	**0.041**
Heart rate (bpm)	70 (65–85)	75 (65–85)	0.676
C-reactive protein (mg/L)	1.03 (0.40–3.22)	1.13 (0.49–5.10)	0.508
Fibrinogen (g/L)	3.4 ± 0.7	3.7 ± 0.8	0.229
D-dimer (mg/L FEU)	0.31 (0.22–0.41)	0.28 (0.20–0.37)	0.142
Fasting plasma glucose (mmol/L)	4.9 (4.7–5.4)	6.9 (5.9–8.6)	**< 0.001**
HbA1c (%)	5.1 (4.8–5.4)	6.3 (5.7–7.3)	**< 0.001**
Triglyceride (mmol/L)	1.15 (0.81–1.56)	1.78 (1.11–2.62)	**< 0.001**
Total cholesterol (mmol/L)	5.19 ± 0.90	5.32 ± 1.22	0.624
Low-density lipoprotein (mmol/L)	3.02 ± 0.80	3.03 ± 1.02	0.977
High-density lipoprotein (mmol/L)	1.56 ± 0.41	1.40 ± 0.37	0.082

HCG – healthy control group; DG – diabetes group; BP – blood pressure; bpm – beats *per* minute; FEU – fibrinogen equivalent units; HbA1c – glycosylated haemoglobin.
Results are given as mean ± standard deviation or median (interquartile range).
Bold values indicate the significance level of $p < 0.05$.

Table 2

Levels of serum biomarkers in the healthy control group (HCG) and the diabetes group (DG)

Biomarkers	HCG (n = 29)	DG (n = 45)	*p*-value
IL-1ß (pg/mL)	10.3 ± 7.1	13.1 ± 5.5	0.068
IL-6 (pg/mL)	12 (10–18)	19 (14–24)	**0.002**
IL-1α (pg/mL)	37 (16.5–49)	46 (38–57)	**0.003**
E-selectin (pg/mL)	1,522 (1,219–1,970.5)	2,036 (1,766–2,584.5)	**< 0.001**

IL – interleukin. Results are given as mean ± standard deviation or median (interquartile range).
Bold values indicate the significance level of $p < 0.05$.

Table 3

Echocardiographic characteristics of study participants

Parameters	HCG (n = 29)	DG (n = 45)	*p*-value
LVEF (%)	67 (61–70)	64 (61–66)	0.138
LVEDD (mm)	5.0 ± 0.5	5.1 ± 0.4	0.199
LVESD (mm)	3.0 (2.8–3.3)	3.3 (3.1–3.5)	**0.041**
IVSD (cm)	0.9 (0.9–1.1)	1.1 (1.0–1.1)	**< 0.001**
LWD (cm)	0.9 (0.8–1.0)	1.0 (0.9–1.1)	**< 0.001**
PWD (cm)	0.9 (0.8–1.0)	1.0 (0.9–1.1)	**< 0.001**
E wave (m/s)	0.77 (0.59–0.84)	0.70 (0.61–0.81)	0.626
A wave (m/s)	0.65 (0.56–0.66)	0.77 (0.66–0.89)	**< 0.001**
E/A ratio	1.24 (0.92–1.49)	0.84 (0.72–1.15)	**< 0.001**
Septal e′ (m/s)	0.10 (0.07–0.11)	0.07 (0.06–0.09)	**< 0.001**
Septal a′ (m/s)	0.09 (0.08–0.09)	0.10 (0.09–0.11)	**0.001**
E/e′ ratio	8.2 ± 1.4	9.6 ± 1.7	**0.001**
LAVI	16.5 ± 3.0	18.7 ± 3.9	**0.014**
LAV (mL)	31 (27–37)	36 (32–42.25)	**0.004**
DT (m/s)	200.2 ± 47.6	223.8 ± 40.9	**0.030**
IVRT (ms)	91.1 ± 12.9	100.8 ± 15.0	**0.007**
TRpV (m/s)	2.58 ± 0.25	2.62 ± 0.26	**0.024**

HCG – healthy control group; DG – diabetes group; LVEF – left ventricular ejection fraction; LVEDD – left ventricular end-diastolic diameter; LVESD – left ventricular end-systolic diameter; IVSD – interventricular septum diameter; LWD – lateral wall diameter; PWD – posterior wall diameter; LAVI – left atrial volume indexed; LAV – left atrial volumen; DT – deceleration time; IVRT – isovolumic relaxation time; TRpV – tricuspid regurgitation peak velocity.

Results are given as mean ± standard deviation or median (interquartile range).

Bold values indicate the significance level of $p < 0.05$.

Patients with diabetes had significantly higher levels of DD parameters: E/e′ ratio (mean: 9.6 vs. 8.2, $p = 0.001$), LAVI (mean: 18.7 vs. 16.5, $p = 0.014$), IVRT (mean: 100.8 ms vs. 91.1 ms, $p = 0.007$), TRpV (mean: 2.62 m/s vs. 2.58 m/s, $p = 0.024$), and DT (mean 223.8 m/s vs. 200.2 m/s, $p = 0.030$), compared to HCG. However, levels of E/A ratio (median: 0.84 vs. 1.24, $p < 0.001$) and septal e′ (median: 0.07 m/s vs. 0.10 m/s, $p < 0.001$) were significantly lower in patients with diabetes (Table 3).

Correlation between echocardiographic parameters and pro-inflammatory cytokines

There was no significant correlation between analyzed cytokines and diastolic function parameters in HCG. However, some significant correlations were revealed between E-selectin and septal a′ ($p = 0.014$), E-selectin and A wave ($p = 0.041$), and IL-1α and septal a′ ($p = 0.028$) (Table 4).

When talking about the parameters essential for the assessment of DD in DG, there was a statistically significant positive correlation between IL-6 level and E/e′ ratio (r = 0.424, $p = 0.028$), IL-6 and LAVI (r = 0.248, $p = 0.037$), IL-1β and E/e′ ratio (r = 0.265, $p = 0.026$), and between IL-1α and LAVI (r = 0.241, $p = 0.043$). There was also a significant negative correlation between IL-6 level and E/A ratio (r = -0.281, $p = 0.018$), E-selectin and E/A ratio (r = -0.245, $p = 0.040$), and IL-6 and septal e′ (r = -0.301, $p = 0.011$) (Table 4).

There was a statistically significant positive correlation between IL-6 and LVEDD ($p = 0.004$), IL-6 and LVESD ($p < 0.001$), and IL-6 and wall thickness [IVSD ($p = 0.006$), LWD ($p = 0.010$), PWD ($p = 0.007$)]. A significant positive correlation was found between IL-1α and septal a′ ($p = 0.003$) and between IL-1α and wall thickness [LWD ($p = 0.049$), PWD ($p = 0.041$)] as well. Moreover, there is a significant positive correlation between E-selectin and septal a′ ($p < 0.001$) and between E-selectin and wall thickness [IVSD ($p = 0.039$), LWD ($p = 0.013$), PWD ($p = 0.006$)]. There was a significant negative correlation between IL-6 level and LV EF ($p < 0.001$) (Table 4).

In the exploratory analysis, out of 16 patients with echocardiographic signs of DD, 14 were from DG and only 2 were from HCG. Those participants with LVDD [16 (21.6%)] had significantly higher IL-6 (median: 20.5 vs. 16 pg/mL, $p = 0.003$) and IL-1β (mean: 15.0 vs. 11.4 pg/mL, $p = 0.036$) compared to the ones with normal diastolic function [58 (78.4%)] (Table 5). There was no statistically significant difference in E-selectin and IL-1α between DG and HCG.

Table 4

Correlation coefficients between inflammatory markers and echocardiographic parameters between the healthy control group (HCG) and the diabetes group (DG)

Parameters	HCG (n = 29) / DG (n = 45)			
	IL-β	IL-6	IL-1α	E-selectin
LVEF	-0.130/0.218	-0.354/-0.433*	-0.191/-0.183	-0.124/-0.33
LVEDD	-0.104/0.027	0.341/0.355*	0.098/0.130	0.020/0.103
LVESD	-0.011/0.227	0.348/0.453*	0.093/0.196	0.033/0.105
IVSD	-0.137/0.059	0.026/0.324*	0.142/0.232	-0.055/0.245*
LWD	-0.059/0.001	0.226/0.302*	0.303/0.235*	0.054/0.293*
PWD	-0.089/-0.011	0.250/0.318*	0.313/0.243*	0.107/0.322*
E/A ratio	0.211/-0.075	0.103/-0.281*	-0.170/-0.152	-0.158/-0.245*
DT	0.024/0.018	-0.015/0.177	0.190/0.068	-0.093/-0.020
E/e′ ratio	0.146/0.265*	0.031/0.424*	0.071/0.151	-0.141/0.171
LAV	-0.094/0.119	0.022/0.069	0.101/-0.012	0.018/-0.203
IVRT	-0.131/0.065	0.011/0.158	0.168/0.066	0.145/0.131
LAVI	-0.027/0.160	0.170/0.248*	0.225/0.241*	0.110/0.031
Septal e′	0.118/-0.184	0.091/-0.301*	-0.096/-0.115	0.046/-0.100
Septal a′	0.128/0.068	0.167/0.271	0.424*/0.343*	0.468*/0.433*
E wave	0.069/-0.141	-0.040/-0.209	-0.214/0.160	-0.008/0.256
A wave	-0.012/0.084	0.141/0.131	0.206/-0.030	0.382*/0.229
TRpV	0.068/0.100	-0.204/-0.187	-0.138/-0.202	0.040/-0.188

For abbreviations, see Tables 2 and 3.
Units of measurement of all presented parameters are given in Tables 2 and 3. *$p < 0.05$.

Table 5

Levels of serum biomarkers in participants with and without diastolic dysfunction (DD)

Biomarkers	Without DD (n = 58)	With DD (n = 16)	Values
IL-1β (pg/mL)	11.4 ± 6.0	15.0 ± 5.9	t = -2.135, **p = 0.036**
IL-6 (pg/mL)	16 (11–19.5)	20.5 (15.0–33.5)	U = 239.5, **p = 0.003**
IL-1α (pg/mL)	40.9 ± 15.0	47.9 ± 14.4	t = -1.757, p = 0.083
E-selectin (pg/mL)	1,929 (1,507–2,331)	1,766 (1,541.5–2,599.5)	U = 456.5, p = 0.922

IL – interleukin.
Results are given as mean ± standard deviation or median (interquartile range).
Bold values indicate the significance level of $p < 0.05$.

Discussion

The connection between T2DM, chronic inflammation, and cardiomyopathy has been known for many decades. Some pro-inflammatory cytokines, chemokines, selectins, and specific adhesive molecules were exceedingly discussed among scientific and professional circles as likely significant. They were studied as potential diagnostic and prognostic markers or even therapeutic targets. It is a common fact that low-grade chronic inflammation precedes T2DM and that the immune system is connected to every single stage of its development in various ways [21–23]. It is known that chronic hyperglycemia with hyperinsulinism, as a main characteristic of these disorders, is followed by changes in serum levels of different cytokines [5]. Additionally, inflammation is implicated in the process of remodeling myocardium with consequent HF in patients with T2DM [11, 24–31]. The estimated prevalence of DCM in the diabetic population is approximately 16.9% [32]. Nowadays, DCM is defined as a specific cardio-muscular dysfunction in patients with T2DM, characterized by the development of myocardial interstitial fibrosis, cardiomyocyte hypertrophy, and apoptosis, occurring independently of arterial hypertension, coronary artery disease, valvular heart disease, and other structural ones [33]. The subclinical period of the disease is long and asymptomatic, and it is characterized by the presence of LV DD, a precursor of systolic HF in DCM [25–28]. Evaluation of systolic function is usually implied in cardiac mechanics, but DD has proven to be its essential element [34]. Over the past decades, inflammation stood out as one of the principal features in the pathogenesis of systolic heart disease [9, 11, 29]. However, there has not been much data on the role of inflammation in the development of DD.

Our results showed a significant difference among many parameters (anthropometric, biochemical, and echosonographic) and the monitored cytokines and selectin between DG and HCG. Patients with T2DM had higher BMI, WC, BP values, morning glycemia, HbA1c, and TG, which is in accordance with previous research [35–37]. They are considered a manifestation of a poorly led lifestyle and depict not only the consequences of T2DM but also its causes. Moreover, many studies indicate that similar differences in the mentioned parameters were noticed much earlier, even in the prediabetes stage [22, 35, 36, 38]. Our examination also revealed that DG had significantly higher levels of IL-6, IL-1α, and E-selectin than HCG. Such results unequivocally indicate the importance of the pro-inflammatory factor in the development of T2DM and are partially in agreement with earlier research [5, 9, 36, 39]. IL-6 is a pro-inflammatory cytokine produced by numerous cells such as activated leukocytes, macrophages, monocytes, endothelial cells, vascular smooth muscle cells, fibroblasts, and adipocytes [30, 39]. Statistically higher values of IL-6 in DG in our study are in agreement with many known results [40–44]. This cytokine was proved to incite hyperglycemia and compensatory hyperinsulinemia in murine models and humans [40]. Spranger et al. [41] noted that the IL-6 plasma levels are associated with the development of T2DM. In a study published in 2019, the authors suggested a connection between HbA1c and serum levels of IL-6 in patients with T2DM [42]. Tuttolomondo et al. [43] explained the positive correlation between IL-6 and vascular complications of T2DM. Direct connection was found among IL-6, IL-1β, HbA1c, and FPG [44].

In our research, the IL-1α values were significantly higher in DG than HCG ($p = 0.003$), with IL-1β showing a trend towards higher values ($p = 0.068$). Similar results were obtained in previously published data [45–51]. The IL-1 family of cytokines is primarily connected to innate immunity and contains pro-inflammatory components but also has elements that antagonize and regulate inflammation [45, 46]. Innate immunity is manifested by inflammation, which functions as a host defense mechanism but can also be detrimental to survival if uncontrolled. There is also evidence proving the key role of the innate system in the process of destroying pancreatic β-cells and impaired insulin secretion [47, 48]. There is a small number of studies that have dealt with the correlation between IL-1α and T2DM. Physiologically, IL-1α is expressed in epithelial and mesenchymal cells (including cardiomyocytes) and is released upon injury or cell death. When released from necrotic cells, IL-1α triggers a large amount of inflammatory reactions [49]. On the other hand, even though IL-1β is the most researched member of the IL-1 family, the results of the studies that dealt with its connection with T2DM were very inconsistent [45]. A meta-analysis from 2022 showed that patients with T2DM have non-significantly higher values of IL-1β than healthy controls, thus excluding this cytokine as a significant T2DM marker [50]. A recent meta-analysis from 2024 stated a clear connection between elevated levels of IL-6 and IL-1β with T2DM and its complications [51].

There is a lack of evidence confirming that elevated E-selectin values accelerate T2DM. Increased levels of E-selectin have been previously associated with insulin resistance and hyperinsulinemia [52]. Previous studies claimed that E-selectin and other specific adhesion molecules had been connected to microvascular complications of T2DM as markers of endothelial dysfunction [53, 54]. Furthermore, higher E-selectin values in circulation had been interpreted as early markers of the forthcoming microvascular (neuropathic and retinopathic) T2DM complications [36, 55]. Ekelund et al. [56] indicated that the levels of E-selectin may serve as a predictive biomarker for the development of diabetic retinopathy in patients with T2DM.

The prevalence of HF in diabetic patients is very high (~30%) [57]. It is also the main cause of hospitalization and an important predictor of increased mortality [58]. The results of our study, observing only echocardiographic parameters, are all in agreement with the listed epidemiological data [57, 58]. Echocardiographic parameters, such as wall thickness (IVSD, LWD, PWD), LVESD, LAV, A wave, and septal a′, had statistically higher values in DG patients. Usually, the four phases of diastole include isovolumic relaxation, rapid filling (E wave), slow filling (diastasis), and active filling (A wave) [20]. The main pathophysiological consequence of advanced DD is elevated filling pres-

sure [59]. In our study, values of almost every followed DD parameter had a statistically significant difference between the followed groups. TRpV, E/e′ ratio, LAVI, IVRT, and DT were significantly higher in DG, while E/A ratio and septal e′ had much lower levels compared to HCG. Since all patients had preserved LV EF, it is important to underline that the majority of subjects with DD were from DG. However, when interpreting these results, apart from the existence of T2DM, we must take into account the fact that the subjects had higher BMI and WC, higher BP values, and TG.

The results of our study indicate several significant correlations between the analyzed biomarkers and many myocardial indices in DG, which were not detected in the HCG. The correlations between the monitored cytokines and echosonographic parameters of DD strongly point out the important role of inflammation in the process of DCM formation (positive correlations: between IL-6 and LAVI, IL-6 and E/e′ ratio, IL-1β and E/e′ ratio, IL-1α and LAVI; negative correlations: between IL-6 and E/A ratio, IL-6 and septal e′, E-selectin and E/A).

A very strong correlation between the IL-6 values and the DD parameters in DG is not surprising. IL-6 is a significant cytokine with an important role in numerous heart diseases and a spectrum of functions [2]. Some authors pointed out a connection between IL-6 and many myocardial indices [PWD, IVSD, left ventricle mass (LVM), LVM index (LVMI), relative wall thickness (RWT), and LVEDD] [9]. What might have served as a predictive marker for HF were higher IL-6 levels associated with reduced systolic function in apparently healthy individuals [60]. By infusing IL-6 in rats, both DD and clinically consequential HFpEF can be induced [61]. According to the observations, HFpEF patients have a higher circulating IL-6 level compared with the ones with asymptomatic hypertension, and patients with HF have a higher IL-6 level compared with the healthy [62, 63]. Circulating IL-6 levels were connected to the escalating severity of congestive HF [63]. In their study, Toczylowski et al. [64] reported a connection between the high level of IL-6 with LV hypertrophy and systolic dysfunction. In patients with acute HF, higher IL-6 levels were detected at 30 days. Such values were connected to all-cause mortality at 48–72 hrs [65]. Physical exercise has been noticed to reduce IL-6 and improve overall function in patients with HF [66]. The signal transducing receptor subunit glycoprotein 130 (gp130) levels for IL-6 have been connected to total, cardiovascular, and HF-induced mortality [67]. There is a positive correlation between IL-6 and systolic and diastolic functions of the heart, which increase the functional and structural morbidity of the cardiac muscles, according to Zhuravlyova and Sokolnikova [68].

We also detected a positive correlation between IL-1β and E/e' ratio and between IL-1α and LAVI, as well as a negative correlation between E-selectin and E/A in DG patients. IL-1α and IL-1β are primarily pro-inflammatory cytokines and are dominantly involved in the pathophysiology of heart diseases [69, 70]. IL-1β is one of the most studied cytokines as a possible therapeutic target for a number of heart diseases. However, IL-1β levels are in general affected by many factors such as age or BMI, but also many genetic, hormonal, and environmental ones [50]. The results of the study of the IL-1 receptor blocker (anakinra) effect on the heart are significant. A study on animal models found that IL-1 receptor blockade after myocardial infarction prevents deterioration of systolic and diastolic function [71]. In D-HART Pilot Study, a two-week anakinra treatment was used on patients with stable HFpEF and proved systematic inflammation. The treatment resulted in statistically significant reduction in sistematic inflammation and an increase in aerobic exercise tolerance [72]. In the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS), using canakinumab (the anti-IL-1β monoclonal antibody) caused a decrease of 15% in mortality risk [73], as well as a decrease in the percentage of HF-related hospitalization [74].

Conclusion

The results of our study can be summarized by highlighting the following four facts. The values of IL-6, E-selectin, and IL-1α, as well as the echocardiographic parameters of DD, were statistically significantly higher in participants with T2DM. The absolute majority of participants who met the criteria for DD were from diabetes group. A correlation was detected between the monitored cytokine and selectin values and the parameters of DD in diabetes group, as well as the absence of such correlation in the healthy control group. Statistically significantly higher IL-6 and IL-1β levels have been detected in participants with proven DD.

This information suggests a mutual relationship between heart diastolic function disorders and chronic inflammation in individuals with T2DM and normal left ventricular systolic function. There is a clear possibility of using the elevated values of certain cytokines or selectins as non-invasive tests in the early selection of patients with T2DM for echocardiographic examination. Further clinical and experimental research is necessary to fully explain the relationship between DD and inflammation in patients with T2DM, but also the pro- and anti-inflammatory role of cytokines in the earliest stages of the disease and their interaction with metabolic and immune elements.

REFERENCES

1. *Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N,* et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). Eur Heart J 2013; 34(39): 3035–87. Erratum in: Eur Heart J 2014; 35(27): 1824.

2. *Zhang H, Dhalla NS*. The Role of Pro-Inflammatory Cytokines in the Pathogenesis of Cardiovascular Disease. Int J Mol Sci 2024; 25(2): 1082.
3. *American Diabetes Association*. Standards of Medical Care in Diabetes-2020 Abridged for Primary Care Providers. Clin Diabetes 2020; 38(1): 10–38.
4. *Joseph JJ, Deedwania P, Acharya T, Aguilar D, Bhatt DL, Chyun DA,* et al. Comprehensive Management of Cardiovascular Risk Factors for Adults With Type 2 Diabetes: A Scientific Statement From the American Heart Association. Circulation 2022; 145(9): e722–59.
5. *Saxena M, Srivastava N, Banerjee M*. Association of IL6, TNF-α and IL-10 gene polymorphisms with type 2 diabetes mellitus. Mol Biol Rep 2013; 40(11): 6271–9.
6. *Grossmann V, Schmitt VH, Zeller T, Panova-Noeva M, Schulz A, Laubert-Reh D,* et al. Profile of the immune and inflammatory response in individuals with prediabetes and type 2 diabetes. Diabetes Care 2015; 38(7): 1356–64.
7. *Nadeem A, Mumtaz S, Naveed AK, Aslam M, Siddiqui A, Lodhi GM,* et al. Gene-gene, gene-environment, gene-nutrient interactions and single nucleotide polymorphisms of inflammatory cytokines. World J Diabetes 2015; 6(4): 642–7.
8. *Wang M, Li Y, Li S, Lv J*. Endothelial Dysfunction and Diabetic Cardiomyopathy. Front Endocrinol (Lausanne) 2022; 13: 851941.
9. *Ghanem SE, Abdel-Samiee M, Torky MH, Gaafar A, Mohamed SM, Salah Eldin GMM,* et al. Role of resistin, IL-6 and NH2-terminal portion proBNP in the pathogenesis of cardiac disease in type 2 diabetes mellitus. BMJ Open Diabetes Res Care 2020; 8(1): e001206.
10. *Kaur N, Guan Y, Raja R, Ruiz-Velasco A, Liu W*. Mechanisms and Therapeutic Prospects of Diabetic Cardiomyopathy Through the Inflammatory Response. Front Physiol 2021; 12: 694864.
11. *Fathelbab M, Fahmy EM, Elshormilisy AA, Gaafar AE, Waly NE*. A putative role for oxidative stress in pathophysiology of diabetic cardiomyopathy. Egypt J Obes Diabetes Endocrinol 2017; 3(3): 95–9.
12. *Ofstad AP*. Myocardial dysfunction and cardiovascular disease in type 2 diabetes. Scand J Clin Lab Invest 2016; 76(4): 271–81.
13. *Fang ZY, Schull-Meade R, Downey M, Prins J, Marwick TH*. Determinants of subclinical diabetic heart disease. Diabetologia 2005; 48(2): 394–402.
14. *Ceriello A, Catrinoiu D, Chandramouli C, Cosentino F, Dombrowsky AC, Itzhak B,* et al. Heart failure in type 2 diabetes: Current perspectives on screening, diagnosis and management. Cardiovasc Diabetol 2021; 20(1): 218.
15. *Jia G, DeMarco VG, Sowers JR*. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. Nat Rev Endocrinol 2016; 12(3): 144–53.
16. *Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA,* et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr 2009; 22(2): 107–33.
17. *Jia G, Hill MA, Sowers JR*. Diabetic Cardiomyopathy: An Update of Mechanisms Contributing to This Clinical Entity. Circ Res 2018; 122(4): 624–38.
18. *Liu X, Yang ZG, Gao Y, Xie LJ, Jiang L, Hu BY,* et al. Left ventricular subclinical myocardial dysfunction in uncomplicated type 2 diabetes mellitus is associated with impaired myocardial perfusion: a contrast-enhanced cardiovascular magnetic resonance study. Cardiovasc Diabetol 2018; 17(1): 139.
19. *Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V,* et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J 2020; 41(2): 255–323. Erratum in: Eur Heart J 2020; 41(45): 4317.
20. *Kossaify A, Nasr M*. Diastolic dysfunction and the new recommendations for echocardiographic assessment of left ventricular diastolic function: summary of guidelines and novelties in diagnosis and grading. J Diagn Med Sonogr 2019; 35(4): 317–25.
21. *Marinković DM, Dragović T, Stanojević I, Djurić P, Dejanović B, Rakočević J,* et al. Low-grade inflammation and inflammatory mediators in individuals with prediabetes. Vojnosanit Pregl 2024; 81(9): 547–54.
22. *Brahimaj A, Ligthart S, Ghanbari M, Ikram MA, Hofman A, Franco OH,* et al. Novel inflammatory markers for incident prediabetes and type 2 diabetes: the Rotterdam Study. Eur J Epidemiol 2017; 32(3): 217–26.
23. *Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A,* et al. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes 2003; 52(7): 1799–805.
24. *Piccini JP, Klein L, Gheorghiade M, Bonow RO*. New insights into diastolic heart failure: role of diabetes mellitus. Am J Med 2004; 116 Suppl 5A: 64S–75S.
25. *Füth R, Dinh W, Bansemir L, Ziegler G, Bufe A, Wolfertz J,* et al. Newly detected glucose disturbance is associated with a high prevalence of diastolic dysfunction: double risk for the development of heart failure? Acta Diabetol 2009; 46(4): 335–8.
26. *Karwi QG, Ho KL, Pherwani S, Ketema EB, Sun Q, Lopaschuk GD*. Concurrent diabetes and heart failure: Interplay and novel therapeutic approaches. Cardiovasc Res 2022; 118(3): 686–715. Erratum in: Cardiovasc Res 2022; 118(7): 1850.
27. *Abudureyimu M, Luo X, Wang X, Sowers JR, Wang W, Ge J,* et al. Heart failure with preserved ejection fraction (HFpEF) in type 2 diabetes mellitus: From pathophysiology to therapeutics. J Mol Cell Biol 2022; 14(5): mjac028.
28. *Riehle C, Bauersachs J*. Key inflammatory mechanisms underlying heart failure. Herz 2019; 44(2): 96–106.
29. *Chou CH, Hung CS, Liao CW, Wei LH, Chen CW, Shun CT,* et al. IL-6 trans-signalling contributes to aldosterone-induced cardiac fibrosis. Cardiovasc Res 2018; 114(5): 690–702.
30. *Avagimyan A, Fogacci F, Pogosova N, Kakturskiy L, Kogan E, Urazova O,* et al. Diabetic Cardiomyopathy: 2023 Update by the International Multidisciplinary Board of Experts. Curr Probl Cardiol 2024; 49(1 Pt A): 102052.
31. *Vig H, Ravinandan AP, Vishwas HN, Tyagi S, Rathore S, Wal A,* et al. An Insight into the Pathogenesis of Diabetic Cardiomyopathy Along with the Novel Potential Therapeutic Approaches. Curr Diabetes Rev 2024; 20(1): e020523216416.
32. *Dandamudi S, Slusser J, Mahoney DW, Redfield MM, Rodeheffer RJ, Chen HH*. The prevalence of diabetic cardiomyopathy: A population-based study in Olmsted County, Minnesota. J Card Fail 2014; 20(5): 304–9.
33. *Paolillo S, Marsico F, Prastaro M, Renga F, Esposito L, De Martino F,* et al. Diabetic cardiomyopathy: definition, diagnosis, and therapeutic implications. Heart Fail Clin 2019; 15(3): 341–7.
34. *Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Dokainish H, Edvardsen T,* et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2016; 17(12): 1321–60.
35. *Wang Z, Shen XH, Feng WM, Ye G, Qiu W, Li B*. Analysis of Inflammatory Mediators in Prediabetes and Newly Diagnosed Type 2 Diabetes Patients. J Diabetes Res 2016; 2016: 7965317.
36. *Tiftikcioglu BI, Duksal T, Bilgin S, Kose S, Zorlu Y*. Association between the levels of IL-6, sE-selectin and Distal sensory nerve conduction studies in patients with prediabetes. Eur Neurol 2016; 75(3–4): 124–31.

37. *Al-Shukaili A, Al-Ghafri S, Al-Marhoobi S, Al-Abri S, Al-Lawati J, Al-Maskari M.* Analysis of inflammatory mediators in type 2 diabetes patients. Int J Endocrinol 2013; 2013: 976810.
38. *Weaver JR, Odanga JJ, Breathwaite EK, Treadwell ML, Murchinson AC, Walters G,* et al. An increase in inflammation and islet dysfunction is a feature of prediabetes. Diabetes Metab Res Rev 2021; 37(6): e3405.
39. *Ridker PM, Rane M.* Interleukin-6 Signaling and anti-interleukin-6 therapeutics in cardiovascular disease. Circ Res 2021; 128(11): 1728–46.
40. *Tsigos C, Papanicolaou DA, Kyrou I, Defensor R, Mitsiadis CS, Chrousos GP.* Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. J Clin Endocrinol Metab 1997; 82(12): 4167–70.
41. *Spranger J, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, Ristow M,* et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 2003; 52(3): 812–7.
42. *Sari MI, Tala ZZ, Wahyuni DD.* Association between Glycated Hemoglobin with the Levels of Serum Proinflammatory Cytokines and Antioxidants in Patients with Type 2 Diabetes Mellitus in Universitas Sumatera Utara Hospital. Open Access Maced J Med Sci 2019; 7(5): 715–20.
43. *Tuttolomondo A, La Placa S, Di Raimondo D, Bellia C, Caruso A, Lo Sasso B,* et al. Adiponectin, resistin and IL-6 plasma levels in subjects with diabetic foot and possible correlations with clinical variables and cardiovascular co-morbidity. Cardiovasc Diabetol 2010; 9: 50.
44. *Abd El-Hameed AM, Eskandrani AA, Salah Abdel-Reheim E, Abdel Moneim A, Addaleel W.* The amelioration effect of antidiabetic agents on cytokine expression in patients with type 2 diabetes mellitus. Saudi Pharm J 2024; 32(5): 102029.
45. *Dinarello CA.* Overview of the IL-1 Family in innate inflammation and acquired immunity. Immunol Rev 2018; 281(1): 8–27.
46. *Szekely Y, Arbel Y.* A Review of Interleukin-1 in Heart Disease: Where do we stand today? Cardiol Ther 2018; 7(1): 25–44.
47. *Sokolova M, Sahraoui A, Høyem M, Øgaard J, Lien E, Aukrust P,* et al. NLRP3 inflammasome mediates oxidative stress-induced pancreatic islet dysfunction. Am J Physiol Endocrinol Metab 2018; 315(5): E912–23.
48. *King BC, Kulak K, Krus U, Rosberg R, Golec E, Wozniak K,* et al. Complement component C3 is highly expressed in human pancreatic islets and prevents β cell death via ATG16L1 interaction and autophagy regulation. Cell Metab 2019; 29(1): 202–10.
49. *Di Paolo NC, Shayakhmetov DM.* Interleukin-1α and the inflammatory process. Nat Immunol 2016; 17(8): 906–13.
50. *Alfadul H, Sabico S, Al-Daghri NM.* The role of interleukin-1β in type 2 diabetes mellitus: A systematic review and meta-analysis. Front Endocrinol (Lausanne) 2022; 13: 901616.
51. *Jin Z, Zhang Q, Liu K, Wang S, Yan Y, Zhang B,* et al. The association between interleukin family and diabetes mellitus and its complications: An overview of systematic reviews and meta-analyses. Diabetes Res Clin Pract 2024; 210: 111615.
52. *Matsumoto K, Miyake S, Yano M, Ueki Y, Tominaga Y.* High serum concentrations of soluble E-selectin in patients with impaired glucose tolerance with hyperinsulinemia. Atherosclerosis 2000; 152(2): 415–20.
53. *Blum A, Pastukh N, Socea D, Jabaly H.* Levels of adhesion molecules in peripheral blood correlat with stages of diabetic retinopathy and may serve as bio markers for microvascular complications. Cytokine 2018; 106: 76–9.
54. *Siddiqui K, George TP, Mujammami M, Isnani A, Alfadda AA.* The association of cell adhesion molecules and selectins (VCAM-1, ICAM-1, E-selectin, L-selectin, and P-selectin) with microvascular complications in patients with type 2 diabetes: A follow-up study. Front Endocrinol (Lausanne) 2023; 14: 1072288.
55. *Zheng H, Sun W, Zhang Q, Zhang Y, Ji L, Liu X,* et al. Proinflammatory cytokines predict the incidence of diabetic peripheral neuropathy over 5 years in Chinese type 2 diabetes patients: A prospective cohort study. EclinicalMedicine 2020; 31: 100649.
56. *Ekelund C, Dereke J, Nilsson C, Landin-Olsson M.* Are soluble E-selectin, ICAM-1, and VCAM-1 potential predictors for the development of diabetic retinopathy in young adults, 15–34 years of age? A Swedish prospective cohort study. PLoS One 2024; 19(6): e0304173.
57. *Boonman-de Winter LJ, Rutten FH, Cramer MJ, Landman MJ, Liem AH, Rutten GE,* et al. High prevalence of previously unknown heart failure and left ventricular dysfunction in patients with type 2 diabetes. Diabetologia 2012; 55(8): 2154–62.
58. *Vaur L, Gueret P, Lievre M, Chabaud S, Passa P.* Development of congestive heart failure in type 2 diabetic patients with microalbuminuria or proteinuria: Observations from the DIABHYCAR (type 2 DIABetes, Hypertension, CArdiovascular Events and Ramipril) study. Diabetes Care 2003; 26(3): 855–60. Erratum in: Diabetes Care 2003; 26(8): 2489.
59. *Paulus WJ, Tschöpe C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE,* et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007; 28(20): 2539–50.
60. *Yan AT, Yan RT, Cushman M, Redheuil A, Tracy RP, Arnett DK,* et al. Relationship of interleukin-6 with regional and global left-ventricular function in asymptomatic individuals without clinical cardiovascular disease: Insights from the multi-ethnic study of atherosclerosis. Eur Heart J 2010; 31(7): 875–82.
61. *Meléndez GC, McLarty JL, Levick SP, Du Y, Janicki JS, Brower GL.* Interleukin 6 mediates myocardial fibrosis, concentric hypertrophy, and diastolic dysfunction in rats. Hypertension 2010; 56(2): 225–31.
62. *Collier P, Watson CJ, Voon V, Phelan D, Jan A, Mak G,* et al. Can emerging biomarkers of myocardial remodelling identify asymptomatic hypertensive patients at risk for diastolic dysfunction and diastolic heart failure? Eur J Heart Fail 2011; 13(10): 1087–95.
63. *Fedacko J, Singh RB, Gupta A, Hristova K, Toda E, Kumar A,* et al. Inflammatory mediators in chronic heart failure in North India. Acta Cardiol 2014; 69(4): 391–8.
64. *Toczylowski K, Hirnle T, Harasiuk D, Zabielski P, Lewczuk A, Dmitruk I,* et al. Plasma concentration and expression of adipokines in epicardial and subcutaneous adipose tissue are associated with impaired left ventricular filling pattern. J Transl Med 2019; 17(1): 310.
65. *Perez AL, Grodin JL, Chaikijurajai T, Wu Y, Hernandez AF, Butler J,* et al. Interleukin-6 and outcomes in acute heart failure: An ASCEND-HF substudy. J Card Fail 2021; 27(6): 670–6.
66. *Fernandes-Silva MM, Guimarães GV, Rigaud VO, Lofrano-Alves MS, Castro RE, Cruz LGdB,* et al. Inflammatory biomarkers and effect of exercise on functional capacity in patients with heart failure: Insights from a randomized clinical trial. Eur J Prev Cardiol 2017; 24(8): 808–17.
67. *Askevold ET, Nymo S, Ueland T, Gravning J, Wergeland R, Kjekshus J,* et al. Soluble glycoprotein 130 predicts fatal outcomes in chronic heart failure: analysis from the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA). Circ Heart Fail 2018; 6(1): 91–8.
68. *Zhuravlyova L, Sokolnikova N.* 0149: the relationship between resistin, interleukin-6 level and diastolic dysfunction in patients with type 2 diabetes mellitus. Arch Cardiovasc Dis Suppl 2015; 7(1): 33.

69. *Jia C, Chen H, Zhang J, Zhou K, Zhuge Y, Niu C,* et al. Role of pyroptosis in cardiovascular diseases. Int Immunopharmacol 2019; 67: 311–8.
70. *Peh ZH, Dihoum A, Hutton D, Arthur JSC, Rena G, Khan F,* et al. Inflammation as a therapeutic target in heart failure with preserved ejection fraction. Front Cardiovasc Med 2023; 10: 1125687.
71. *Toldo S, Mezzaroma E, Bressi E, Marchetti C, Carbone S, Sonnino C,* et al. Interleukin-1β blockade improves left ventricular systolic/diastolic function and restores contractility reserve in severe ischemic cardiomyopathy in the mouse. J Cardiovasc Pharmacol 2014; 64(1): 1–6.
72. *Van Tassell BW, Arena R, Biondi-Zoccai G, Canada JM, Oddi C, Abouzaki NA,* et al. Effects of interleukin-1 blockade with anakinra on aerobic exercise capacity in patients with heart failure and preserved ejection fraction (from the D-HART pilot study). Am J Cardiol 2014; 113(2): 321–7.
73. *Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C,* et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 2017; 377(12): 1119–31.
74. *Everett BM, Cornel JH, Lainscak M, Anker SD, Abbate A, Thuren T,* et al. Anti-inflammatory therapy with canakinumab for the prevention of hospitalization for heart failure. Circulation 2019; 139(10): 1289–99.

Received on October 3, 2024
Revised on December 28, 2024
Accepted on January 15, 2025
Online First March 2025

ORIGINAL ARTICLE

UDC: 159.95:[616.895.4:616.895.8
DOI: https://doi.org/10.2298/VSP240923020L

Neuropsychological disorders in patients with schizophrenia and depression

Neuropsihološki poremećaji obolelih od shizofrenije i depresije

Ivana Leposavić*, Vesna Dukanac†

***University Clinical Center of Serbia, Psychiatry Clinic, Belgrade, Serbia;**
†Academy of Human Development, Belgrade, Serbia

Abstract

Background/Aim. Neuropsychological deficits among persons with psychotic disorders are identified clinically but also through many investigations. Comparison of patients with schizophrenia and depressive patients with healthy persons showed that both groups of patients are significantly impaired on the cognitive level compared to healthy persons. The aim of this study was to compare the neuropsychological functioning between patients with schizophrenia and depressive patients with psychotic symptoms (DPS) in remission, as well as between these two groups of patients and healthy persons. **Methods**. The study included 90 right-handed persons divided into three groups: 30 inpatients with schizophrenia, 30 inpatients with DPS, and 30 healthy persons. For examining neuropsychological functions of all participants, the following tests were applied: Wechsler's Individual Test of Intelligence (with subtests: Information, Digit Span, Arithmetic, Similarities, Picture Completion, Block Design, Digit Symbol), Mini-Mental State Examination, Trail Making Test, Rey-Osterrieth Complex Figure test, Hooper Visual Organization Test, phonemic, i.e., verbal fluency task, Rey Auditory Verbal Learning Test, and Wisconsin Card Sorting Test. **Results**. In the group of patients with schizophrenia, dysexecutive syndrome and reduced attention were singled out in several domains as basic deficits. Disturbances in abstract thinking and verbal fluency appeared together with dysexecutive syndrome, while attention disorders cause secondary damage to short-term memory, recognition in verbal modality, and information processing speed. In the neuropsychological profile of the DPS group, mild disturbances in the domain of visual search speed and information processing speed were noted. Impaired attention negatively affected the proper carrying out of other neuropsychological functions, while this phenomenon specifically impacted executive functions, visual gnosis, and processing speed among the DPS group. **Conclusion**. Neuropsychological disorders of patients with schizophrenia manifest in a larger number of cognitive domains and are more severe than those of the DPS group. In the DPS group, mild neurocognitive disorders were registered. Lack of motivation and effort during testing contributes to cognitive disturbances in the DPS group.

Key words:
depression; cognition disorders; neuropsychological tests; schizophrenia.

Apstrakt

Uvod/Cilj. Neuropsihološki deficiti osoba obolelih od psihotičnih poremećaja identifikuju se klinički, ali i mnogim istraživanjima. Poređenje obolelih od shizofrenije i depresije sa zdravim osobama pokazalo je da su obe grupe bolesnika značajno oštećene na kognitivnom planu, u odnosu na zdrave osobe. Cilj rada bio je da se uporedi neuropsihološko funkcionisanje između obolelih od shizofrenije i obolelih od depresije sa simptomima psihoze (DPS) u remisiji, kao i između ove dve grupe bolesnika i zdravih osoba. **Metode**. Ispitivanjem je obuhvaćeno 90 desnorukih osoba, svrstanih u tri grupe: 30 hospitalizovanih osoba obolelih od shizofrenije, 30 hospitalizovanih osoba obolelih od DPS i 30 zdravih osoba. Za ispitivanje neuropsihološke funkcije svih učesnika primenjeni su sledeći testovi: Vekslerov individualni test inteligencije (sa subtestovima: Informacije, Brojevi, Aritmetika, Sličnosti, Dopune, Kocka mozaik, Šifra), Kratko ispitivanje mentalnog statusa, Test trasiranja puta, Rej-Osteritov test složene figure, Huperov test vizuelne organizacije, zadatak fonemske, tj. verbalne fluentnosti, Rejov test auditivnog verbalnog učenja, Viskonsin test sortiranja karata. **Rezultati**. U grupi obolelih od shizofrenije kao bazični deficiti izdvojili su se disegzekutivni sindrom i redukovana pažnja u nekoliko domena. U sklopu disegzekutivnog sindoma nastale su i teškoće na planu apstraktnog mišljenja i verbalne fluentnosti, dok su smetnje pažnje sekundarno oštetile kratkoročnu memoriju, prepoznavanje u verbalnom modalitetu i brzinu obrade informacija. U neuropsihološkom profilu

Correspondence to: Vesna Dukanac, Academy of Human Development, Terazije 34, 11 000 Belgrade, Serbia. E-mail: vesna.dukanac@gmail.com

DPS grupe uočeni su blagi poremećaji u domenu brzine vizuelnog pretraživanja i brzine obrade informacija. Narušena pažnja negativno je uticala na pravilno odvijanje ostalih neuropsiholoških funkcija, dok je fenomen posebno uticao na egzekutivne funkcije, vizuelnu gnoziju i brzinu obrade informacija kod DPS grupe. **Zaključak**. Neuropsihološki poremećaji obolelih od shizofrenije ispoljavaju se u većem broju kognitivnih domena i težeg su stepena nego u grupi DPS. U grupi DPS registrovani su neurokognitivni poremećaji u umerenom stepenu. Nedostatak motivisanosti i uloženog napora tokom testiranja doprinosi kognitivnim smetnjama u DPS grupi.

Ključne reči:
depresija; saznanje, poremećaji; testovi, neuropsihološki; shizofrenija.

Introduction

Psychotic disorders are often connected with cognitive deficits. These deficits are registered through numerous studies that deal with the cognitive functioning of patients with schizophrenia (SCH) and patients with depression (DP) [1, 2]. More than 30 years ago, neurodevelopmental hypotheses [3, 4] emerged as an explanation for this phenomenon among patients with SCH, which was supported in later examinations [5, 6]. According to this concept, SCH is a neurodevelopmental disorder where abnormalities of the nervous system do not predominate until they interact with brain development processes, particularly in the frontal plane. Regarding depression, a model called the diathesis-stress model [7, 8] was presented. It explains that longer or repeated stress among adults, based on a hyperactive stress response system (due to early trauma), leads to a continuous increase of neuropeptide neurotransmitters, which is the basis for depression and anxiety.

Both groups of patients, with SCH and depression, were significantly more damaged compared to the group of healthy persons (HP) [9]. In addition, SCH is associated with a wide range of cognitive disorders (CDs), while according to meta-analytic studies, CDs among psychotic DP are mildly present [10].

It was demonstrated that the most prominent disorder among patients with SCH is information processing speed [11]. This deficit is conjoined with impaired immediate verbal memory already during the first episode of SCH [12]. Generally, patients with SCH display more severe disorders in all cognitive domains compared with DP [13].

Compared with the control group of HP, DP show weaker functioning of working, verbal, and visual memory, and disturbed information processing speed [14, 15]. Compared to the patients with SCH, those with post-acute depression show better functioning in the domain of verbal memory, fluency, and selective attention [16].

On cognitive levels, disturbed information processing speed is common among both disorders. Reductions in prefrontal cortex gray matter, which may be related to cognitive impairment, have also been reported in both disorders [17].

On the other hand, the results of the research, where they compared the cognitive functioning of patients with SCH and DP, are somewhat heterogeneous [18] because the severity of the clinical picture and stage of the disease have a significant impact on neuropsychological functioning. Here is a high probability of occurrence of neuropsychological disorders among those with psychotic disorders [19].

The aim of this study was to determine whether there are differences in cognitive functioning between patients with SCH and those with depression with psychotic symptoms (DPS) in the remission phase. Moreover, the aim was to determine the presence of differences between both patient groups and the HP on the level of cognitive functioning.

Methods

The study included three groups of right-handed subjects only because nobody was left-handed. Furthermore, determining the dominant hemisphere is more precise among the right-handed population because the vast majority of them have their left hemisphere as dominant for speech (at around 95–99% of cases). Among the left-handed population, the left hemisphere is dominant in a lesser number of cases (60–70%), and bilateral representation of speech is more common among the left-handed than the right-handed subjects [20].

The first group comprised 30 patients with SCH, the second 30 patients with DPS, and the third 30 HP. Both groups of patients were hospitalized in the Psychiatry Clinic of the University Clinical Center of Serbia. For this investigation, Ethics Committee Approval was obtained from the University Clinical Center of Serbia (No. 420/2, from 26 December 2024). Patients gave their written consent for hospitalization upon arriving at the clinic, and they confirmed weekly their written consent for applying diagnostic and therapeutic procedures.

Patients were diagnosed according to the International Classification of Diseases, 10th revision (ICD-10) criteria and were tested during clinical remission. Remission was confirmed by applying the Positive and Negative Syndrome Scale (PANSS) in the group of patients with SCH and the Hamilton Rating Scale for Depression (HRSD) in the group of DP, where the average score ± standard deviation was 51.32 ± 2.51 and 5.20 ± 2.10, respectively.

Neuropsychological testing was carried out in the scope of relevant diagnostic procedures. Exclusion criteria implied the presence of organic psycho-syndromes, neurological illnesses, substance or alcohol abuse, and disorders such as head trauma, brain insult, and epilepsy. Inclusion criteria constituted persons who never had organic psycho-syndromes, neurological illnesses or disorders (head injuries, epilepsy), and those who did not suffer from alcohol or substance abuse.

The applied neuropsychological battery comprised the following tests: Wechsler's Individual Test of Intelligence

(WITI), which is a version of the Wechsler Adult Intelligence Scale in Serbian language standardized on the Serbian population [21] (subtests: Information, Digit Span, Arithmetic, Similarities, Picture Completion, Block Design, Digit Symbol), Mini-Mental State Examination (MMSE) [22], Trail Making Test (TMT) [23], Rey-Osterrieth Complex Figure (ROCF) test [24], Hooper Visual Organization Test (HVOT) [25], phonemic, i.e., verbal fluency (VF) task [26] (adapted Benton Controlled Oral Word Association Test and given with three letters: s, k, l), Rey Auditory Verbal Learning Test (RAVLT) [27], and Wisconsin Card Sorting Test (WCST) [28].

WITI is a composite test consisting of six verbal and five non-verbal subtests. The test evaluates the current intellectual status of adults and adolescents. Verbal, non-verbal, and total intelligence quotients are derived from test scores. Subtest Information examines general knowledge that most people should have. Digit Span is a test of attention span and immediate memory, i.e., short-term memory. Subtest Arithmetic examines the ability to calculate and concentrate, while results are also influenced by immediate memory and calculi (arithmetic operations in the narrow sense). Subtest Similarities examines abstract thinking, i.e., finding a mutual category of objects. The Picture Completion subtest investigates visual gnosis and is based on visual perception, visual organization, and conclusions, as well as on long-term memory. Block Design subtest is a test of constructional and visuospatial abilities, i.e., it examines constructional praxis in three dimensions. The Digit Symbol subtest is an attention and processing speed test. Manual proficiency, sharpness of vision, and visual-motor coordination are significant as well.

MMSE is dedicated to evaluating cognitive falls. The test is a composite of many simple tasks.

TMT consists of two parts, A and B. Part A evaluates mostly attention, i.e., concentration, visual perception, and visual search speed. Besides the aforementioned, part B evaluates complex visual search speed, which is part of executive functions.

RAVLT in verbal modality (VM) examines learning, recognizing, and recalling. In the scope of all these functions, immediate memory, learning strategy, creation of learning curve, proactive and retroactive interference, tendencies towards confabulation, and retention evaluation are being determined.

In the first part of ROCF, constructional praxis in two dimensions is being examined. In the second part of the test, learning and postponed memory, as well as evoking in visual modality, are being examined.

HVOT examines visual-perceptive analysis and conceptual reorganization of fragmented objects. Achievements on this test mostly depend on visual-spatial organization, visual gnosis, and conceptual abilities.

Achievements in VF depend on the ability to formulate a specific strategy for remembering words and intact divergent thinking that allows finding more correct answers to the same task.

WCST is the most famous test for revealing perseveration and mental rigidity. Success on this test depends on perception organization, experience with adequately remembered material, and other aspects of thinking.

Data processing was conducted using standard statistical procedures in SPSS (Version 16.0.1). Group differences were analyzed using Analysis of Variance (ANOVA), which is appropriate for this sample size given that the central limit theorem supports the approximation of normality in the sampling distribution of the mean, even with imperfectly distributed original data sources [29]. The value of $p \leq 0.05$ is considered statistically significant

Results

The study included a total of 90 respondents divided into three groups: patients with SCH, DPS, and HP. Each group (n = 30) had an equal number of men and women. They were similar in terms of age ($p > 0.05$), education level ($p > 0.05$), and gender (Chi-square = 1.97, $p > 0.05$) (Table 1). Education level was determined according to the number of years and months of schooling.

On all applied WITI subtests, significant differences between groups were noted (Table 2). Achievements on subtests Information ($p < 0.01$), Similarities ($p < 0.01$), Picture Completion ($p < 0.01$), and Digit Symbol ($p < 0.01$) are significantly lower in both patient groups compared to the control group, while patients with SCH had significantly lower values compared to patients with DPS. These subtests are indicators of the level of general knowledge, abstract thinking, visual gnosis, and information processing speed. Subtests Digit Span ($p < 0.01$) and Arithmetic ($p < 0.01$), which represent attention span, short-term memory, and concentration, differ significantly between the group with SCH and the other two groups. On the subtest Block Design that examines construction praxis in three dimensions, the achievements of the two patient groups are significantly lower compared to the control group ($p < 0.01$).

Significant differences between groups were registered on the MMSE, by which the general cognitive level was examined. Patients with SCH had significantly lower achievements compared to the other two groups ($p < 0.01$) (Table 3). The time needed to complete the task, visual search speed – TMT A ($p < 0.01$), was significantly prolonged in both patient groups compared with the HP group, while patients with SCH required significantly more time for this task compared with the DPS group. Complex visual search speed –

Table 1

Main demographic characteristics of respondents of all groups

Parameter	Groups			F test	*p*-value
	SCH	DPS	HP		
Age	42.398 ± 7.248	43.38 ± 8.76	41.73 ± 6.54	0.50	> 0.05
Education level	13.178 ± 2.035	13.59 ± 2.32	13.57 ± 2.52	0.42	> 0.05

SCH – schizophrenia; DPS – depression with psychotic symptoms; HP – healthy persons.
Values are given as mean ± standard deviation.

Table 2

Achievements of respondents of all groups on Wechsler's Individual Test of Intelligence (WITI) subtests

Subtests	Groups			F test	*p*-value
	SCH	DPS	HP		
Information	12.97 ± 6.72	16.57 ± 2.01	19.38 ± 6.60	10.25	< 0.01
Digit Span	13.07 ± 3.49	15.70 ± 3.16	16.77 ± 3.73	13.45	< 0.01
Arithmetic	10.49 ± 2.85	12.76 ± 2.32	12.90 ± 2.77	10.77	< 0.01
Similarities	11.58 ± 4.08	14.60 ± 5.59	17.52 ± 3.03	13.93	< 0.01
Picture Completion	11.09 ± 2.99	12.83 ± 2.91	14.65 ± 1.84	14.45	< 0.01
Block Design	18.41 ± 7.73	23.20 ± 14.11	30.17 ± 2.00	10.46	< 0.01
Digit Symbol	23.78 ± 11.62	29.00 ± 1.77	39.38 ± 2.50	11.31	< 0.01

Values are given as mean ± standard deviation.
For abbreviations, see Table 1.

Table 3

Achievements of respondents of all groups on neuropsychological tests

Tests	Groups			F test	*p*-value
	SCH	DPS	HP		
MMSE	26.28 ± 1.80	27.93 ± 1.19	28.08 ± 1.24	7.36	< 0.01
TMT A	70.00 ± 10.60	62.17 ± 24.22	52.41 ± 16.31	14.22	< 0.01
TMT B	137.73 ± 34.13	128.07 ± 19.36	114.41 ± 57.25	3.65	< 0.01
ROCF C	27.24 ± 2.83	28.24 ± 3.91	28.75 ± 5.91	1.53	> 0.05
ROCF 40'	12.06 ± 2.94	13.15 ± 4.32	15.41 ± 8.21	3.69	< 0.05
HVOT	21.19 ± 4.00	20.65 ± 4.12	21.55 ± 2.38	0.69	> 0.05
VF s	9.83 ± 5.50	10.83 ± 1.63	11.60 ± 1.80	3.47	< 0.05
VF k	12.07 ± 1.40	11.00 ± 4.200	12.80 ± 1.02	6.39	< 0.05
VF l	8.93 ± 1.40	8.07 ± 3.10	8.90 ± 1.31	2.25	> 0.05
RAVLT t	44.05 ± 4.24	43.52 ± 7.88	46.50 ± 7.77	2.48	> 0.05
RAVLT e	7.58 ± 1.73	7.86 ± 2.60	7.90 ± 1.08	0.43	> 0.05
RAVLT r	11.39 ± 1.36	11.93 ± 1.10	12.52 ± 2.18	3.50	< 0.05
WCST ca	3.23 ± 1.61	4.73 ± 1.20	5.01 ± 1.06	4.15	< 0.05
WCST fms	2.34 ± 1.39	1.36 ± 0.91	1.27 ± 0.52	14.99	< 0.01
WCST per	53.10 ± 24.15	42.48 ± 21.46	30.37 ± 26.97	10.25	< 0.01

MMSE – Mini-Mental State Examination; TMT A – Trail Making Test (TMT) that evaluates attention, i.e., concentration, visual perception, and visual search speed; TMT B – TMT that evaluates complex visual search speed in addition; ROCF C – copying of the Ray-Osterrieth Complex Figure (ROCF); ROCF 40' – postponed visual memorizing of the ROCF; HVOT – Hooper Visual Organization Test; VF s – verbal fluency: overall number of collective nouns that begin with letter s; VF k – verbal fluency: overall number of collective nouns that begin with letter k; VF l – verbal fluency: overall number of collective nouns that begin with letter l; RAVLT t – total number of repeated words in five attempts in the Rey Auditory Verbal Learning Test (RAVLT); RAVLT e – number of repeated words after 30 min (evocation) in the RAVLT; RAVLT r – number of correctly recognized words (recognition) in the RAVLT; WCST ca – categories achieved in the Wisconsin Card Sorting Test (WCST); WCST fms – failures to maintain set in the WCST; WCST per – perseverative responses in the WCST. For other abbreviations, see Table 1.
Values are given as mean ± standard deviation.

TMT B ($p < 0.01$), drastically differentiated the group with SCH from the HP group. Both patient groups had significantly lower achievements on VF s and VF k in the domain of highly frequent words compared to the HP group ($p < 0.05$). Examining executive functions showed significant differences between groups. Both groups of patients had drastically lower working memory – WCST ca ($p < 0.05$), as well as drastically increased number of perseverative answers – WCST per ($p < 0.01$), compared to the control group of HP. On the other hand, the SCH group had significantly lower WCST ca and WCST per compared to the DPS group. Visual memory – ROCF 40' ($p < 0.05$), the ability to recognize previously learned material – RAVLT r ($p < 0.05$), that reflect the learning capacity, as well as prolonged attention – WCST fms ($p < 0.01$), were drastically lower in the SCH group compared to the other two groups.

Discussion

Significant differences in the achievement of most of the applied tests were identified among the SCH group in clinical remission. Results show that the most noticeable deficits in this group include information processing speed, level of general knowledge, abstract thinking, visual gnosis, and constructional praxis in three dimensions because their achievements are significantly lower compared with the control group on tests that evaluate these functions. To the same degree, the following examined attention modalities were

disrupted: attention span, concentration, prolonged attention, visual and complex visual search speed, followed by one or a couple of peculiarities in the scope of complex mental, motor, or perceptual activity. Disruptions of short-term memory and a tendency for perseveration are also significant among SCH patients. To a mild degree, working memory (the capacity of learned content in VM and phonemic/VF, i.e., verbal divergent thinking that leads in different ways towards a goal) was reduced.

In the active phase of illness, the presence of incoherent speech was observed among a certain number of patients with SCH included in the investigation. Although this characteristic does not imply a disorder of language functions, such as aphasia, difficulties of VF may be, to some extent, the consequence of a lack of coherence, i.e., a lack of structure in the discourse.

However, although the cognitive level is significantly lower in this group, from the clinical point of view and the perspective of everyday functioning, these patients do not have drastic CDs because their MMSE score is in normal ranges. This is also supported by the preserved capacity of verbal learning and remembrance, constructional praxis in two dimensions, mental rotation, and phonemic/VF in the domain of low-frequency words.

Differences in neuropsychological profiles between the two groups of patients are worse for patients with SCH in terms of levels of general knowledge, abstract thinking, visual gnosis, short-term memory, and recognition in VM, as well as in executive functions.

Furthermore, several attention modalities are reduced in the SCH group compared with the DPS group: simple attention (span), concentration, information processing speed, visual search speed, and prolonged attention.

By analyzing neuropsychological disturbances in the SCH group, dysexecutive syndrome (disrupted working memory and increased number of perseverative answers) and reduced attention in a couple of domains (simple, prolonged attention, concentration, and visual search speed) were noted as basic deficits. As part of dysexecutive syndrome, difficulties in abstract thinking and VF occur as well. On the other hand, attention disorders secondarily disrupt short-term memory, recognition on VM, and information processing speed.

Two groups of patients function similarly in the domain of constructional praxis in three dimensions – complex conceptual memory, visual memory, and VF.

Mild CDs were registered in the DPS group. Their achievements were significantly lower on a smaller number of tests compared to the HP group. Besides that, their results are either drastically better or similar to those of the SCH group.

General cognitive level, short-term memory, simple attention (attention span), and concentration among DPS patients are preserved. Moreover, complex visual search speed, visual memory, and recognition in VM are being carried out without difficulties. Their executive functions (working memory, perseverative answers), information processing speed, and visual search speed are damaged to a mild degree compared to the SCH group. This pattern of achievement is applied to abstract thinking as well as visual gnosis and the level of general knowledge.

In the neuropsychological profile of patients with DPS, mild disturbances in the domain of attention, visual search speed, and information processing speed are being singled out. In general, disrupted attention negatively influences the proper carrying out of other neuropsychological functions, while among the depression group, this phenomenon specifically influences executive functions, visual gnosis, and processing speed.

Besides the fact that the patients with DPS were investigated in the remission phase, during testing, they did not put enough effort into working on tests, and their motivation for this kind of examination oscillated, with the tendency to get discouraged when faced with more difficult challenges. More precisely, psychogenic factors contributed to lower achievements in this group. This was specifically brought to light on the tasks that examined general knowledge because these tasks included searching and recalling information stored in long-term memory.

Neuropsychological disturbances that were registered among the groups of patients with SCH and DPS are in harmony with previous investigations [30] and can be considered permanent [31, 32], keeping in mind that these difficulties were registered in remission, i.e., when symptoms of mental disorders were gone. Given that the research included middle-aged people, the possibility of a further deterioration of neuropsychological functions as a cause of aging cannot be ruled out, which leads to a longer duration of the disease. To check this assumption, longitudinal studies that would enable tracking of cognitive flows of these mental disorders are necessary.

Given that this is a cross-sectional study represents a certain limitation to our investigation. That being said, the patients are not retested in later stages of treatment after discharge. Therefore, conclusions on potential variations over time in their cognitive profiles cannot be made. In order to come to a more precise conclusion, longitudinal monitoring in terms of retesting is necessary.

Conclusion

Neuropsychological disturbances of patients with schizophrenia are being demonstrated in a more significant number of cognitive domains and are at higher levels compared to patients with depression.

Among the patients with schizophrenia in clinical remission, dysexecutive syndrome, disrupted information processing speed, and disrupted attention in a couple of domains were noted. Executive difficulties were followed by compromised abstract thinking and disrupted verbal fluency. As a consequence, attention difficulties reduce short-term memory, recognition in visual memory, and information processing speed.

Among the patients with depression with psychotic symptoms in clinical remission, neurocognitive disorders of a mild degree were registered. Disrupted visual search

speed and information processing speed secondarily disrupt executive functions and visual gnosis. Lack of motivation and effort during testing contributes to cognitive deficits.

The results of this investigation can serve as a basis for planning neuropsychological rehabilitation of patients with schizophrenia and patients with depression with psychotic symptoms in clinical remission in terms of designing principal, general, and special methods of treatment of cognitive dysfunctions. Special methods can apply an individualized approach to every patient according to their neuropsychological deficits.

REFERENCES

1. *Huang YC, Lee Y, Lee CY, Lin PY, Hung CF, Lee SY,* et al. Defining cognitive and functional profiles in schizophrenia and affective disorders. BMC Psychiatry 2020; 20(1): 39.
2. *Krabbendam L, Arts B, van Os J, Alamen A.* Cognitive functioning in patients with schizophrenia and bipolar disorder: a quantitative review. Schizophr Res 2005; 80(2–3): 137–49.
3. *Weinberger DR.* The pathogenesis of schizophrenia: a neurodevelopmental theory. In: *Nasrallah RA, Weinberger DR*, editors. The Neurology of Schizophrenia. Amsterdam: Elsevier; 1986. p. 387–405.
4. *Murray RM, Lewis SW.* Is schizophrenia a neurodevelopmental disorder? Br Med J (Clin Res Ed) 1987; 295(6600): 681–2.
5. *Nour MM, Howes OD.* Interpreting the neurodevelopmental hypothesis of schizophrenia in the context of normal brain development and ageing. Proc Natl Acad Sci U S A 2015; 112(21): E2745.
6. *Owen MJ, O'Donovan MC, Thapar A, Craddock N.* Neurodevelopmental hypothesis of schizophrenia. Br J Psychiatry 2011; 198(3): 173–5.
7. *Monroe SM, Simons AD.* Diathesis-stress theories in the context of life stress research: implications for the depressive disorders. Psychol Bull 1991; 110(3): 406–25.
8. *Ingram RE, Luxton DD.* Vulnerability-Stress Models. In: *Hankin BL, Abela JRZ*, editors. Development of psychopathology: A vulnerability-stress perspective. Thousand Oaks, CA: Sage Publications Inc; 2005. p. 32–46.
9. *Annette S, Stephan G, Mueser KT, Martin H, Elisabeth R, Ulrich G,* et al. A 2-year longitudinal study of neuropsychological functioning, psychosocial adjustment and rehospitalisation in schizophrenia and major depression. Eur Arch Psychiatry Clin Neurosci 2020; 270(6): 699–708.
10. *Ma M, Zhang Y, Zhang X, Yan H, Zhang D, Yue W.* Common and Distinct Alterations of Cognitive Function and Brain Structure in Schizophrenia and Major Depressive Disorder: A Pilot Study. Front Psychiatry 2021; 12: 705998.
11. *Fazilat-Pour M, Sharif-Pour L, Arjmand SA.* A comparison of selective attention processing in major depressive disorder and schizophrenia. J Pract Clin Psychol 2017; 5(3): 217–25.
12. *Mesholam-Gately RI, Giuliano AJ, Goff KP, Faraone SV, Seidman LJ.* Neurocognition in first-episode schizophrenia: a meta-analytic review. Neuropsychology 2009; 23(3): 315–36.
13. *Reichenberg A, Harvey PD, Bowie CR, Mojtabai R, Rabinowitz J, Heaton RK,* et al. Neuropsychological function and dysfunction in schizophrenia and psychotic affective disorders. Schizophr Bull 2009; 35(5): 1022–9.
14. *Zaremba D, Schulze Kalthoff I, Förster K, Redlich R, Grotegerd D, Leehr EJ,* et al. The effects of processing speed on memory impairment in patients with major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2019; 92: 494–500.
15. *Lee RS, Hermens DF, Porter MA, Redoblado-Hodge MA.* A meta-analysis of cognitive deficits in first-episode Major Depressive Disorder. J Affect Disord 2012; 140(2): 113–24.
16. *Schaub A, Neubauer N, Mueser KT, Engel R, Möller HJ.* Neuropsychological functioning in inpatients with major depression or schizophrenia. BMC Psychiatry 2013; 13(1): 203.
17. *Jiang Y, Duan M, Chen X, Zhang X, Gong J, Dong D,* et al. Aberrant prefrontal-thalamic-cerebellar circuit in schizophrenia and depression: evidence from a possible causal connectivity. Int J Neural Syst 2019; 29(5): 1850032.
18. *Zaninotto L, Guglielmo R, Calati R, Ioime L, Camardese G, Janiri L,* et al. Cognitive markers of psychotic unipolar depression: a meta-analytic study. J Affect Disord 2015; 174: 580–8.
19. *Sheffield JM, Karcher NR, Barch DM.* Cognitive deficits in psychotic disorders: a lifespan perspective. Neuropsychol Rev 2018; 28(4): 509–33.
20. *Pavlović D.* Specialization of the hemispheres. In: Neuropsychology with the basics of behavioral neurology. Belgrade: Kaligraf; 2011. p. 82–7. (Serbian)
21. *Berger J, Marković M, Mitić M.* Wechsler's Individual Test of Intelligence. Belgrade: Center for Applied Psychology, Serbian Psychological Society; 1991. (Serbian)
22. *Folstein MF, Folstein SE, McHugh PR.* Mini Mental State. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12(3): 189–98.
23. *Reitan RM.* Validity of the Trail Making test as an indication of organic brain damage. Percept Mot Skills 1958; 8: 271–6.
24. *Rey A.* The psychological examination of cases of traumatic encephalopathy. Archives de Psychologie 1941; 28: 286–340. (French)
25. *Hooper HE.* Hooper Visual Organization Test Manual. Los Angeles, CA: Western Psychological Services; 1983.
26. *Benton AL, Hamsher K.* Multilingual Aphasia Examination Manual. Iowa City: University of Iowa; 1976.
27. *Rey A.* The Clinical Psychological Examination. Paris: Presses Universitaires de France; 1964. (French)
28. *Berg EA.* A simple objective technique for measuring flexibility in thinking. J Gen Psychol 1948; 39:15–22.
29. *Schmider E, Ziegler M, Danay E, Beyer L, Bühner M.* Is It Really Robust? Reinvestigating the robustness of ANOVA against violations of the normal distribution assumption. Methodology 2010; 6(4): 147–51.
30. *Gkintoni E, Skokou M, Gourzis P.* Integrating Clinical Neuropsychology and Psychotic Spectrum Disorders: A Systematic Analysis of Cognitive Dynamics, Interventions, and Underlying Mechanisms. Medicina (Kaunas) 2024; 60(4): 645.
31. *Zanelli J, Mollon J, Sandin S, Morgan C, Dazzan P, Pilecka I,* et al. Cognitive Change in Schizophrenia and Other Psychoses in the Decade Following the First Episode. Am J Psychiatry 2019; 176(10): 811–9. Erratum in: Am J Psychiatry 2019; 176(12): 1051.
32. *Neu P, Gooren T, Niebuhr U, Schlattmann P.* Cognitive impairment in schizophrenia and depression: A comparison of stability and course. Appl Neuropsychol Adult 2019; 26(3): 215–28.

Received on September 23, 2024
Revised on November 28, 2024
Revised on December 31, 2024
Accepted on January 15, 2025
Online First April 2025

ORIGINAL ARTICLE

UDC: 616-006.44-085-036.8
DOI: https://doi.org/10.2298/VSP231207016P

Effects of monoclonal antibody daratumumab combined with ixazomib-based treatment regimen on survival of patients with relapsed/refractory multiple myeloma

Efekti daratumumab monoklonskog antitela u kombinaciji sa režimom lečenja zasnovanim na iksazomibu na preživljavanje obolelih od relapsnog/refraktornog multiplog mijeloma

[1]Lang Peng[*], [1]Jiawen He[†], Ying Zheng[†], Qingfang Zeng[‡]

[*]The Fourth Hospital of Changsha, Changsha, Hunan Province, China; [†]Department of Pharmacy, The 903rd Hospital of Chinese PLA Joint Logistics Support Force, Hangzhou, Zhejiang Province, China; [‡]Internal Medicine Area Three, Ganzhou Cancer Hospital, Ganzhou, Jiangxi Province, China

[1]The two authors contributed equally to this study.

Abstract

Background/Aim. Combination therapy with daratumumab and ixazomib has been previously used for the treatment of multiple myeloma (MM), but treatment outcomes of these drugs and safety have not yet been confirmed. The aim of the study was to assess the effects of monoclonal antibody daratumumab in combination with an ixazomib-based treatment regimen on the survival of patients with relapsed/refractory MM (RRMM). **Methods.** A retrospective study included the clinical data of 65 RRMM patients admitted from March 2016 to March 2019. The patients were divided according to different treatment regimens into two groups: Group A, with 31 patients, treated with a combination of ixazomib, dexamethasone, lenalidomide, and Group B, with 34 patients, treated with a combination of ixazomib, dexamethasone, lenalidomide, and daratumumab. Treatment outcomes, adverse reactions, quality of life, and survival were compared. **Results.** Groups A and B showed no significant differences in the objective response rate (70.97% vs. 85.29%) or the type and grade of adverse reactions ($p = 0.161$). The scores of all dimensions of the World Health Organization Quality of Life Brief Version of group B were higher than those of group A after treatment ($p < 0.05$). There was no significant difference in the one-year or three-year survival rate between group A (64.52%, 19.35%) and group B (73.53%, 32.35%) ($p = 0.432$ and $p = 0.234$, respectively). Still, group B had a significantly higher two-year survival rate than that of group A (61.76% vs. 35.48%) ($p = 0.034$). **Conclusion.** The combination of daratumumab and ixazomib-based treatment regimen helps improve the survival and quality of life of RRMM patients without increasing the incidence rate of adverse reactions during treatment.

Key words:
antineoplastic combined chemotherapy protocols; drug-related adverse effect and adverse reactions; immunotherapy; multiple myeloma; quality of life; survival; treatment outcome.

Apstrakt

Uvod/Cilj. Kombinovana terapija daratumumabom i iksazomibom je ranije korišćena za lečenje multiplog mijeloma (MM), ali rezultati lečenja ovim lekovima i bezbednost još uvek nisu potvrđeni. Cilj rada bio je da se procene efekti daratumumab monoklonskog antitela u kombinaciji sa režimom lečenja zasnovanim na iksazomibu na preživljavanje obolelih od relapsnog/refraktornog MM (RRMM). **Metode.** Retrospektivnom studijom obuhvaćeni su klinički podaci 65 obolelih od RRMM, primljenih u periodu od marta 2016. do marta 2019. godine. Bolesnici su podeljeni prema različitim režimima lečenja na dve grupe: na grupu A, koja je obuhvatila 31 bolesnika lečenih kombinacijom iksazomiba, deksametazona i lenalidomida i grupu B koja je obuhvatila 34 bolesnika lečenih kombinacijom iksazomiba, deksametazona, lenalidomida i daratumumaba. Upoređivani su ishodi lečenja, neželjene reakcije, kvalitet života i preživljavanje bolesnika. **Rezultati.** Nisu pokazane značajne razlike u objektivnoj stopi odgovora između grupa A i B (70,97% vs. 85,29%) niti u vrsti ili stepenu neželjenih reakcija ($p = 0,161$). Skorovi svih

Correspondence to: Qingfang Zeng, Internal Medicine Area Three, Ganzhou Cancer Hospital, Ganzhou 341 000, Jiangxi Province, China. E-mail: zengqfgch@dh-edu.cn

dimenzija Upitnika Svetske zdravstvene organizacije o kvalitetu života – kratka verzija (*World Health Organization Quality of Life Brief Version*) grupe B bili su viši od skorova grupe A posle lečenja ($p < 0,05$). Nije bilo značajne razlike u jednogodišnjoj i trogodišnjoj stopi preživljavanja između grupe A (64,52%, 19,35%) i grupe B (73,53%, 32,35%) ($p = 0,432$ i $p = 0,234$, redom). Ipak, grupa B imala je značajno višu stopu preživljavanja od grupe A (61,76% vs. 35,48%) ($p = 0,034$). **Zaključak.** Kombinacija daratumumaba i režima lečenja zasnovanog na iksazomibu poboljšava preživljavanje i kvalitet života obolelih od RRMM, bez povećanja stope incidencije neželjenih reakcija tokom lečenja.

Ključne reči:
lečenje kombinovanjem antineoplastika, protokoli; neželjena dejstva i neželjene reakcije; imunoterapija; multipli mijelom; kvalitet života; preživljavanje; lečenje, ishod.

Introduction

Multiple myeloma (MM) is a malignant tumor of plasma cells that cannot be cured at the moment by any treatment regimen. Most MM patients are in a remission-relapse-retreatment loop during treatment, and the disease eventually progresses into relapsed/refractory MM (RRMM) [1]. The diagnosis and treatment of RRMM aim to prolong the survival of patients and improve their quality of life (QoL).

Proteasomes are crucial for the degradation of proteins and the regulation of various signaling pathways [2]. The proliferation of tumor cells in MM patients has a close relationship with the signaling pathway regulating proteasomes [3]. The main pathway for the degradation of 80% of proteins lies in the ubiquitin-proteasome system. Proteasome activity is a determinant of the proliferation of myeloma cells, and this process can produce numerous proteins to increase the cell burden. In turn, these myeloma cells can activate the ubiquitin-proteasome system to maintain the protein homeostasis, which further induces dysfunction [4]. Hence, proteasomes may be the drug target of MM.

Proteasome inhibitors, immunomodulators, and hormones are commonly used in the maintenance treatment of RRMM. Ubiquitin-conjugating enzyme E2K (UBE2K) participates in the synthesis of K48-linked ubiquitin chains, which can be the target of some drugs used in the treatment of RRMM [5]. Inhibiting UBE2K expression can suppress myeloma cell proliferation, block the cell cycle, trigger cell apoptosis, and increase the production of reactive oxygen species, which can also regulate the genes related to mitosis and apoptosis. Ixazomib is a reversible proteasome inhibitor exhibiting high selectivity and anti-myeloma activity [6]. It can suppress chymotrypsin activity and induce the accumulation of ubiquitinated proteins by selectively binding to the β5 subunit of 20S proteasome, thereby impeding the proliferation and differentiation of tumor cells and playing an anti-myeloma role [7]. Wang et al. [8] reported that ixazomib shortened myeloma cell survival and facilitated cell apoptosis in a dose-dependent manner. Ixazomib can also extend the progression-free survival (PFS) of adult RRMM patients by 5.9 months [9]. In addition, Dimopoulos et al. [10] demonstrated that maintenance therapy with ixazomib prolonged the PFS of MM patients.

At present, ixazomib is approved for use in combination therapy with dexamethasone and lenalidomide. Daratumumab is a human monoclonal antibody specific for CD38, a key target for myeloma cells [11]. It was initially approved as monotherapy for RRMM and later for use in combination with other new myeloma therapies due to favorable toxic traits [12]. Li et al. [13] reported that 29.4% of MM patients selected the combined therapy with daratumumab and ixazomib. Nevertheless, their treatment outcomes and safety still need further validation.

In this study, the effect of daratumumab combined with an ixazomib-based treatment regimen on the survival of RRMM patients was assessed, aiming to provide more options and guidance for clinical maintenance treatment.

Methods

General data

A retrospective study included the clinical data of 65 RRMM patients treated from March 2016 to March 2019. According to different treatment regimens, these patients were assigned into two groups: group A with 31 patients and group B with 34 patients.

In group A, there were 19 males and 12 females, aged 42–69 years, with a mean of 55.48 ± 4.70 years. In terms of RRMM types, there were 8 cases of immunoglobulin (Ig) A, 18 cases of IgG, 2 cases of IgM, 1 case of lambda (λ) light chain, and 2 cases of kappa (κ) light chain. According to the Durie-Salmon staging system [14], the patients were classified into stage III (n = 15) and stage IIIA (n = 16). There were 6 cases in Revised International Staging System (R-ISS) Stage I, 14 in Stage II, and 11 in Stage III. Besides, 10 cases received one treatment line, 15 cases received two treatment lines, and 6 cases received three or more treatment lines. PFS and overall survival (OS) at the moment of starting the treatment were 18.2 ± 3.5 months and 42.5 ± 6.7 months, respectively.

Group B consisted of 20 males and 14 females aged 40–72 years, with a mean of 56.34 ± 4.02 years. Classified by RRMM types, there were 11 cases of IgA, 20 cases of IgG, 1 case of IgM, 1 case of IgD, and 1 case of λ light chain. Classified by the Durie-Salmon staging system, there were 17 cases in stage IIIA and 17 in stage IIIB. There were 7 cases in R-ISS Stage I, 16 in Stage II, and 11 in Stage III. Additionally, 11 cases received one treatment line, 16 cases received two treatment lines, and 7 cases received three or more treatment lines. The PFS and OS at the moment of starting treatment were 18.7 ± 3.8 months and 43.1 ± 6.3 months, respectively.

The general data displayed no statistically significant differences between the two groups ($p > 0.05$).

Inclusion and exclusion criteria

The inclusion criteria were the following: patients with RRMM diagnosed based on the diagnostic criteria of MM in the Guidelines for the Diagnosis and Management of Multiple Myeloma [15]; those with loci showing minimal response (MR) after receiving at least one of previous treatment regimens; those whose tumor progressed during treatment or within 60 days after the last treatment, or those whose tumor response rate was ≤ 25% after treatment; those aged ≥ 18 years; those with sufficient bone marrow reserves; those with complete clinical data.

The exclusion criteria were as follows: patients with plasma cell leukemia; those with monoclonal protein changes; those who were treated with daratumumab and ixazomib; those with abnormal organ enlargement; those with congestive heart failure; those complicated with myelodysplastic syndrome, uncontrollable hypertension, or hyperglycemia; those with an expected survival of < 3 months.

Therapeutic methods

Both groups received ixazomib-based treatment with a 28-day treatment model. Group A took orally 4 mg of ixazomib (4 mg, Takeda Pharma A/S) on the 1st, 8th, and 15th day, dexamethasone (20 mg, Chengdu Tiantaishan Pharmaceutical Co., Ltd.) on the 1st, 8th, 15th, and 22nd day, respectively, and lenalidomide (25 mg, Chia Tai Tianqing Pharmaceutical Group Co., Ltd. – CTTQ) once every three days from the 1st to 21st day. In addition to the medications for group A, 16 mg/kg daratumumab (15 mL, Cilag AG) was infused intravenously into group B on the 1st, 8th, 15th, and 22nd day, which was conducted once every two weeks from the 9th week and once every four weeks from the 25th week.

Observation of indicators

The treatment outcomes were evaluated according to the MM evaluation criteria [16]. The response status of loci was classified into complete response (CR), strict CR (sCR), very good partial response – PR (VGPR), PR, MR, stable disease, and progressive disease. Total objective response rate (ORR) = percentage of CR + sCR + VGPR + PR cases.

Adverse reactions (AR) were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 published by the National Cancer Institute USA [17], and the safety of ixazomib and daratumumab was evaluated.

The World Health Organization Quality of Life Brief Version (WHO QOL-BREF) was utilized to assess QoL before treatment and one year after treatment. The WHO QOL-BREF measured four specific domains and one general domain, involving 26 questions, among which 24 questions were used to evaluate physical health (7 items), psychological health (6 items), social relationship (3 items), and environmental health (8 items), and the remaining 2 questions were employed to evaluate general health. Each domain scored 0–100 points, and the higher the score, the better QoL [18].

Survival was assessed after three years of outpatient or telephone follow-up until April 2022. One-, two-, and three-year survival rates of the patients were recorded.

Statistical analysis

All data were statistically analyzed using SPSS 23.0 software. Measurement data were compared between two groups using the independent samples *t*-test. Count data were expressed as percentages and compared with the Chi-squared (χ^2) test. The Kaplan-Meier method was used for survival analysis. The difference was statistically significant as $p < 0.05$.

Results

Treatment outcomes of patients in both groups are shown in Table 1. ORR showed no significant difference between groups A and B (70.97% vs. 85.29%) ($\chi^2 = 1.969$; $p = 0.161$).

There were no significant differences in the type or grade of AR between groups A and B ($p > 0.05$) (Table 2).

Prior to treatment, the QOL-BREF score was not significantly different between groups A and B ($p > 0.05$). After treatment, the physical health, psychological health, social relationship, environmental health, and general QoL scores in group B were significantly higher than those in group A ($p < 0.05$) (Table 3).

No statistically significant difference was detected in the one- or three-year survival rate between group A (64.52% and 19.35%) and group B (73.53% and 32.35%) ($p = 0.432$ and $p = 0.234$, respectively). Still, group B had a significantly higher two-year survival rate than that of group A (61.76% vs. 35.48%) ($p = 0.034$) (Table 4). Moreover, the median follow-up time was 20.32 months in group A and 24.74 months in group B, and the OS was compared between the two groups using the log-rank test, showing no significant difference ($\chi^2 = 2.154$, $p = 0.142$) (Figure 1).

Table 1

Treatment outcomes of patients in both groups

Group	CR	sCR	VGPR	PR	MR	SD	ORR
A (n = 31)	0 (0.00)	3 (9.68)	8 (25.81)	11 (35.48)	6 (19.35)	3 (9.68)	22 (70.97)
B (n = 34)	0 (0.00)	5 (14.71)	12 (35.29)	12 (35.29)	3 (8.82)	2 (5.88)	29 (85.29)
χ^2							1.969
p							0.161

CR – complete response; SCR – strict CR; VGPR – very good PR; PR – partial response; MR – minimal response; SD – stable disease; ORR – objective response rate.
Values are given as numbers (percentages).

Table 2

Type and grade of adverse reactions

Parameter	Grade 1–2		Grade 3–4		Total incidence rate		χ^2	p
	Group A	Group B	Group A	Group B	Group A	Group B		
Neutropenia	9 (29.03)	11 (32.35)	5 (16.13)	5 (14.71)	14 (45.16)	16 (47.06)	0.023	0.878
Lymphopenia	11 (35.48)	13 (38.24)	3 (9.68)	4 (11.76)	14 (45.16)	17 (50.00)	0.152	0.696
Thrombocytopenia	8 (25.81)	9 (26.47)	2 (6.45)	1 (2.94)	10 (32.26)	10 (29.41)	0.062	0.804
Cardiotoxicity	8 (25.81)	9 (26.47)	2 (6.45)	1 (2.94)	10 (32.26)	10 (29.41)	0.062	0.804
Anemia	11 (35.48)	10 (29.41)	3 (9.68)	4 (11.76)	14 (45.16)	14 (41.18)	0.105	0.746
Nausea	18 (58.06)	20 (58.82)	7 (22.58)	9 (26.47)	25 (80.65)	29 (85.29)	0.249	0.618
Vomiting	15 (48.39)	14 (41.18)	5 (16.13)	4 (11.76)	20 (64.52)	18 (52.94)	1.430	0.232
Peripheral neuropathy	5 (16.13)	5 (14.71)	1 (3.23)	0 (0.00)	6 (19.35)	5 (14.71)	0.249	0.618
Diarrhea	4 (12.90)	5 (14.71)	1 (3.23)	2 (5.88)	5 (16.13)	7 (20.59)	0.214	0.643
Constipation	7 (22.58)	6 (17.65)	1 (3.23)	3 (8.82)	8 (25.81)	9 (26.47)	0.004	0.951
Fatigue	19 (61.29)	21 (61.76)	8 (25.81)	11 (32.35)	27 (87.10)	32 (94.12)	0.954	0.329

Values are given as numbers (percentages).
Note: Group A consists of 31 patients and Group B of 34 patients.

Table 3

WHO QOL-BREF scores before and after treatment

Parameter	Values	t	p
Physical health			
Group A			
BT	36.86 ± 4.20	0.944	0.349
AT	59.63 ± 5.07		
Group B			
BT	35.89 ± 4.08	2.725	0.008
AT	63.25 ± 5.59		
Psychological health			
Group A			
BT	30.72 ± 3.47	0.541	0.590
AT	46.82 ± 4.75		
Group B			
BT	31.19 ± 3.53	2.858	0.006
AT	50.32 ± 5.09		
Social relationship			
Group A			
BT	32.75 ± 3.59	1.009	0.317
AT	31.89 ± 3.28		
Group B			
BT	61.58 ± 5.42	3.792	< 0.001
AT	66.91 ± 5.87		
Environmental health			
Group A			
BT	45.51 ± 4.82	0.553	0.582
AT	46.18 ± 4.93		
Group B			
BT	69.72 ± 6.45	2.286	0.026
AT	73.50 ± 6.84		
General health			
Group A			
BT	38.93 ± 4.05	0.099	0.921
AT	39.03 ± 4.10		
Group B			
BT	60.36 ± 5.03	3.316	0.002
AT	64.75 ± 5.59		

WHO QOL-BREF – World Health Organization Quality of Life Brief Version; BT – before treatment; AT – after treatment. Values are given as mean ± standard deviation.

For the results before vs. after treatment in the same group, $p < 0.05$ was considered statistically significant.

Table 4

Survival of patients

Group	1-year survival	2-year survival	3-year survival
A (n = 31)	20 (64.52)	11 (35.48)	6 (19.35)
B (n = 34)	25 (73.53)	21 (61.76)	11 (32.35)
χ^2	0.618	4.481	1.418
p	0.432	0.034	0.234

Values are given as numbers (percentages).

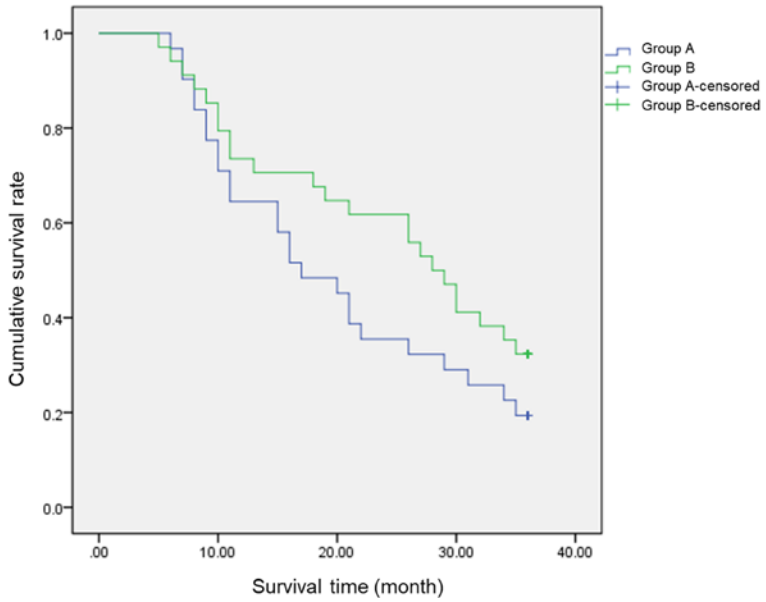


Fig. 1 – Survival curves of group A and group B.
Group A and B censored: moment when monitoring was terminated.

Discussion

In this study, groups A and B showed no significant difference in the treatment outcomes, which may be attributed to the small sample size. However, the two-year survival rate and WHO QOL-BREF score of group B were significantly higher than those of group A, which suggested that the combination of daratumumab and ixazomib-based treatment regimen can improve the prognosis of RRMM patients regarding both survival and QoL. These findings are consistent with those reported by Stege et al. [19], who observed prolonged survival and improved QoL in patients receiving ixazomib + daratumumab maintenance therapy. It is possible that daratumumab directly binds to CD38 on the surface of myeloma cells and triggers their death through multiple mechanisms [20]. As reported by Saltarella et al. [21], daratumumab resisted MM activity through the mechanisms of cytotoxicity mediated by antibody-dependent cells, antibody-dependent cell phagocytosis, complement-dependent cytotoxicity, and immunomodulation.

Ixazomib-based treatment regimen has good drug resistance in general, and the common AR include neutropenia, lymphopenia, thrombocytopenia, fatigue, nausea, and vomiting [22]. The main reason is that treatment with chemotherapy drugs for a period can inhibit the hematopoietic function of the bone marrow owing to the toxic effect [23]. Herein, we found no significant increase in the overall AR after the combination of daratumumab with an ixazomib-based treatment regimen. Similarly, Maouche et al. [24] found favorable resistance profiles with ixazomib, dexamethasone, and lenalidomide in the treatment of RRMM. Hence, the combination was safe and reliable, with most drug-related adverse events within a controllable range.

Nevertheless, this study is limited. The sample size is relatively small, so larger multicenter studies are required to investigate the observed trends further.

Conclusion

The combination of daratumumab with ixazomib-based treatment regimen can improve the survival rate and quality of life of relapsed/refractory multiple myeloma patients without leading to an obvious increase in the incidence rate of adverse reactions during treatment.

Acknowledgement

This study was not financially supported.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Zheng Z, Lin K.* A comparison of the efficacy and safety of ixazomib and lenalidomide combined with dexamethasone in the treatment of multiple myeloma. Am J Transl Res 2021; 13(5): 5248–55.
2. *Hájek R, Minařík J, Straub J, Pour L, Jungova A, Berdeja JG,* et al. Ixazomib-lenalidomide-dexamethasone in routine clinical practice: effectiveness in relapsed/refractory multiple myeloma. Future Oncol 2021; 17(19): 2499–512.
3. *Takakuwa T, Yamamura R, Ohta K, Kaneko H, Imada K, Nakaya A,* et al. Outcomes of ixazomib/lenalidomide/dexamethasone for multiple myeloma: A multicenter retrospective analysis. Eur J Haematol 2021; 106(4): 555–62.
4. *Terpos E, Ramasamy K, Maouche N, Minarik J, Ntanasis-Stathopoulos I, Katodritou E,* et al. Real-world effectiveness and safety of ixazomib-lenalidomide-dexamethasone in relapsed/refractory multiple myeloma. Ann Hematol 2020; 99(5): 1049–61.
5. *Davies F, Rifkin R, Costello C, Morgan G, Usmani S, Abonour R,* et al. Real-world comparative effectiveness of triplets containing bortezomib (B), carfilzomib (C), daratumumab (D), or ixazomib (I) in relapsed/refractory multiple myeloma (RRMM) in the US. Ann Hematol 2021; 100(9): 2325–37.
6. *Boiten HJ, Buijze M, Zweegman S, Levin MD.* Ixazomib Treatment of IgA Multiple Myeloma with Hyperviscosity Syndrome. Clin Lymphoma Myeloma Leuk 2020; 20(11): e832–5.
7. *Goldsmith SR, Foley N, Schroeder MA.* Daratumumab for the treatment of multiple myeloma. Drugs Today (Barc) 2021; 57(10): 591–605.
8. *Wang Q, Dong Z, Su J, Huang J, Xiao P, Tian L,* et al. Ixazomib inhibits myeloma cell proliferation by targeting UBE2K. Biochem Biophys Res Commun 2021; 549: 1–7.
9. *Tzogani K, Florez B, Markey G, Caleno M, Olimpieri OM, Melchiorri D,* et al. European Medicines Agency review of ixazomib (Ninlaro) for the treatment of adult patients with multiple myeloma who have received at least one prior therapy. ESMO Open 2019; 4(5): e000570.
10. *Dimopoulos MA, Špička I, Quach H, Oriol A, Hájek R, Garg M,* et al. Ixazomib as Postinduction Maintenance for Patients With Newly Diagnosed Multiple Myeloma Not Undergoing Autologous Stem Cell Transplantation: The Phase III TOURMALINE-MM4 Trial. J Clin Oncol 2020; 38(34): 4030–41. Erratum in: J Clin Oncol 2022; 40(8): 919.
11. *Offidani M, Corvatta L, Morè S, Nappi D, Martinelli G, Olivieri A,* et al. Daratumumab for the Management of Newly Diagnosed and Relapsed/Refractory Multiple Myeloma: Current and Emerging Treatments. Front Oncol 2021; 10: 624661.
12. *Durie BG, Salmon SE.* A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer 1975; 36(3): 842–54.
13. *Li J, Bao L, Xia Z, Wang S, Zhou X, Ding K,* et al. Ixazomib-based frontline therapy in patients with newly diagnosed multiple myeloma in real-life practice showed comparable efficacy and safety profile with those reported in clinical trial: a multicenter study. Ann Hematol 2020; 99(11): 2589–98.
14. *Chen WM.* The guidelines for the diagnosis and management of multiple myeloma in China (2017 revision): interpretation of treatment of relapsed/refractory multiple myeloma. Zhonghua Nei Ke Za Zhi 2017; 56(11): 799–800. (Chinese)
15. *Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P,* et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol 2016; 17(8): e328–46.
16. *Rajkumar SV.* Multiple myeloma: 2020 update on diagnosis, risk-stratification and management. Am J Hematol 2020; 95(5): 548–67. Erratum in: Am J Hematol 2020; 95(11): 1444.
17. *National Cancer Institute.* Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 [Internet]. U.S. Department of health and human services; 2017 [cited 2018 Nov 8; accessed 2025 Jan 13]. Available from: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf
18. *Kumar P, Sen RK, Aggarwal S, Jindal K, Rajnish RK.* Assessment and reliability of the World Health Organisation quality of life (WHO QOL-BREF) questionnaire in total hip replacement patients. J Clin Orthop Trauma 2020; 11(Suppl 5): S756–9.
19. *Stege CAM, Nasserinejad K, van der Spek E, Bilgin YM, Kentos A, Sohne M,* et al. Ixazomib, Daratumumab, and Low-Dose Dexamethasone in Frail Patients With Newly Diagnosed Multiple Myeloma: The Hovon 143 Study. J Clin Oncol 2021; 39(25): 2758–67.
20. *Ziff M, Lawson G, De-Silva D, Cheesman S, Kyriakou C, Mahmood S,* et al. Ixazomib with lenalidomide and dexamethasone for patients with relapsed multiple myeloma: impact of 17p deletion and sensitivity to proteasome inhibitors from a real world data-set. Leuk Lymphoma 2021; 62(5): 1243–6.
21. *Saltarella I, Desantis V, Melaccio A, Solimando AG, Lamanuzzi A, Ria R,* et al. Mechanisms of Resistance to Anti-CD38 Daratumumab in Multiple Myeloma. Cells 2020; 9(1): 167.
22. *Mateos MV, Nahi H, Legiec W, Grosicki S, Vorobyev V, Spicka I,* et al. Subcutaneous versus intravenous daratumumab in patients with relapsed or refractory multiple myeloma (COLUMBA): a multicentre, open-label, non-inferiority, randomised, phase 3 trial. Lancet Haematol 2020; 7(5): e370–80. Erratum in: Lancet Haematol 2020; 7(10): e710.
23. *Chong LL, Soon YY, Soekojo CY, Ooi M, Chng WJ, de Mel S.* Daratumumab-based induction therapy for multiple myeloma: A systematic review and meta-analysis. Crit Rev Oncol Hematol 2021; 159: 103211.
24. *Maouche N, Kishore B, Jenner MW, Boyd K, Bhatti Z, Bird SA,* et al. Ixazomib, lenalidomide, and dexamethasone is effective and well tolerated in multiply relapsed (≥2nd relapse) refractory myeloma: a multicenter real world UK experience. Leuk Lymphoma 2021; 62(6): 1396–404.

Received on December 7, 2023
Revised on August 23, 2024
Revised on November 20, 2024
Revised on January 10, 2025
Accepted on January 15, 2025
Online First March 2025

ORIGINAL ARTICLE

UDC: 616.98:578.828]:[615.371:616.9-036.21
DOI: https://doi.org/10.2298/VSP241206022M

Humoral response to anti-COVID-19 immunization and SARS-CoV-2 infection in HIV-infected persons

Humoralni odgovor na anti-COVID-19 imunizaciju i SARS-CoV-2 infekciju kod osoba inficiranih HIV-om

Daniela Marić*†,Vanja Andrić*†, Snežana Brkić*†, Dragana Žuvić*‡, Romana Mijović*‡, Aleksandra Bulović§

University of Novi Sad, *Faculty of Medicine, †Department of Infectious Diseases, Novi Sad, Serbia; University Clinical Center of Vojvodina, ‡Center for Laboratory Medicine, §Clinic for Infectious Diseases, Novi Sad, Serbia

Abstract

Background/Aim. At the beginning of the coronavirus disease 2019 (COVID-19) pandemic, human immunodeficiency virus (HIV)-infected persons (HIP) were considered to be at an increased risk of more severe forms of the disease. Although vaccination of HIP is deemed essential, data on the humoral response to both infection and vaccination in this population are inconsistent, particularly when comparing different vaccine types. The aim of this study was to examine factors that could influence severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) anti-spike protein antibody titers in HIP after vaccination and/or exposure to the virus. **Methods.** The study included all HIP who came for routine check-ups to the Center for HIV/AIDS of the University Clinical Center of Vojvodina, Serbia, from April to December 2022 and who had received at least two doses of the vaccine or had a positive history of COVID-19. Data on age, duration of antiretroviral therapy (ART), nadir and current $CD4^+$ and $CD8^+$ T-cell counts, and type of vaccine were collected from medical records and the national database. Immunoglobulin G (IgG) antibodies against SARS-CoV-2 spike protein were determined in the sera of HIP using the AdviseDx SARS-CoV-2 IgG II assay. **Results.** The research included 226 HIP with undetectable viremia, in 96.3% of cases, the CD4 T-lymphocyte count was over 350 cells/mm^3. Out of 171 HIP who received at least two doses of a vaccine, 64 (37.4%) were both vaccinated and had COVID-19 and 107 (62.6%) were vaccinated and had no evidence of COVID-19. Among the vaccinated participants, 62% received three doses and 38% received two vaccine doses. Regarding the type of vaccine, 59.6% of participants received a messenger ribonucleic acid (mRNA) vaccine, 25.1% an inactivated vaccine, and 15.3% received a vector vaccine. A better humoral response was observed in the mRNA compared to the inactivated vaccines and in three compared to two doses in the case of mRNA vaccines. Age and duration of ART negatively correlated with antibody titers, while the number of CD8 T-cells had a positive correlation. **Conclusion.** The study showed the immunogenicity and safety of full vaccination against COVID-19 in HIP with any of the available vaccines.

Key words:
covid-19; hiv; immunity, humoral; immunoglobulin g; sars-cov-2; t-lymphocytes; vaccination; vaccines.

Apstrakt

Uvod/Cilj. Na početku pandemije izazvane koronavirusom 2019 (*coronavirus disease 2019* – COVID-19), smatralo se da su osobe inficirane virusom humane imunodeficijencije (HIV) u većem riziku od razvoja težih formi ove bolesti. Iako se vakcinacija HIV-inficiranih smatra neophodnom, podaci o humoralnom odgovoru na infekciju i vakcinaciju u ovoj populaciji su nedosledni, posebno kada se porede različite vrste vakcina. Cilj ove studije bio je da se istraže faktori koji bi kod osoba inficiranih HIV-om mogli da utiču na titar antitela specifičnih za *spike* protein koronavirusa 2 izazivača teškog akutnog respiratornog sindroma (*severe acute respiratory syndrome coronavirus 2* – SARS-CoV-2) nakon vakcinacije i/ili nakon izlaganja virusu. **Metode.** U studiju su bile uključene sve HIV-inficirane osobe koje su došle na rutinski pregled u Centar za HIV/AIDS, Univerzitetskog kliničkog centra Vojvodine, Srbija, od aprila do decembra 2022. godine i koje su primile najmanje dve doze vakcine i/ili su preležale COVID-19. Podaci o starosti, trajanju antiretrovirusne terapije (ART), najnižem i trenutnom broju $CD4^+$ i $CD8^+$ T-ćelija i podaci o tipu vakcine prikupljeni su iz medicinske dokumentacije i nacionalne baze podataka. Imunoglobulin G (IgG) antitela protiv SARS-CoV-2 *spike* proteina određivana su u serumima osoba inficiranih HIV-om

Correspondence to: Daniela Marić, University of Novi Sad, Faculty of Medicine, Department of Infectious Diseases, Hajduk Veljkova 3, 21 000 Novi Sad, Serbia. E-mail: daniela.maric@mf.uns.ac.rs

korišćenjem AdviseDx SARS-CoV-2 IgG II testa. **Rezultati.** U istraživanje je bilo uključeno 226 HIV-inficiranih osoba sa nedetektabilnom viremijom, u 96,3% slučajeva, broj CD4 T-limfocita bio je preko 350 ćelija/mm^3. Od 171 ispitanika koji su primili najmanje dve doze vakcine, 64 (37,4%) je bilo i vakcinisano i imalo COVID-19 a 107 (62,6%) je bilo samo vakcinisano i nije imalo COVID-19. Među vakcinisanim ispitanicima, tri doze primilo je 62%, a njih 38% je primilo dve doze vakcine. Kada je u pitanju tip vakcine, 59,6% primilo je vakcinu na bazi informacione ribonukleinske kiseline (*messenger ribonucleic acid* – mRNA), 25,1% inaktivisanu vakcinu, a 15,3% vektorsku vakcinu. Bolji humoralni odgovor je pokazan u slučajevima mRNK vakcine u odnosu na inaktivisanu vakcinu i kod onih koji su primili tri doze u odnosu na dve u slučaju mRNK vakcina. Godine starosti i trajanje ART su bili u negativnoj korelaciju, a broj CD8 T-ćelija u pozitivnoj korelaciji sa titrima antitela. **Zaključak.** Studija je pokazala imunogenost i bezbednost potpune vakcinacije protiv COVID-19 kod osoba inficiranih HIV-om bilo kojom od dostupnih vakcina.

Ključne reči:
covid-19; hiv; imunitet, humoralni; imunoglobulin g; sars-cov-2; limfociti t; vakcinacija; vakcine.

Introduction

In late 2019, a new virus spread rapidly worldwide, resulting in a global pandemic. The virus was designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease it caused was named coronavirus disease 2019 (COVID-19). Early in the pandemic, human immunodeficiency virus (HIV)-infected persons (HIP) were defined as a population with vaccination priority. Large cohort studies from the UK, the USA, and South Africa, including data reported to the World Health Organization from across the world, identified a higher risk of death and hospitalization from COVID-19 in this population [1–6]. Even so, many questions on humoral response to infection and vaccination in HIP remain unanswered.

Traditionally considered immunodeficient, HIP are a very heterogeneous population, with numerous factors influencing humoral response to vaccines and infections. There are distinct challenges in mounting effective immune responses in HIP, particularly in response to vaccination. The primary deficiency in HIP is the depletion of CD4$^+$ T-cells, which are critical for orchestrating the immune response. Not only does this T-cell deficiency affect cellular immunity, but it also impairs the function of B cells, which are crucial for producing antibodies in response to infections and vaccinations, including those against SARS-CoV-2[5].

In the pre-registration studies of messenger ribonucleic acid (mRNA) and vector vaccines against COVID-19, HIP were very poorly represented in the studied populations [7, 8]. The effectiveness and safety of inactivated vaccines were not tested before their use in HIP, so the recommendations were based on data from previously used inactivated vaccines in this population.

The response to COVID-19 in Serbia involved a combination of public health measures, healthcare system adaptation, government policies, and international collaboration. Serbia's approach evolved throughout the pandemic as the country faced successive waves of infection, adjusting strategies to curb the spread of the virus and mitigate its impact on society and the economy. By March 2021, four different vaccines became available for Serbian citizens: mRNA vaccine Pfizer-BioNTech/BNT162b2 (Comirnaty®), inactivated vaccine Sinopharm/BBIBP-CorV (Vero Cell®), and two vector vaccines Gam-COVID-Vac (Sputnik V®) and Oxford/AstraZeneca ChAdOx1-S/nCoV-19, AZD1222 (Vaxzevria®). All of the vaccines were also available for HIP, with the choice of vaccine left to the patients or their caregivers, with no clear protocols for their use in this population. To our knowledge, all three vaccine types against COVID-19 (inactivated, vector, and mRNA) are hardly ever offered to HIP from a single cohort.

The aim of this study was to examine factors influencing humoral response to vaccination against COVID-19 and natural exposure to the SARS-CoV-2 in the cohort of HIP.

Methods

We conducted a cross-sectional, observational study on 226 participants at the Center for HIV/acquired immunodeficiency syndrome (AIDS), Clinic for Infectious Diseases, University Clinical Center of Vojvodina, Novi Sad, Serbia. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Novi Sad (No. 01-39/189/1, from March 31, 2022).

All HIP who had routine check-ups between April 1 and December 30, 2022, and either received at least two doses of the anti-COVID-19 vaccine and/or were previously diagnosed with COVID-19 were included in the study. The exclusion criteria were hepatitis B and/or C co-infections and unwillingness to participate in the study. All HIP who met the criteria signed the informed consent and were then included in the study. Remnant serum samples were collected from all the participants who underwent routine outpatient laboratory testing for the measurement of immunoglobulin G (IgG) antibody titer.

Relevant data on the HIV infection were collected using participants' medical records. The data included their current age, estimated duration of the HIV infection, nadir and current CD4$^+$ and CD8$^+$ T-cell counts, and current polymerase chain reaction (PCR) HIV RNA viral load. Data on vaccine type and date of the vaccination against COVID-19 were collected using the national database. Data on events associated with adverse effects of the vaccines were collected from the participants in a short interview during their visit to

the Clinic. Data on thrombosis after vaccination were collected from the medical records of HIP.

Which of the available vaccines will HIP receive is based on the individual's decision after obtaining advice from a general practitioner or their HIV physician. At the time of the study, HIP were recommended to receive three doses of the vaccine, i.e., primary series plus a booster.

Determination of IgG antibodies against SARS-CoV-2 spike protein was performed using the AdviseDx SARS-CoV-2 IgG II assay [9]. The assay is a chemiluminescent microparticle immunoassay (CMIA) intended for the qualitative and semi-quantitative detection of IgG antibodies to the receptor-binding domain of the S1 subunit of the spike protein of SARS-CoV-2 in serum and plasma. The resulting chemiluminescent reaction was measured as a relative light unit, which was then translated into antibody concentration in arbitrary units (AU/mL). Samples with > 12 AU/mL, 10–12 AU/mL, and < 10 AU/mL were considered positive, indeterminate, and negative for IgG, respectively.

Statistical analysis data was performed using SPSS 20.0 software. Frequencies and percentages were used to describe and analyze the sample in order to represent the delineation of certain categories or answers. Descriptive statistical methods were used to measure central tendencies (arithmetic mean), variability (standard deviation), and extreme values (minimum and maximum) of the analyzed numerical features.

Before proceeding with further statistical analyses, the distribution of the scores of the applied scales was verified. Transformation of the predictor and criterion variables was then performed using normalization and standardization, given that the initial distributions significantly deviated from the normal. In further analysis, the *t*-test was used to compare means between two groups, and analysis of variance (ANOVA) was used to compare means across three or more groups. Spearman correlation was used to assess relationships between continuous variables.

Results

Demographic data

Out of 550 HIP undergoing treatment, 226 (41.2%) were included in the research. The average age of the participants was 41.3 years (Table 1).

All participants were on antiretroviral therapy (ART) and had undetectable viremia for at least six months prior to this study. Most participants (61%) were on integrase strand transfer inhibitor (INSTI)-based treatment, 30% were on non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART, while 9% were on protease inhibitor (PI)-based treatment. HIP who were on unconventional or salvage treatments were not included in the study. The average duration of ART was 5.6 years. Regarding the CD4+ T-cell count, 19.7% of the participants had previously AIDS, while 48.0% of the subjects had a nadir $CD4^+$ T-cell count of less than 350 cells/mm^3. The current $CD4^+$ T-cell count was between 200 and 350 cells/mm^3 in only 3.7% of the subjects, while all other subjects (96.3%) had a CD4 T-lymphocyte count over 350 cells/mm^3. The current CD4/CD8 ratio was above 1.0 in 32%, while in the rest of the participants, this ratio was below 1.0, potentially indicating persistent inflammation in these participants.

Regarding ways of transmission, all the subjects included in the study have been infected by the sexual route, and none were infected due to intravenous drug use.

Vaccination against COVID-19

Of 226 participants in the study, 171 (75%) had been vaccinated with at least two vaccine doses. All vaccinated participants received a homologous primary series of one vaccine type. In individuals who received booster doses, the same type of vaccine was used as in the primary series.

Out of all vaccinated participants, 64 (37.4%) were both vaccinated and previously diagnosed with COVID-19, and 107 (62.6%) were vaccinated but were not previously diagnosed with COVID-19 (Table 2). The most commonly received vaccine, in 59.6% of vaccinated participants, was the mRNA. The second most commonly received was the inactivated vaccine, taken up by 25.1% of participants, while the rest (15.3%) received one of the two available vector vaccines. According to the results of the interviews and medical records, no significant adverse effects were observed with any type of vaccine. In the group analysis, there was no statistically significant difference in age, ART duration, and immunological parameters among the vaccine type groups.

Regarding the number of doses, 62% of vaccinated HIP received three doses and 38% received two vaccine doses (Table 2). Full vaccination with three vaccine doses was

Table 1

General characteristics of the cohort

Parameters	Mean ± SD	Min–Max
Age (years)	41.3 ± 12.0	20–74
Nadir $CD4^+$ T-cell count (cells/mm^3)	401.3 ± 298.4	3–1,514
Nadir $CD8^+$ T-cell count (cells /mm^3)	1,147.7 ± 709.3	149–8,299
Current $CD4^+$ T-cell count (cells /mm^3)	1,075.5 ± 991.7	270–14,422
Current $CD8^+$ T-cell count (cells /mm^3)	1,351.8 ± 484.0	259–2,200
Current CD4/CD8 ratio	0.8 ± 0.3	0.2–2.51
Duration of ART (years)	5.5 ± 4.5	0.5–23
SARS-CoV-2 anti-spike IgG titer (AU/mL)	8,832.1 ± 29,527.3	9.0–42,000

SD – standard deviation; ART – antiretroviral therapy; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; IgG – immunoglobulin G; AU – arbitrary units; min – minimum; max – maximum.

Table 2

Comparison of variables based on the number and type of the received vaccine and registered COVID-19

Variables	Registered COVID-19	n	Age (years)	Years of ART	Nadir CD4 (cells/mm^3)	Nadir CD8 (cells/mm^3)	IgG anti-spike antibody titer (AU/mL)
Three vaccine doses ‡							
mRNA	yes	30	41.5	5.9	391.4	1,205.5	16,526.3 ± 13,838.3*†
	no	28	43.1	6.0	404.0	1,441.1	12,260.7 ± 1,1474.0
inactivated	yes	7	40.0	7.3	320.1	945.1	4,257.5 ± 4,004.8*
	no	23	49.1	8.1	263.6	1,022.7	7,315.2 ± 6,599.0
vector	yes	5	39.0	5.0	481.2	1,229.0	11,411.5 ± 11,194.3
	no	13	44.9	5.9	430	1,091.2	9,714.0 ± 9,606.0
Two vaccine doses							
mRNA	yes	14	35.5	4.8	533.0	998.9	5,534.6 ± 2,850.5†
	no	30	36.9	3.6	494.3	1,083.3	4,763.8 ± 4,344.1
inactivated	yes	4	41.7	4.2	584.0	1,562.5	7,507.2 ± 2,995.7**
	no	9	36.8	4.1	432.1	946.0	1,812.5 ± 1,703.0**
vector	yes	4	40.5	4.25	434.0	970.5	2,047.2 ± 1,784.2
	no	4	33.3	2.3	610.0	1,242.3	10,497.0 ± 8,786.0
Not vaccinated‡	yes	55	41.8	5.1	366.9	1,002.8	1,330.5 ± 1,765.1

n – number of participants; *signifficant difference between the three-dose mRNA vaccine group vs. the three-dose inactivated vaccine group ($p = 0.04$); †signifficant difference between the three-dose mRNA vaccine group vs. the two-dose mRNA vaccine group ($p = 0.005$); **signifficant difference between the two-dose inactivated vaccine with no registered COVID-19 group vs. the two-dose inactivated vaccine with registered COVID-19 group ($p = 0.0016$); ‡signifficant difference between all groups vaccinated with three doses vs. the non-vaccinated group ($p < 0.005$).
COVID-19 – coronavirus disease 2019; mRNA – messenger ribonucleic acid; ART – antiretroviral therapy; AU – arbitrary units; IgG – immunoglobulin G.
All values are given as mean ± standard deviation or number.

achieved in 56.8% of individuals in the mRNA group, in 60.0% of the vector vaccine group, and in 69.7% of the inactivated group.

In the group analysis, booster dose was more likely to be received by HIP previously diagnosed with AIDS, who were over 40 years of age and on ART for more than 5 years ($p = 0.05$).

In total, 55 (24.3%) participants included in the study had not been vaccinated against COVID-19. There were no statistically significant differences between vaccinated and unvaccinated participants in age, ART duration, nadir or current CD4$^+$ and CD8$^+$ T-cell counts, or current CD4/CD8 ratio.

SARS-CoV-2 anti-spike IgG antibody titer

The lowest values of antibody titers were seen in persons who had COVID-19 and were not vaccinated against the disease. Significant differences in antibody titers were seen among unvaccinated HIP compared to all vaccinated HIP with three doses, regardless of the type of vaccine ($p < 0.005$).

The highest values of titers were seen in HIP who received the mRNA vaccine. In individuals who both had COVID-19 and received a vaccine, the titer in the mRNA vaccine group was statistically higher than that in the inactivated vaccine group ($p = 0.04$). However, no differences were found between the mRNA group and the vector vaccine group. In the analysis of HIP who were vaccinated but did not have COVID-19, the humoral response did not differ among the vaccine types.

Regarding the number of vaccine doses, the greatest benefit from the booster was found in the mRNA vaccine group. In this group, there was a great difference in antibody titers between the persons who received a booster compared to the persons who received two vaccine doses ($p = 0.005$). No such boosting effect was seen in the remaining two types of vaccines. The greatest effect of COVID-19 on antibody titer was observed in the inactivated vaccine group. In this group, there was a statistically significant difference in the titers between the persons who had and did not have the diseases ($p = 0.0016$).

In all vaccinated participants, antibody titer had a slight but significant negative correlation with age ($r = -0.90$, $p = 0.04$). The correlation between age and antibody titer was stronger in participants who were both vaccinated against and diagnosed with COVID-19 ($r = -0.27$, $p = 0.036$). ART duration negatively correlated with antibody titer in vaccinated individuals who were also diagnosed with COVID-19 ($r = -0.39$, $p = 0.02$). The correlation was absent between those who were vaccinated but never infected, and vice versa.

Nadir and current CD4$^+$ T-cell count, as well as nadir and current CD4/CD8 ratio, did not correlate with antibody titers in vaccinated participants. Furthermore, there were no statistically significant differences in antibody titers between individuals whose nadir CD4$^+$ T-cell count was below 200 cells/mL^3 and those who were never immunocompromised. On the other hand, nadir CD8 levels had a positive impact on antibody titers.

The time since the last vaccine dose was, on average, 96 days (min 60, max 150). The time since COVID-19 in

vaccinated HIP was, on average, 94 days (min 18, max 206). Neither time period correlated with antibody titers.

Similar results were observed in the unvaccinated HIP group. The antibody titer in this group did not correlate with the time since COVID-19 diagnosis (mean 109, min 25, max 256). In addition, antibody titers did not correlate with other factors, such as nadir and current $CD4^+$ and $CD8^+$ T-cell counts, age, and ART duration.

Discussion

Based on previous experience with other causes of atypical pneumonia, such as influenza, SARS-CoV-2 infection was initially considered especially dangerous for HIP. As efforts to combat the virus intensified, the development and deployment of vaccines emerged as crucial actions in controlling the spread and mitigating the impact of COVID-19. In this study, we compared humoral responses in HIP who had suppressed viral replication at the time of study with good immunological status but who received different types of anti-COVID-19 vaccine and a number of doses.

Unlike HIV infection, which is fatal in the absence of ART, COVID-19 has a variable clinical course, especially in immunocompromised individuals [10, 11]. Although SARS-CoV-2 infection was mild in the majority of cases, there have been reports of poor outcomes due to the development of acute respiratory distress syndrome. In our study, the poorest humoral response to the infection was in HIP who were not vaccinated, which supports the concern that the immune response to SARS-CoV-2 may be partly inadequate in this population.

Due to widely available ART, HIP are an aging population. One of the factors that stood out as a negative predictor for poorer humoral response to vaccines in our study was advanced age. Age negatively correlated with antibody titers in all vaccinated participants in our study. However, the correlation was not present in those who developed humoral immune response through natural exposure to the virus. This result is not surprising, as previous studies identified advanced age as a significant negative modulator of humoral response following two-doses of anti-COVID-19 vaccine, warranting a three-dose vaccination regimen in older individuals [12, 13].

Many studies examined the effect of CD4 T-cell count on immunogenicity in HIP. In a review by Søndergaard et al. [13], a lower serological response was associated with HIP with a lower $CD4^+$ T-cell count in 26 out of 59 studies (44%). Similarly, in the meta-analysis by Zhou et al. [14], the seroconversion was 4.6 times higher in HIP with higher $CD4^+$ T-cell counts than in HIP with lower CD4 counts. We did not find any correlation between $CD4^+$ T-cell count and antibody titer, although none of the patients had a current $CD4^+$ T-cell count below 200 cells/mL^3.

HIV infection is characterized by a profound disruption of the immune system, and even in HIP with fully suppressed viral load, up to 30% of patients will suffer incomplete immune recovery [15, 16]. Therefore, it was essential to investigate T-cell count in relation to antibody titers in the participants of this study. There was no correlation between $CD4^+$ T-cell count and CD4/CD8 ratio and the antibody titer levels, regardless of the type of vaccine. However, we found that the nadir $CD8^+$ T-cell count correlated with the level of humoral response to vaccination, indicating the importance of these elements in the cellular response to immunization. We did not find any studies that specifically examined nadir CD8 as a predictor of humoral response to anti-COVID-19 vaccines. Nevertheless, there is plenty of data on the importance of $CD8^+$ T-cell response to pathogens and vaccination. Activated $CD8^+$ T-cells can induce apoptotic death of virus-infected cells by producing tumor necrosis factor-alpha and interferon-gamma (IFN-γ) [17–19]. Early mRNA vaccine studies revealed that all arms of adaptive immunity respond to immunization, including $CD8^+$ T-cells that produce IFN-γ [20–22]. Recent studies suggest that IFN-γ production from $CD8^+$ T-cells enhances cellular and humoral immune responses following immunization [23, 24]. Based on these and similar studies, it is indicated that individuals with higher nadir CD8+ T-cell counts may have a better immune response, including that toward vaccines.

Regarding the number of doses, more than half of the participants (62%) received three doses of the vaccine. Based on the results of our study, there was a significant increase in antibody titer after the third dose in the case of the mRNA vaccine. We did not find similar boosting effects in the other two vaccine types. Still, in agreement with many previous studies and national recommendations, we would also recommend giving three doses of the vaccine [25–27].

Regarding the type of vaccine, at the time of vaccination of our cohort, there were no specific guidelines on which type of vaccine is better for HIP. The data were just coming out on the supreme immunogenicity of the mRNA vaccine in the general population, but some HIP were hesitant to receive the novel type of vaccine. No patients reported serious adverse effects with any of the vaccines, and all patients seroconverted. Most participants (59.6%) received the mRNA vaccine, a quarter of the participants received the inactivated vaccine (25.1%), and 15.3% received the vector vaccine. We showed a statistically significant difference in antibody titers between the mRNA vaccine compared to the inactivated vaccine in persons who previously had COVID-19.

The favorable humoral response to the mRNA vaccine found in our study is in accordance with most real-world studies on vaccination in HIP. Several meta-analyses [14, 27, 28] clearly showed the supreme immunogenicity of the mRNA vaccine in HIP.

A limitation of our study was its cross-sectional nature. As HIP received the last vaccine dose 3 to 6 months before IgG titer measurement, a follow-up study of titer values might show the immunogenicity of different types of vaccines and the degree of long-term protection conferred by different types of vaccines. Another limitation of the study was the great variability of the antibody titers, which clearly shows that individual variation in humoral response to vaccination goes far beyond the factors investigated in this study.

Conclusion

All available vaccines against coronavirus disease 2019 have led to seroconversion in our cohort. Although there was significant variation from the mean in achieved antibody titers, it appears that age and time since introduction to antiretroviral therapy may be factors that have a negative influence on humoral response to immunization. Attention is needed in older patients and those who have been on antiretroviral therapy for a long time, who may therefore need more booster doses and more frequent antibody measurements. Our findings are in agreement with general recommendations for the safe and effective use of the messenger ribonucleic acid vaccine in persons infected with human immunodeficiency virus.

REFERENCES

1. *Barbera LK, Kamis KF, Rowan SE, Davis AJ, Shehata S, Carlson JJ,* et al. HIV and COVID-19: review of clinical course and outcomes. HIV Res Clin Pract 2021; 22(4): 102–18.
2. *Ambrosioni J, Blanco JL, Reyes-Urueña JM, Davies MA, Sued O, Marcos MA,* et al. Overview of SARS-CoV-2 infection in adults living with HIV. Lancet HIV 2021; 8(5): e294–305.
3. *Baskaran V, Lawrence H, Lansbury LE, Webb K, Safavi S, Zainuddin NI,* et al. Co-infection in critically ill patients with COVID-19: an observational cohort study from England. J Med Microbiol 2021; 70(4): 001350.
4. *Del Amo J, Polo R, Moreno S, Jarrín I, Hernán MA.* SARS-CoV-2 infection and coronavirus disease 2019 severity in persons with HIV on antiretroviral treatment. AIDS 2022; 36(2): 161–8.
5. *Yang X, Sun J, Patel RC, Zhang J, Guo S, Q Zheng,* et al. Associations between HIV infection and clinical spectrum of COVID-19: a population level analysis based on US National COVID Cohort Collaborative (N3C) data. Lancet HIV 2021; 8(11): e690–700.
6. *Bertagnolio S, Thwin SS, Silva R, Nagarajan S, Jassat W, Fowler R,* et al. Clinical features of, and risk factors for, severe or fatal COVID-19 among people living with HIV admitted to hospital: analysis of data from the WHO Global Clinical Platform of COVID-19. Lancet HIV 2022; 9(7): e486–95.
7. *Madhi SA, Koen AL, Izu A, Fairlie L, Cutland CL, Baillie V,* et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in people living with and without HIV in South Africa: an interim analysis of a randomised, double-blind, placebo-controlled, phase 1B/2A trial. Lancet HIV 2021; 8(9): e568–80. Erratum in: Lancet HIV 2022; 9(12): e822.
8. *Ruddy JA, Boyarsky BJ, Bailey JR, Karaba AH, Garonzik-Wang JM, Segev DL,* et al. Safety and antibody response to two-dose SARS-CoV-2 messenger RNA vaccination in persons with HIV. AIDS 2021; 35(14): 2399–401.
9. *Maine GN, Krishnan SM, Walewski K, Trueman J, Sykes E, Sun Q.* Clinical and analytical evaluation of the Abbott AdviseDx quantitative SARS-CoV-2 IgG assay and comparison with two other serological tests. J Immunol Methods 2022; 503: 113243.
10. *Popovska Jovičić B, Raković I, Pavković A, Marković V, Petrović S, Gavrilović J,* et al. Significance of initial clinical laboratory parameters as prognostic factors in patients with COVID 19. Vojnosanit Pregl 2022; 79(9): 849–56.
11. *Gojković Z, Djokanović D, Nikić G, Jović-Djokanović O, Mavija Z, Rakita I,* et al. COVID-19 infection in patients with malignant diseases. Vojnosanit Pregl 2020; 77(11): 1235–6.
12. *Brumme ZL, Mwimanzi F, Lapointe HR, Cheung PK, Sang Y, MC Duncan,* et al. Humoral immune responses to COVID-19 vaccination in people living with HIV receiving suppressive antiretroviral therapy. NPJ Vaccines 2022; 7(1): 28.
13. *Søndergaard MH, Thavarajah JJ, Churchill Henson H, Wejse CM.* SARS-CoV-2 vaccine immunogenicity for people living with HIV: a systematic review and meta-analysis. HIV Med 2024; 25(1): 16–37.
14. *Zhou Q, Liu Y, Zeng F, Meng Y, Liu H, Deng G.* Correlation between CD4 T-cell counts and seroconversion among COVID-19 vaccinated patients with HIV: a meta-analysis. Vaccines (Basel) 2023; 11(4): 789.
15. *Kelley CF, Kitchen CM, Hunt PW, Rodriguez B, Hecht FM, Kitahata M,* et al. Incomplete peripheral CD4+ cell count restoration in HIV-infected patients receiving long-term antiretroviral treatment. Clin Infect Dis 2009; 48(6): 787–94.
16. *Gazzola L, Tincati C, Bellistré GM, d'Arminio Monforte A, Marchetti G.* The absence of CD4+ T cell count recovery despite receipt of virologically suppressive highly active antiretroviral therapy: clinical risk, immunological gaps, and therapeutic options. Clin Infect Dis 2009; 48(3): 328–37.
17. *Harty JT, Tvinnereim AR, White DW.* CD8+ T cell effector mechanisms in resistance to infection. Annu Rev Immunol 2000; 18: 275–308.
18. *Schmidt ME, Varga SM.* The CD8 T Cell Response to Respiratory Virus Infections. Front Immunol 2018; 9: 678.
19. *Oberhardt V, Luxenburger H, Kemming J, Schulien I, Ciminski K, Giese S,* et al. Rapid and stable mobilization of CD8+ T cells by SARS-CoV-2 mRNA vaccine. Nature 2021; 597(7875): 268–73.
20. *Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, M Vormehr,* et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 2020; 586(7830): 594–9. Erratum in: Nature 2021; 590(7844): E17.
21. *Sahin U, Muik A, Vogler I, Derhovanessian E, Kranz LM, Vormehr M,* et al. BNT162b2 vaccine induces neutralizing antibodies and poly-specific T cells in humans. Nature 2021; 595(7868): 572–7.
22. *Li C, Lee A, Grigoryan L, Arunachalam PS, Scott MK, M Trisal,* et al. Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech BNT162b2 vaccine. Nat Immunol 2022; 23(4): 543–55.
23. *Borriello F, Poli V, Shrock E, Spreafico R, Liu X, Pishesha N,* et al. An adjuvant strategy enabled by modulation of the physical properties of microbial ligands expands antigen immunogenicity. Cell 2022; 185(4): 614–29.e21.
24. *Mullender C, da Costa KA, Alrubayyi A, Pett SL, Peppa D.* SARS-CoV-2 immunity and vaccine strategies in people with HIV. Oxf Open Immunol 2022; 3(1): iqac005.
25. *Levy I, Rahav G.* The effect of HIV on COVID-19 vaccine responses. Curr Opin HIV AIDS 2023; 18(3): 135–41.
26. *Bessen C, Plaza-Sirvent C, Simsek A, Bhat J, Marheinecke C, Urlaub D,* et al. Impact of SARS-CoV-2 vaccination on systemic immune responses in people living with HIV. Front Immunol 2022; 13: 1049070.
27. *Augello M, Bono V, Rovito R, Tincati C, Marchetti G.* Immunologic interplay between HIV/AIDS and COVID-19: adding fuel to the flames? Curr HIV/AIDS Rep 2023; 20(2): 51–75.
28. *Ouzounakis P, Frantzana A, Iliadis C, Mihalache A, Alefragkis D, Kourkouta L.* HIV infection and vaccinations. World J Adv Res Rev 2023 ; 17(3): 101–6.

Received on December 6, 2024
Revised on January 14, 2025
Accepted on January 29, 2025
Online First April 2025

ORIGINAL ARTICLE

UDC: 655.413:616.314
DOI: https://doi.org/10.2298/VSP240728018K

A bibliometric analysis of 3D printing in endodontics

Bibliometrijska analiza 3D štampanja u endodonciji

Anil Ozgun Karatekin

Uskudar University, Faculty of Dentistry, Department of Endodontics, Istanbul, Turkey

Abstract

Background/Aim. In recent years, owing to the development of the three-dimensional (3D) printing method, managing complex clinical cases and educational and standardized experimental models has become possible, with a sharp increase in publications from 2018. The aim of this study was to reveal the bibliometric analysis and trends of the studies published in the field of the use of 3D printing in endodontics in the last 10 years. **Methods.** Studies published between 2014 and 2023 were accessed in the Web of Science Core Collection database, and the data obtained were examined with bibliometric analysis in terms of countries, journals, publication years, publication numbers, publication types, topic tendencies, and citation analysis. **Results.** A total of 128 papers were included in the study. The retrieved papers received an average of 13.59% of citations *per* publication. The first three countries with the highest number of publications were the People's Republic of China, the United States of America (USA), and Germany. At the same time, the first three countries with the highest number of citations were Germany, Switzerland, and USA. In the co-authorship analyses, it can be seen that the highest total link strength is between Germany and Switzerland, while the USA is the country with the highest number of co-authorships. The top five keywords with the highest total link strength were "3D printing", "guided endodontics", "endodontics", "root canal treatments", and cone-beam computed tomography or "CBCT". **Conclusion.** To our knowledge, this study is the first bibliometric analysis of worldwide research on the use of 3D printing in the endodontic field. Such analyses need to be conducted regularly to closely monitor the development of this research field.

Key words:
bibliometrics; cone-beam computed tomography; database; endodontics; printing, three-dimensional.

Apstrakt

Uvod/Cilj. Poslednjih godina, zahvaljujući razvoju metode trodimenzionalnog (3D) štampanja, postalo je moguće rešavanje složenih kliničkim slučajeva i primena edukativnh i standardizovanh eksperimentalnih modela, uz nagli porast publikacija od 2018. godine. Cilj ove studije bio je da prikaže bibliometrijsku analizu i trendove istraživanja objavljenih u oblasti upotrebe 3D štampe u endodonciji u poslednjih 10 godina. **Metode.** Studijama objavljenim između 2014. i 2023. godine pristupalo se u bazi podataka *Web of Science Core Collection*, a dobijeni podaci ispitani su bibliometrijskom analizom u pogledu zemalja, časopisa, godina izdavanja, broja publikacija, tipova publikacija, tendencija tema i analize citata. **Rezultati.** U studiju je bilo uključeno ukupno 128 radova. Preuzeti radovi su u proseku dobili 13,59% citata po publikaciji. Prve tri zemlje sa najvećim brojem publikacija bile su Narodna Republika Kina, Sjedinjene Američke Države (SAD) i Nemačka. Istovremeno, prve tri zemlje sa najvećim brojem citata bile su Nemačka, Švajcarska i SAD. Najveća ukupna snaga povezanosti koautora pokazana je između Nemačke i Švajcarske, dok je SAD država sa najvećim brojem koautorstava. Top pet ključnih reči sa najvećom ukupnom snagom povezanosti bile su "3D štampanje", "vodjena endodoncija", "endodoncija", tretmani kanala korena i kompjuterizovana tomografija konusnog zraka (*cone-beam computed tomography* – "CBCT"). **Zaključak.** Prema našim saznanjima, ova studija je prva bibliometrijska analiza svetskih istraživanja o upotrebi 3D štampe u endodontskoj oblasti. Takve analize treba redovno sprovoditi kako bi se pomno pratio razvoj ove istraživačke oblasti.

Ključne reči:
bibliometrija; tomografija, kompjuterizovana, konusna; baze podataka; endodoncija; štampanje, trodimenzionalno.

Correspondence to: Anil Ozgun Karatekin, Uskudar University, Faculty of Dentistry, Department of Endodontics, Saray Mahallesi, Ahmet Tevfik Ileri Caddesi 5, 34 768, Umraniye, Istanbul, Turkiye. E-mail: anilozgun.karatekin@uskudar.edu.tr

Introduction

Three-dimensional (3D) printing technology in dentistry is used, along with treatment planning and surgical guidance, to produce dental models for orthognathic surgery, implant surgery, oral and maxillofacial surgery, orthodontics, and prosthetic appliances [1]. Using 3D printing, high-resolution models can be produced from resin with an automatic production process and layered on top of each other, with a resolution of 16–32 μm for each layer. Rapid prototyping of natural teeth is very promising and has particular potential for inclusion in endodontic education. In addition, it allows the standardization of important samples in laboratory studies such as root canal instrumentation, filling, and retreatment [2].

Working on models obtained by 3D printing in endodontics is of great importance in terms of preclinical education and gaining experience for clinicians by working with replicas of teeth in different variations. In literature, the 3D guided endodontics technique has been studied increasingly in different clinical situations and indications (access cavity preparation, calcified canals, endodontic surgery, fiber post removal, teeth with developmental anomalies, and/or complicated root canal formation) [3–6].

Bibliometric methods or analysis are now considered a field of scientific expertise and, in many fields, have become an integral part of research evaluation methodology. Visualizing a specific field or subject in a certain systematic way and analyzing the relationship between authors or publications can be called bibliometric mapping or scientific mapping [7]. When explaining the relationship between bibliometric analysis and citations, scientific mapping comes to the fore. Scientific mapping and bibliometric analyses that enable us to recognize the relevant field have reached a high-quality stage. Advanced bibliometric analyses are seen as an indispensable element in the evaluation of research [8].

The aim of this study was to reveal the bibliometric analysis and trends of the studies published in the field of the use of 3D printing in endodontics in the last 10 years. In this way, it aims to increase the awareness of the authors and subjects that make up the discipline and how to direct the studies of the researchers in this field against the increasing number of papers.

Methods

Data collection tools and process

A search was made on January 1, 2024, on the Web of Science (WoS) Core Collection (WoSCC) database, using keywords “3D printing, root canal”, “3D printing, endodontics”, “replica, root canal”, and “replica, endodontics”. The data obtained were examined with bibliometric analysis in terms of countries, journals, publication years, publication numbers, publication types, subject tendencies, citation analysis, as well as the universities that supported the research.

When the keywords are entered as a subject in the WoSCC database, publications containing the words in the title, abstract, and keywords are scanned and ranked. With this screening, 128 studies published between 2014 and 2023 were accessed and analyzed.

Bibliometric analysis and mapping

Some of the data obtained as a result of the research were arranged in tabular form and expressed as percentages and frequencies. The findings of the research were analyzed using the descriptive analysis technique. Density and network maps were used with the help of the bibliometric mapping program VOSviewer 1.6.18, one of the software programs developed for bibliometric purposes [9].

Results

Main information of the collection

In the search, a total of 128 publications were reached, with the keywords specified in the last ten years. General information on the publications is presented in Table 1.

Table 1

General information of the publications

Publications	number (%)
Document type	
article	113 (88.28)
review article	13 (10.15)
proceeding paper	5 (3.90)
early access	41 (32.03)
Web of Science Categories	
Dentistry Oral Surgery Medicine	95 (74.21)
Materials Science Multidisciplinary	10 (7.81)
Education Scientific Disciplines	7 (5.46)
Materials Science Biomaterials	7 (5.46)
Physics Applied	7 (5.46)
Web of Science Index	
Science Citation Index Expanded	111 (86.71)
Emerging Sources Citation Index	15 (11.71)
Conference Proceedings Citation Index – Science	5 (3.90)
Social Sciences Citation Index	2 (1.56)
Total number of citations	1,739
Average citations *per* document, %	13.59

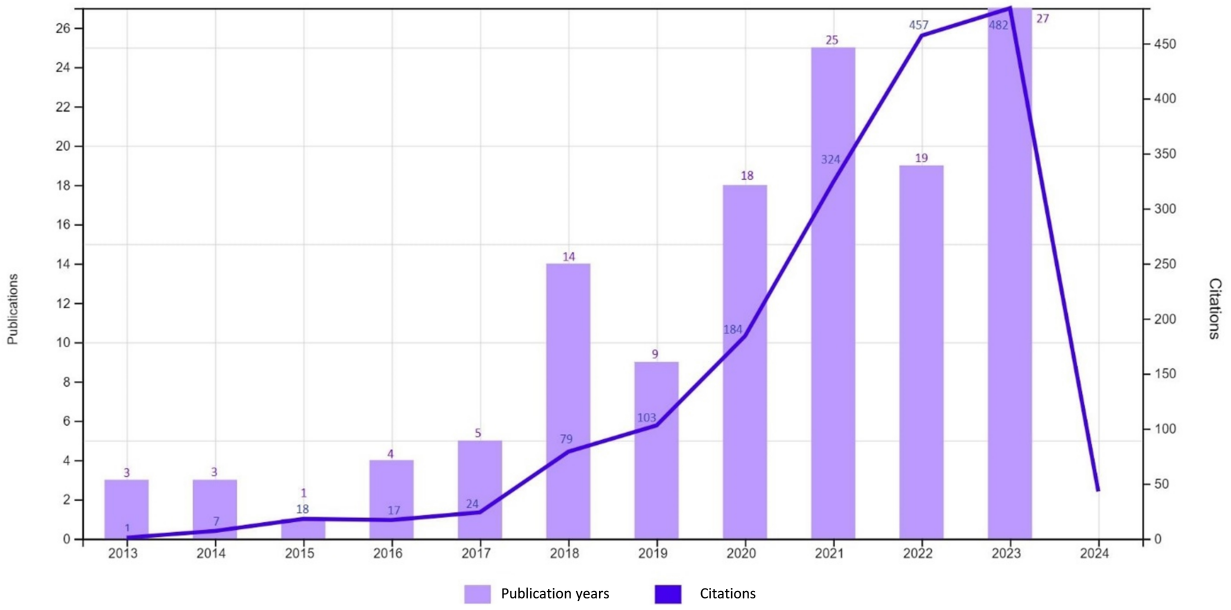


Fig. 1 – Times cited and publications over time.

Number of articles, review articles, and proceeding papers accounted for 113 (88.28%), 13 (10.15%), and 5 (3.90%), respectively. Forty-one (32.03%) of these publications were early access. When the annual publication numbers are examined, the number of annual publications, which was 3 in 2014, 1 in 2015, 4 in 2016, and 5 in 2017, increased rapidly to 14 in 2018, 18 in 2020, 25 in 2021, and 27 in 2023 (Figure 1). According to WoS categories, the five most published categories were Dentistry Oral Surgery Medicine [95 (74.21%)], Materials Science Multidisciplinary [10 (7.81%)], Education Scientific Disciplines [7 (5.46%)], Materials Science Biomaterials [7 (5.46%)], and Physics Applied [7 (5.46%)]. According to WoS indexes, 111 publications were indexed by Science Citation Index Expanded (SCIE), 15 publications by Emerging Sources Citation Index (ESCI), 5 publications by Conference Proceedings Citation Index – Science (CPCI-S), and 2 publications by Social Sciences Citation Index (SSCI). With an average of 13.59% of citations *per* publication, the collection received a total of 1,739 citations.

Most cited papers

Eleven publications [10–20] with the highest number of citations are listed in Table 2, including additional information about the journal and authors. Upon detailed analysis, it was seen that the ninth most cited article in the WoS search was not related to 3D printing [18], so the eleventh-ranked article was also included in the Table [20]. Five of the publications were Articles [10, 13, 14, 16, 18] and six were Review Articles [11, 12, 15, 17, 19, 20]. The total citation number of these publications was 825 (47.44% of the total citation number of all publications).

The first and fourth most cited publications were consecutively linked studies [10, 13]. Considering the distribution of the subjects, the main focus of the studies was on 3D printed guided endodontics by four publications [10, 13, 16, 17], review of 3D printing in dentistry by two [11, 12], endodontic applications of 3D printing by two [15, 20], 3D printed replicas for endodontic education by one [14], cone-beam computed tomography (CBCT) for 3D reconstruction of artificially created periapical bone defects by one [18], review of advanced biomaterials and techniques including additive manufacturing (3D bioprinting) technologies for oral tissue engineering and regeneration by one [19].

Even the words endodontics and 3D printing were presented in the study by Tian et al. [12], the third most cited study. The study was mainly focused on prosthodontics, surgery, and implantology applications of 3D printing, and endodontics applications were vaguely mentioned. Therefore, this situation shows the deficiency of WoS keyword search for precise access to articles meeting the researcher's search interest.

Global cooperation and the most productive countries

The distribution of countries according to the number of publications and citations is given in Table 3. Considering the number of publications, the first three countries with the highest number of publications are the People's Republic of China (PRC), the United States of America (USA), and Germany. At the same time, the first three countries with the highest number of citations are Germany, Switzerland, and USA. It can be seen in the Table that seven countries have over 100 citations.

There were 10 countries in the country co-authorship mapping. This analysis yields three clusters represented in different colors. The first cluster was identified using green, the second cluster was identified with red, and the third was identified with yellow. According to Figure 2, co-authorship

Table 2

The 10 most cited publications

Number	Publications	Authors	Journal	Type of article	First author's country	Year	Total citations	Average citation/year
1.	Guided endodontics: accuracy of a novel method for guided access cavity preparation and root canal location (Zehnder et al., 2016) [10]	Zehnder, MS; Connert, T; Weiger, R; Krastl, G; Kühl, S	International Endodontic Journal	Article	Switzerland	2016	134	14.89
2.	3D Printing-Encompassing the Facets of Dentistry (Oberoi et al., 2018) [11]	Oberoi, G; Nitsch, S; Edelmayer, M; Janjic, K; Müller, AS; Agis, H	Frontiers in Bioengineering and Biotechnology	Review Article	Austria	2018	95	13.57
3.	A Review of 3D Printing in Dentistry: Technologies, Affecting Factors, and Applications (Tian et al., 2021) [12]	Tian, YY; Chen, CX; Xu, XT; Wang, JY; Hou, XY; Li, KL; Lu, XY; Shi, HY; Lee, ES; Jiang, HB	Scanning	Review Article	People's Republic of China	2021	93	23.25
4.	Microguided Endodontics: a method to achieve minimally invasive access cavity preparation and root canal location in mandibular incisors using a novel computer-guided technique (Connert et al., 2018) [13]	Connert, T; Zehnder, MS; Amato, M; Weiger, R; Kühl, S; Krastl, G	International Endodontic Journal	Article	Switzerland	2018	77	11.00
5.	3D printed replicas for endodontic education (Reymus et al., 2019) [14]	Reymus, M; Fotiadou, C; Kessler, A; Heck, K; Hickel, R; Diegritz, C	International Endodontic Journal	Article	Germany	2019	72	12.00
6.	Endodontic applications of 3D printing (Anderson et al., 2018) [15]	Anderson, J; Wealleans, J; Ray, J	International Endodontic Journal	Review Article	United States of America	2018	71	10.14
7.	3D Computer aided treatment planning in endodontics (van der Meer et al., 2016) [16]	van der Meer, WJ; Vissink, A; Ng, YL; Gulabivala, K	Journal of Dentistry	Article	Netherlands	2016	71	7.89
8.	Clinical applications, accuracy and limitations of guided endodontics: a systematic review (Moreno-Rabié et al., 2020) [17]	Moreno-Rabié, C; Torres, A; Lambrechts, P; Jacobs, R	International Endodontic Journal	Review Article	Belgium	2019	60	10.00
9.	Detection and measurement of artificial periapical lesions by cone-beam computed tomography (Liang et al., 2014) [18]	Liang, YH; Jiang, L; Gao, XJ; Shemesh, H; Wesselink, PR; Wu, MK	International Endodontic Journal	Article	People's Republic of China	2014	55	5.00
10.	Advanced Biomaterials and Techniques for Oral Tissue Engineering and Regeneration-A Review (Matichescu et al., 2020) [19]	Matichescu, A; Ardelean, LC; Rusu, LC; Craciun, D; Bratu, EA; Babucea, M; Leretter, M	Materials	Review Article	Romania	2020	49	9.80
11.	3D imaging, 3D printing, and 3D virtual planning in endodontics (Shah and Chong, 2018) [20]	Shah, P; Chong, BS	Clinical Oral Investigations	Review Article	England	2018	48	6.86

Table 3

Most productive countries according to the number of publications and citations

Country	Total publications	TC (Rank of TC)
People's Republic of China	24	235 (4)
United States of America	21	255 (3)
Germany	17	444 (1)
England	9	208 (5)
South Korea	9	189 (6)
France	8	63 (9)
Switzerland	8	257 (2)
Brazil	7	26 (10)
Canada	7	76 (8)
Belgium	6	165 (7)

TC – total citations

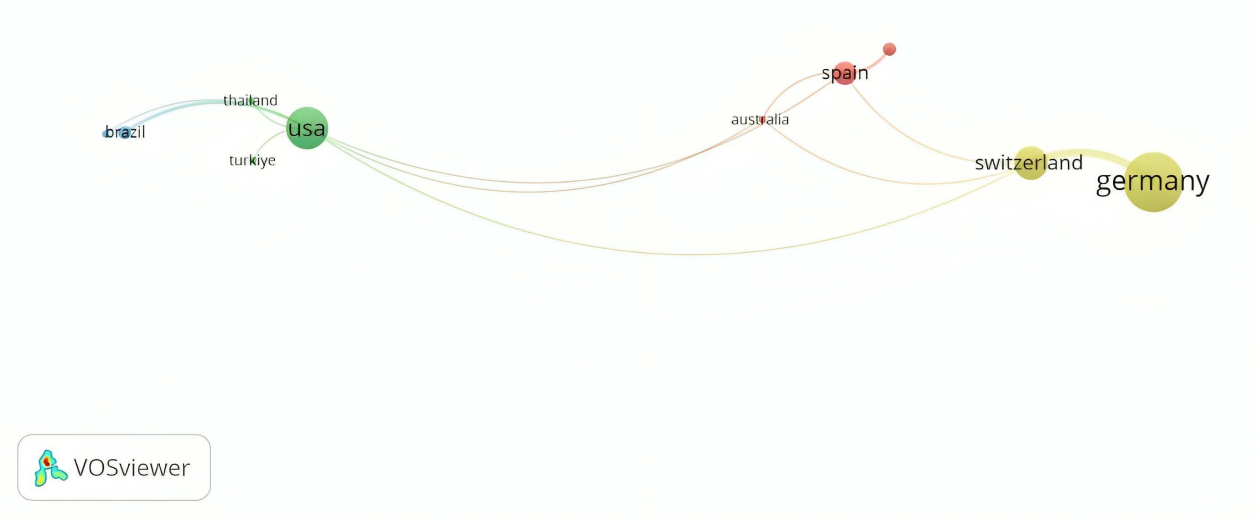


Fig. 2 – Co-authorship analysis of countries.

Table 4

Most productive authors

Author	Institution	Number of articles	Number of citations	H-index
Thomas Connert	University of Basel Univeristy Center for Dental Medicine Basel Eberhard Karls University of Tubingen	6	248	5
Gabriel Krastl	University of Wurzburg University of Birmingham University of Basel	6	248	5
Reinhilde Jacobs	Karolinska Institutet University Hospital Leuven KU Leuven	5	124	3
Marcel Reymus	University of Munich Ludwig Maximilians Univ Munchen	5	146	4
Andres Torres	Siemens EDA Universidade de Santiago de Compostela Universite Catholique Louvain	5	124	3

analysis yields the highest total link strength between Germany and Switzerland, while the USA has the highest number of co-authorships.

Most productive authors

The five most productive authors by number of articles are shown in Table 4. Note that an author may use multiple institutional affiliations, but the information shown in Table 4 was collected from their WoS profiles.

A collaborative network of 26 co-authors having published more than two papers (isolated authors not included). Each node maps to an author. Node size is proportional to the number of articles, while lines between nodes indicate the strength of collaboration. Six groups of co-authorship have been formed. The biggest two clusters consist

of seven authors (red and green), one cluster consists of five authors (blue), one cluster of three authors (yellow), and two clusters of two authors (purple and turquoise) (Figure 3).

Most popular journals

Table 5 shows the top ten journals that have published the most papers related to the given keywords. Only one journal (International Endodontic Journal) published nine articles. One journal does not qualify for the WoSCC (Dentistry Journal), and the remaining nine were qualified for SCIE. Among the top ten journals, three were classified in the first quartile (Q1), three in the second (Q2), two in the third (Q3), and one in the fourth quartile (Q4).

Main topics of the research

Keyword co-occurrence analysis is based on the comparative study of the terms of all keywords in order to determine the close relationship between concepts. Within

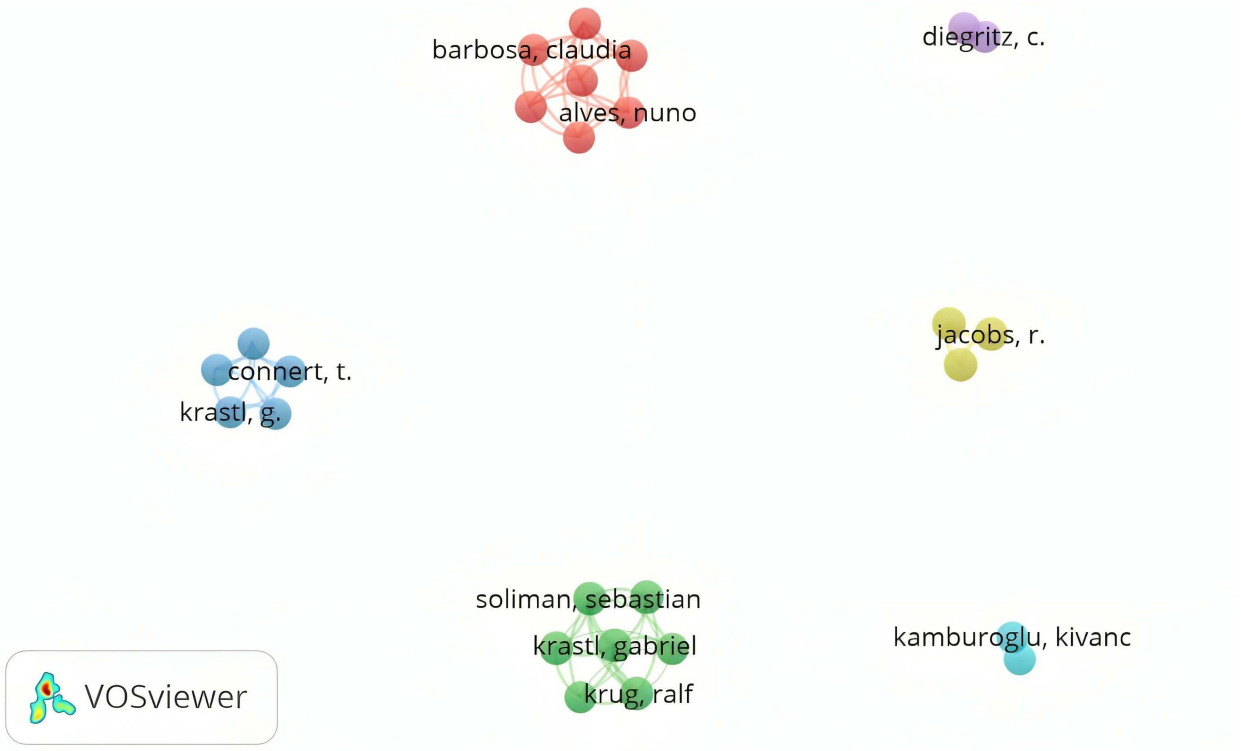


Fig. 3 – Co-authorship network of authors.

Table 5

Most productive journals

Journals	Publishing	Number of articles	Number of citations	WoSCC	Quartile
International Endodontic Journal	Wiley	9	533	SCIE	Q1
Clinical Oral Investigations	Springer	4	67	SCIE	Q2
European Journal of Dental Education	Wiley	4	49	SCIE	Q3
BMC Oral Health	Springer	4	23	SCIE	Q2
Journal of Dentistry	Elsevier	3	71	SCIE	Q1
International Journal of Computerized Dentistry	Quintessence Publishing	3	20	SCIE	Q4
International Journal of Environmental Research and Public Health	MDPI	2	11	SCIE	Q2
Dentistry Journal	MDPI	2	0	N/A	-
Frontiers in Bioengineering and Biotechnology	Frontiers	1	95	SCIE	Q1
Scanning	Hindawi	1	93	SCIE	Q3

WoSCC – Web of Science Core Collection; SCIE – Science Citation Index Expanded; BMC – BioMed Central; MDPI – Multidisciplinary Digital Publishing Institute; N/A – not applicable.

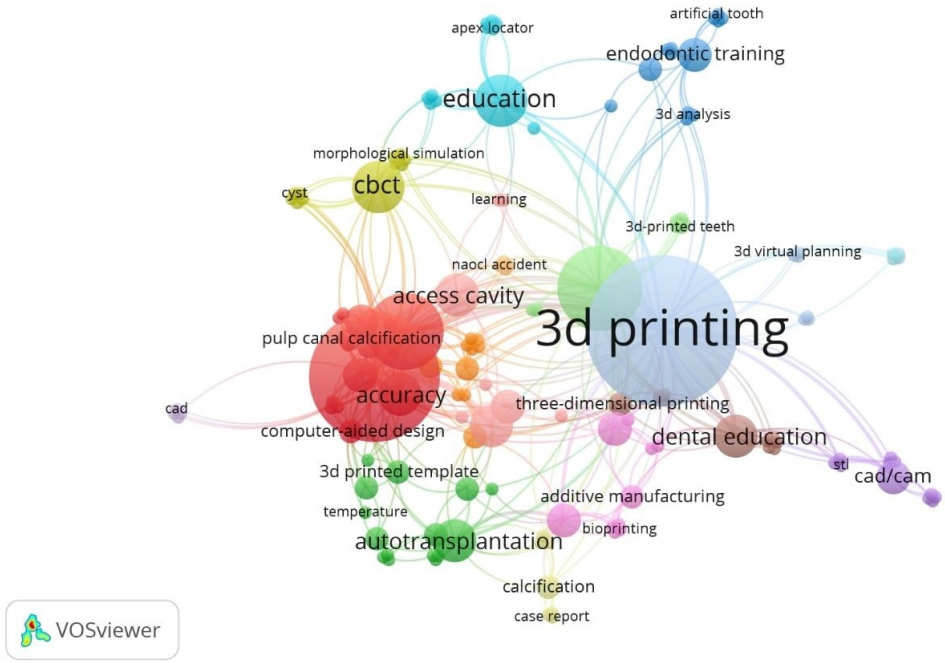


Fig. 4 – Co-occurrence analysis of keywords.

the results obtained by VOSviewer, a visualization of keywords was created in the software and grouped according to the co-occurrence analysis (Figure 4). According to the keyword association analysis, the top five keywords with the highest total link strength were “3D printing”, “guided endodontics”, “endodontics”, “root canal treatments”, and “CBCT”.

Discussion

To the best of our knowledge, this is the first study to use comprehensive bibliographic data from the WoS databases to provide valuable quantitative information about 3D printing in the endodontics field of research. Although this is a new area, this analysis shows that the number of annual publications is gradually increasing, with a sharp increase in 2018 (14 in 2018, 18 in 2020, and 25 in 2021, compared to only 5 publications in 2017).

Most publications are articles, and a small portion are review articles. No book chapters or conference papers on the subject could be found. The subject is open to development, new and current, and it is thought that more reviews, chapters, and conference papers will be produced in the future.

Most productive writers come from developed countries (e.g., USA, Germany, England, PRC). The countries that published the most on the use of 3D printing in endodontics were the PRC and the USA, while the countries that received the most citations were Germany and Switzerland. These high-quality studies from developed countries make great contributions to the global literature.

Dao et al. [21] highlighted that the most productive contributions to Education 4.0 research predominantly emerged from developing countries such as Mexico, Malaysia, and Indonesia rather than developed countries. They emphasized the notable absence of scholars from developed nations, including the USA, the United Kingdom (UK), Canada, the PRC, Japan, and Australia, in key productivity rankings, arguing that strong advancements in Education 4.0 research require collaboration and contributions from scholars in developed economies. In alignment with their findings, our bibliometric analysis also revealed a lack of collaboration in the field of endodontics and 3D printing. However, a significant distinction is evident – all leading contributors in this field are based in developed countries. To bridge this gap, developing countries require access to advanced technological infrastructure and opportunities for collaboration with research institutions in developed economies. Such efforts would enable broader participation and innovation, ultimately fostering the growth of this emerging field.

The largest number of the most cited articles were published in the same journal. This may be related to the high interest of the journal’s editors in this issue and the fact that the mentioned journal publishes a high level of publications on this developing topic. Nine of the ten journals with the most publications are indexed in SCIE, and the quartiles of the journals are generally high. This also increases the value of the studies done on the subject.

The study subjects of Thomas Connert and Gabriel Krastl, the two collaborating authors who have the most publications on this subject (6 publications), are 3D-

printed templates for gaining guided access to root canals [22], guided endodontic treatment in a case of dentin dysplasia with pulp canal calcification on a 3D-printed template [23], and 3D printing in endodontic education [24]. Likewise, Reinhilde Jacobs and Andres Torres, who have many publications on the subject, are two authors in collaboration and have studied the 3D-printed guide to gain access to obliterated root canals [25–27] and augmented reality for guided access cavity preparation in 3D-printed jaws [28]. However, in addition to his studies on guided endodontic access procedures in 3D-printed teeth in collaboration with Gabriel Krastl [22], Marcel Reymus has focused more in his studies on using 3D printing in endodontic education [14, 29, 30] and virtual reality in endodontic education [14].

Cooperation between countries on the subject is limited. In the international cooperation network, only 10 countries are on the map (Figure 2). When looking at country collaborations, the strongest relationship was between Switzerland and Germany. However, it was noteworthy that various countries such as Turkey, Thailand, and Saudi Arabia managed to get on the map even with limited collaborations. Additionally, cooperation between different groups is very weak. This situation highlights the need for much stronger cooperation in the future in order to be able to promote the development of this field. Organizing more meetings and congresses in this emerging field is a good way to consider as these meetings are good for scientists to exchange ideas and build networks for collaboration.

In the analysis of the most popular keywords co-occurrence network, the following keywords attract attention: “guided endodontics”, “access cavity”, “CBCT”, “pulp canal obliteration”, “dental pulp calcification”, and “dental education”. Based on these maps, useful information can be obtained to help identify the research gap and potential research topics, as well as topics of greater interest in the community.

Study limitations

The limitation of the study is the fact that only 128 articles could be accessed in the WoS database. It is thought that new studies that will include other databases, such as the Scopus, and other disciplines will contribute to the holistic perspective. The second limitation is that we only used the VOSviewer tool and not some other bibliographic tools such as BiblioShiny, Science Mapping Analysis Software Tool (SciMAT), or CiteSpace. Using tools other than VOSviewer will help perform other bibliometric analyses that are unavailable in VOSviewer. As seen in the most cited paper analysis, it can also be reported as a limitation that WoS may not always give accurate results in keyword searches.

Furthermore, some important articles related to the subject may be skipped because 3D printing is not included in the keyword and title analysis. For instance, the article in which Ordinola-Zapata et al. [2] investigated the shaping ability of different systems in curved canals of rapid microcomputed tomography based prototyping molar replicas could not be included in the search because 3D and endodontics were not mentioned in the title and keywords. Therefore, future research should be designed with a wider range of keywords.

Conclusion

To our knowledge, this study is the first bibliometric analysis of worldwide research on the use of 3D printing in the endodontic field. With the rapid development of technology today, the number of articles discussing the effects of 3D printing on endodontic clinical cases and its use in education and the construction of standardized novel experimental models will increase. Consequently, such analyses need to be conducted regularly to closely monitor the development of this research field.

REFERENCES

1. *Sun J, Zhang FQ.* The application of rapid prototyping in prosthodontics. J Prosthodont 2012; 21(8): 641−4.
2. *Ordinola-Zapata R, Bramante CM, Duarte MA, Cavenago BC, Jaramillo D, Versiani M.* A. Shaping ability of reciproc and TF adaptive systems in severely curved canals of rapid microCT-based prototyping molar replicas. J Appl Oral Sci 2014; 22(6): 509−15.
3. *Ali A, Arslan H, Jethani B.* Conservative management of Type II dens invaginatus with guided endodontic approach: A case series. J Conserv Dent 2019; 22(5): 503−8.
4. *Connert T, Krug R, Eggmann F, Emsermann I, ElAyouti A, Weiger R,* et al. Guided Endodontics versus Conventional Access Cavity Preparation: A Comparative Study on Substance Loss Using 3-dimensional-printed Teeth. J Endod 2019; 45(3): 327−31.
5. *Giacomino CM, Ray JJ, Wealleans JA.* Targeted Endodontic Microsurgery: A Novel Approach to Anatomically Challenging Scenarios Using 3-dimensional-printed Guides and Trephine Burs-A Report of 3 Cases. J Endod 2018; 44(4): 671−7.
6. *Schwindling FS, Tasaka A, Hilgenfeld T, Rammelsberg P, Zenthöfer A.* Three-dimensional-guided removal and preparation of dental root posts-concept and feasibility. J Prosthodont Res 2020; 64(1): 104−8.
7. *Van Raan T.* Advances in bibliometric analysis: Research performance assessment and science mapping. In: *Blockmans W, Engwall L, Weaire D,* editors. Bibliometrics: Use and abuse in the review of research performance. Vol. 87. London: Portland Press Ltd; 2014. pp. 17−28.
8. *Noyons ECM, Buter RK, van Raan AFJ.* Bibliometric mapping as a science policy tool. London, UK: Proceedings Sixth International Conference on Information Visualisation; 2002. pp. 679−84.
9. *Van Eck NJ, Waltman L.* Software survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics 2010; 84(2): 523−38.
10. *Zehnder MS, Connert T, Weiger R, Krastl G, Kühl S.* Guided endodontics: accuracy of a novel method for guided access cavity preparation and root canal location. Int Endod J 2016; 49(10): 966−72.

11. *Oberoi G, Nitsch S, Edelmayer M, Janjić K, Müller AS, Agis H.* 3D Printing-Encompassing the Facets of Dentistry. Front Bioeng Biotechnol 2018; 6: 172.
12. *Tian Y, Chen C, Xu X, Wang J, Hou X, Li K,* et al. A review of 3D printing in dentistry: Technologies, affecting factors, and applications. Scanning 2021; 2021(1): 9950131.
13. *Connert T, Zehnder MS, Amato M, Weiger R, Kühl S, Krastl G.* Microguided Endodontics: a method to achieve minimally invasive access cavity preparation and root canal location in mandibular incisors using a novel computer-guided technique. Int Endod J 2018; 51(2): 247−55.
14. *Reymus M, Fotiadou C, Kessler A, Heck K, Hickel R, Diegritz C.* 3D printed replicas for endodontic education. Int Endod J 2019; 52(1): 123−30.
15. *Anderson J, Wealleans J, Ray J.* Endodontic applications of 3D printing. Int Endod J 2018; 51(9): 1005−18.
16. *Van der Meer WJ, Vissink A, Ng YL, Gulabivala K.* 3D Computer aided treatment planning in endodontics. J Dent 2016; 45: 67−72.
17. *Moreno-Rabié C, Torres A, Lambrechts P, Jacobs R.* Clinical applications, accuracy and limitations of guided endodontics: a systematic review. Int Endod J 2020; 53(2): 214−31.
18. *Liang YH, Jiang L, Gao XJ, Shemesh H, Wesselink PR, Wu MK.* Detection and measurement of artificial periapical lesions by cone-beam computed tomography. Int Endod J 2014; 47(4): 332−8.
19. *Matichescu A, Ardelean LC, Rusu LC, Craciun D, Bratu EA, Babucea M,* et al. Advanced Biomaterials and Techniques for Oral Tissue Engineering and Regeneration-A Review. Materials (Basel) 2020; 13(22): 5303.
20. *Shah P, Chong BS.* 3D imaging, 3D printing and 3D virtual planning in endodontics. Clin Oral Investig 2018; 22(2): 641−54.
21. *Dao LT, Tran T, Van Le H, Nguyen GN, Trinh TP.* A bibliometric analysis of Research on Education 4.0 during the 2017–2021 period. Educ Inf Technol 2023; 28(3): 2437−53.
22. *Krug R, Reich S, Connert T, Kess S, Soliman S, Reymus M,* et al. Guided endodontics: a comparative in vitro study on the accuracy and effort of two different planning workflows. Int J Comput Dent 2020; 23(2): 119−28.
23. *Krug R, Volland J, Reich S, Soliman S, Connert T, Krastl G.* Guided endodontic treatment of multiple teeth with dentin dysplasia: a case report. Head Face Med 2020; 16(1): 27.
24. *Kolling M, Backhaus J, Hofmann N, Keß S, Krastl G, Soliman S,* et al. Students' perception of three-dimensionally printed teeth in endodontic training. Eur J Dent Educ 2022; 26(4): 653−61.
25. *Torres A, Boelen GJ, Lambrechts P, Pedano MS, Jacobs R.* Dynamic navigation: a laboratory study on the accuracy and potential use of guided root canal treatment. Int Endod J 2021; 54(9): 1659−67.
26. *Torres A, Dierickx M, Coucke W, Pedano MS, Lambrechts P, Jacobs R.* In vitro study on the accuracy of sleeveless guided endodontics and treatment of a complex upper lateral incisor. J Dent 2023; 131: 104466.
27. *Torres A, Shaheen E, Lambrechts P, Politis C, Jacobs R.* Microguided Endodontics: a case report of a maxillary lateral incisor with pulp canal obliteration and apical periodontitis. Int Endod J 2019; 52(4): 540−9.
28. *Farronato M, Torres A, Pedano MS, Jacobs R.* Novel method for augmented reality guided endodontics: An in vitro study. J Dent 2023; 132: 104476.
29. *Ludwig J, Reymus M, Winkler A, Soliman S, Krug R, Krastl G.* Root Maturation of an Immature Dens Invaginatus Despite Unsuccessful Revitalization Procedure: A Case Report and Recommendations for Educational Purposes. Dent J (Basel) 2023; 11(2); 47.
30. *Reymus M, Liebermann A, Diegritz C, Keßler A.* Development and evaluation of an interdisciplinary teaching model via 3D printing. Clin Exp Dent Res 2021; 7(1): 3−10.

Received on July 28, 2024
Revised on December 14, 2024
Revised on January 3, 2025
Accepted on January 15, 2025
Online First March 2025

M E T A - A N A L Y S I S

UDC: 616.31-08:[616.311.2+616.314.17/.28
DOI: https://doi.org/10.2298/VSP241029019H

Recombinant human platelet-derived growth factor-BB combined with grafting materials for the treatment of periodontal intrabony defects: a meta-analysis

Rekombinantni humani trombocitni faktor rasta BB u kombinaciji sa koštanim zamenicima za lečenje parodontalnih intrakoštanih defekata: meta-analiza

[1]Wensong Hao*, [1]Leilei Li†, Kun Huang‡, Xiaodong Sui‡, Yuanyuan Qi†

***Liaocheng University, School of Medicine, Liaocheng, Shandong, China; †Dongying People's Hospital, Dongying, Shandong, China; ‡Liaocheng Traditional Chinese Medicine Hospital, Liaocheng, Shandong, China**

[1] equal contribution of both authors

Abstract

Background/Aim. Periodontitis is a common dental disease affecting around 50% of adult patients globally. It can cause continuous deterioration of periodontal tissue inflammation, leading to irreversible periodontal bone resorption and even tooth loosening. Currently, suitable techniques for managing periodontal intrabony deformities include both allogeneic and autologous bone transplantation, but these methods have certain limitations. The aim of this study was to examine the effect of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) in combination with grafting materials for the treatment of periodontal intraosseous defects using a meta-analysis. **Methods.** In March 2024, electronic databases (Cochrane Library, PubMed, Embase, Web of Science) were searched to gather data from studies on growth factors and grafting materials for the treatment of intrabony periodontal deficiencies. **Results.** A total of 11 articles were fully reviewed, of which nine were included in the meta-analysis. The 9 studies included 313 people, divided into two groups: experimental (n = 156) and control (n = 157). The results of the meta-analysis indicate significantly higher clinical attachment level (CAL) of the experimental group [$p < 0.01$, standardized mean difference (SMD): 0.80; 95% confidence interval (CI): 0.49, 1.10] compared to the control group. Changes in probing depth (PD) of the experimental group were greater ($p < 0.01$, SMD: 0.75; 95% CI: 0.39, 1.11) than in the control group. In addition, bone fill (BF) ($p < 0.01$, SMD: 22.33; 95% CI: 15.71, 28.95) and linear bone growth (LBG) ($p < 0.01$, SMD: 0.87; 95% CI: 0.43, 1.30) of the experimental group were greater. The gingival recession (GR) of the experimental group was lower ($p < 0.05$, SMD: -0.27; 95% CI: -0.51, -0.03) than in the control group. **Conclusion.** The results indicate that the rhPDGF-BB combined with the grafting material can improve CAL, PD, BF, LBG, and GR in periodontal intrabony defects.

Key words:
database; meta-analysis; periodontal diseases; platelet-derived growth factor.

Apstrakt

Uvod/Cilj. Parodontitis je uobičajena bolest zuba koja pogađa oko 50% odraslih pacijenata širom sveta. Može izazvati kontinuirano pogoršanje zapaljenja parodontalnog tkiva, što dovodi do nepovratne resorpcije parodontalne kosti, pa čak i pomeranja (klimanja) zuba. Trenutno, pogodne tehnike za rešavanja parodontalnih intrakoštanih deformiteta uključuju alogenu i autolognu transplantaciju kostiju, ali ove metode imaju određena ograničenja. Cilj rada bio je da se ispita efekat rekombinantnog humanog trombocitnog faktora rasta BB (*recombinant human platelet-derived growth factor*-BB – rhPDGF-BB) u kombinaciji sa koštanim zamenicima za lečenje parodontalnih intrakoštanih defekata primenom meta-analize. **Metode.** U martu 2024. godine izvršena je pretraga elektronskih baza (*Cochrane Library*, *PubMed*, *Embase*, *Web of Science*) kako bi se prikupili podaci iz studija o faktorima rasta i koštanim zamenicima za lečenje intrakoštanih parodontalnih nedostataka. **Rezultati.** Od 11 članaka koji su u potpunosti pregledani njih 9 je uključeno u meta-analizu. Ovih 9 studija je obuhvatilo 313 osoba podeljenih u dve grupe: eksperimentalnu (n = 156) i kontrolnu (n = 157). Rezultati meta-analize ukazuju na značajno viši nivo pripojnog epitela (*clinical attachment level* – CAL) eksperimentalne grupe [$p < 0,01$, *standardized mean difference* (SMD): 0,80; 95% interval poverenja (*confidence interval* – CI): 0,49, 1,10] u poređenju sa kontrolnom grupom.

Correspondence to: Yuanyuan Qi, Dongying People's Hospital, No. 317 Nanyi Road, Dongying District, Dongying 257 091, Shandong, China. E-mail: y44253056@gmail.com

Promene u dubini parodontalnog džepa (*probing depth* – PD) eksperimentalne grupe bile su veće ($p < 0,01$, SMD: 0,75; 95% CI: 0,39, 1,11) od kontrolne grupe. Takodje, koštani ispun (KI) ($p < 0,01$, SMD: 22,33; 95% CI: 15,71, 28,95) i linearni rast kosti (LRK) ($p < 0,01$, SMD: 0,87; 95% CI: 0,43, 1,30) eksperimentalne grupe bili su veći. Recesija gingive (RG) eksperimentalne grupe bila je niža ($p < 0,05$, SMD: -0,27; 95% CI: -0,51, -0,03) u odnosu na kontrolnu grupu. **Zaključak.** Rezultati ukazuju na to da rhPDGF-BB u kombinaciji sa koštanim zamenicima može poboljšati CAL, PD, KI, LRK i RG kod parodontalnih intrakoštanih defekata.

Ključne reči:
baze podataka; meta-analiza; periodontalne bolesti; faktor rasta, trombocitni.

Introduction

Periodontitis is a common dental disease that affects over half of all adults worldwide [1]. It may result in persistent inflammation of the periodontal tissues, irreversible loss of the periodontal bone, and loosening of the teeth [2]. Nowadays, frequent approaches for managing periodontal intrabony flaws consist of allogeneic and autologous bone transplantation, but these methods have certain limitations. For instance, the disadvantages of autologous bone transplantation are increased surgical trauma, longer operation time, limited graft availability, and high incidence rate of the donor site, while allograft bone transplantation may lead to disease transmission and immune rejection [3]. Periodontitis can cause periodontal bone resorption, which can be repaired using bone graft materials [4]. Periapical diseases, cysts, and injuries can all cause blemishes of bones in maxillofacial and oral sections, and bone graft materials are usually used for repair [5–7]. After tooth loss, absorption of the alveolar ridge is inevitable. Bone graft materials can be implanted into the extraction socket at the same time as tooth extraction to delay the absorption of the alveolar ridge. In response to these issues, bone tissue engineering based on biomaterials has great prospects in repairing bone defects [8]. In bone tissue engineering, scaffold materials are one of the three essential components that can act as templates for bone regeneration as well as a base for cell adhesion and proliferation [9]. A scaffold designed for bone tissue creation should be highly biocompatible. In addition, it should have high porosity, biodegradability, and good osteogenic induction activity. Therefore, its mechanical characteristics ought to coincide with the bone tissue of the host [10].

Natural bone tissue regeneration is a multi-stage cascade process, with specific biological factors playing a role at each stage. This inspires us to simulate the natural bone healing process to achieve bone regeneration [11, 12]. Based on the changes in demand for different biological signals during bone healing, precise coordination of drug release is required at each specific regeneration stage. However, this remains a confront in the bone tissue engineering field. The bone repair mechanism is complex, and the use of bioactive substances can enhance the tissue regeneration ability of cells. The growth factor (GF) is a protein secreted by activated bone cells, which can transmit signals to specific receptor target cells. The key role of GFs is to promote cell proliferation and differentiation, hasten the process of bone injuries and flaws healing, and therefore occupy a crucial position. For instance, the introduction of bioactive GFs like bone morphogenetic protein 2 (BMP-2) enhances the tissue renaissance ability of guided bone regeneration membranes. The addition of platelet-derived growth factor (PDGF) was found to have a similar effect [13]. It is known that BMP-2 has a dual effect on bone repair and induces bone formation at low concentrations, while high concentrations can lead to bone loss. So far, however, BMP-2 has been thought to be a potent bone healing activator even today, and its delivery strategy needs further optimization. Yet, surgical data indicate that more transplant materials may be required for posterior foot and ankle joint fusion surgery in some patient groups with various forms of arthritis, and platelet-derived growth factor-BB (PDGF-BB) was authorized as a class III combined medical device/drug in 2015. In terms of specific mechanisms, PDGF can recognize and activate PDGF receptors, recruit multiple cells, carry out signaling pathways transmission, and encourage the proliferation of cells [14]. PDGF has also been proven to induce new blood vessels in the target site through communication reactions with various cells, which is an important aspect of bone repair. Unfortunately, the use of bioactive GFs is restricted by their short half-life, erratic nature, and expensive nature of proteins.

The aim of this study was to examine the effect of recombinant human PDGF-BB (rhPDGF-BB) in combination with grafting materials for the treatment of periodontal intraosseous defects in humans since systematic research on such controlled trials are lacking.

Methods

Study type

This meta-analysis included controlled studies on using GFs in conjunction with grafting materials to repair intrabony periodontal problems.

Intervention types

Two types of subjects were examined – experimental and control. The experimental subjects were administered GF combined with grafting materials in periodontal intrabony defect management, whereas the control subjects were administered GF alone or grafting materials alone.

Outcome measure types

In order for a study to be included in our meta-analysis, at least one of the following outcome markers must be met:

clinical attachment level (CAL), changes in probing depth (PD), bone fill (BF), gingival recession (GR), and linear bone growth (LBG).

Literature retrieval strategy

The deadline for our English search was March 2024. We carried out computer searches of PubMed, Embase, Cochrane Library, and Web of Science electronic databases. The following are a few subject terms created for the English search: "growth factor", "grafting materials", and "periodontal intrabony defects".

Literature screening and data extraction

The screening process used for including or excluding literature is described in the text that follows. Two scholars independently examine the literature. If there are disagreements, they discuss and seek advice from a third party. The scholars read the abstracts and titles before choosing any literature to remove obviously irrelevant material. Afterward, they continue reading the complete book to decide whether or not to incorporate it into the analysis. The third party is engaged in discussions and negotiations to resolve any discrepancies that arise between the two researchers as they separately retrieve pertinent literature data using Microsoft Excel. Some details included in the literature extraction process were average age, gender ratio, sample size, first author, year of publication, intervention measures, and outcome indicators.

Statistical analysis

Stata software version 16 was utilized for the meta-analysis in this study. The odds ratio (OR) is used to show intergroup differences for binary variables, while the weighted mean difference (WMD) or standardized mean difference (SMD) is used to show intergroup differences for continuous variables. The study findings are represented by the 95% confidence interval (CI), and the I^2 index is used to determine the degree of heterogeneity. Depending on how heterogeneous each research is, either a fixed effects model (FEM) analysis or a random effects model (REM) analysis is employed. Additionally, the study's general publication bias is assessed using Egger's and Begg's tests. Sensitivity analysis is performed to identify the cause of any substantial clinical heterogeneity in the research.

Results

Results of the literature search

Two hundred and nineteen pertinent pieces of literature were ultimately searched, and 203 were obtained after screening out duplicate literature. Having perused the abstracts and titles, 11 publications were attained. Further exclusion due to lack of data resulted in the inclusion of 9 articles. In those 9 studies, there were 313 subjects, whom we divided into two groups: experimental (n = 156) and control (n = 157). The basic characteristics of the included studies and a PRISMA flowchart are detailed in Table 1 [15–23] and Figure 1, respectively.

Clinical attachment level

There were a total of 9 literature reports on the CAL of rhPDGF-BB combined with grafting materials in periodontal intrabony defect management. The findings demonstrated that there was a statistically significant difference ($p < 0.01$,

Table 1

Basic characteristics of the included studies

Studies	Cases (n = 313) experimental/ control	Gender (male/ female)	Age (years)	Experimental group	Control group	Outcomes measures
Jayakumar et al. [15]	27/27	25/29	32.6 ± 7.3 / 30.9 ± 5.1	rhPDGF+β-TCP	β-TCP	CAL, PD, BF, LBG
Deshpande et al. [16]	9/9	NA	35.76 ± 7.38	rhPDGF+β-TCP	HA+β-TCP	CAL, PD, BF, GR, LBG
Maroo and. Murthy [17]	15/15	NA	38.4 ± 7.6	rhPDGF+β-TCP	β-TCP	CAL, PD, BF, GR
Qiao et al. [18]	15/16	NA	47.7 ± 13.9	CGF+BPBM	BPBM	CAL, PD, GR
Xu et al. [19]	30/30	NA	55.2 ± 8.3	CGF+Bio-Oss	Bio-Oss	CAL, PD
Saito et al. [20]	16/16	13/19	52.3 ± 10.1 / 50.0 ± 10.9	rhFGF-2+DBBM	rhFGF-2	CAL, PD, GR
Bahammam et al. [21]	15/15	16/14	37.4 ± 4.4 / 40.2 ± 5.9	PRF+HA	HA	CAL, PD
Seshima et al. [22]	16/16	NA	NA	rhFGF-2+DBBM	rhFGF-2	CAL
Priyanka et al. [23]	13/13	NA	33.08 ± 7.70 / 35.23 ± 6.47	rhPDGF-BB+DFDBA	rhPDGF-BB	CAL, PD, LBG

NA – no answer; rhPDGF – recombinant human platelet-derived growth factor; β-TCP – beta-tricalcium phosphate; CAL – clinical attachment level; PD – probing depth; BF – bone fill; GR – gingival recession; LBG – linear bone growth; CGF – concentrated growth factor; BPBM – bovine porous bone mineral; DBBM – deproteinized bovine bone mineral; rhFGF – recombinant human fibroblast growth factor; PRF – platelet-rich fibrin; HA – hydroxyapatite; DFDBA – demineralized freeze-dried bone allograft.
All values are given as mean ± standard deviation or numbers.

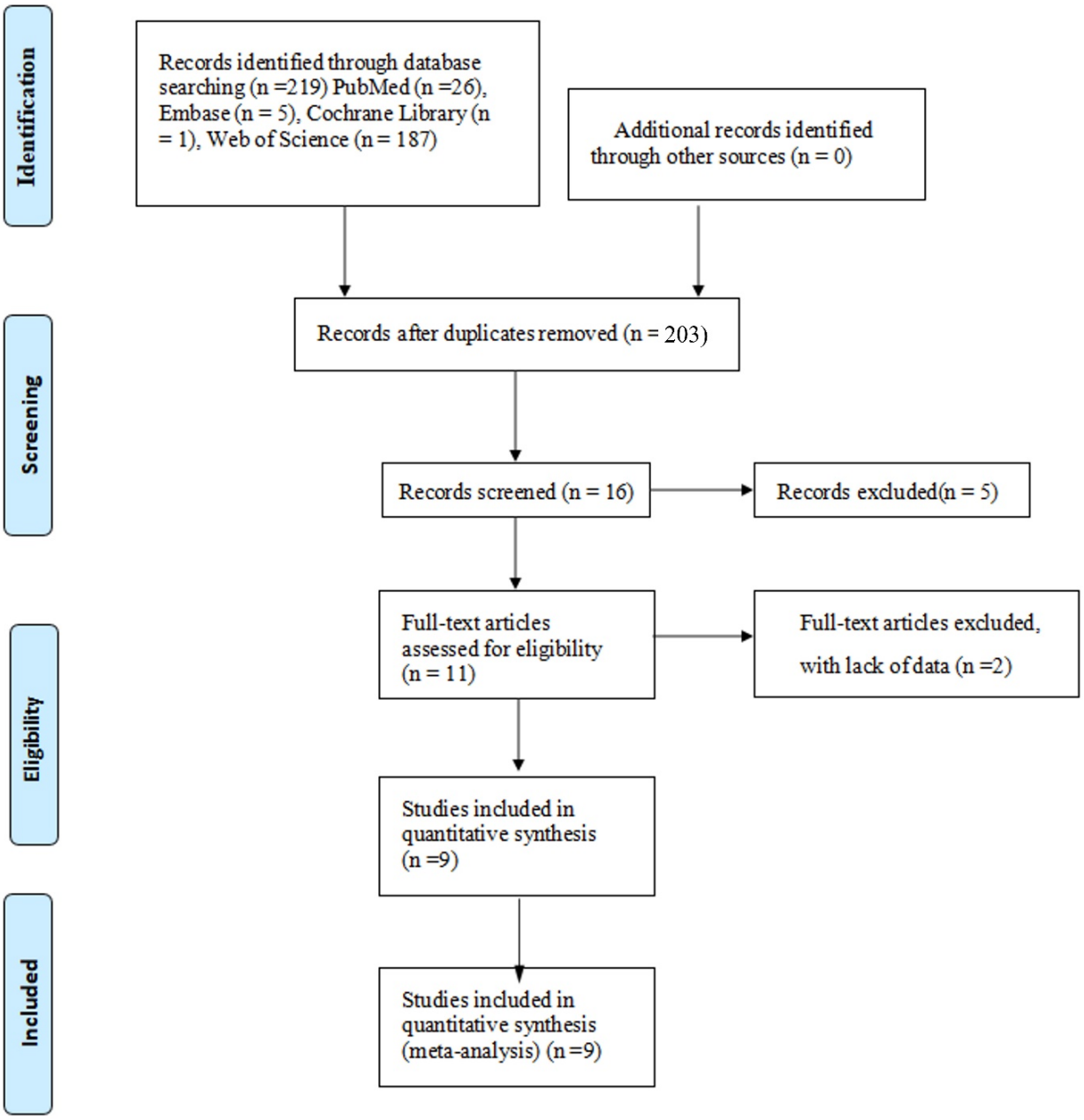


Fig. 1 – Flow chart of growth factor combined and grafting materials in the treatment of periodontal intrabony defects.

SMD: 0.80; 95% CI: 0.49, 1.10) (Figure 2) [15–23]. A funnel plot for the outcome indicators was drawn, and the results show that the funnel plot is symmetrically distributed (Figure 3). I^2 index was 23.6%, with low heterogeneity. This study conducted Egger's and Begg's tests on outcome indicators to govern bias in publications. Begg's and Egger's tests showed $p = 0.118$ and $p = 0.191$, respectively, representing no bias in publication. This indicated that the CAL of patients receiving rhPDGF-BB, pooled with the materials of grafting, was greater than in the control group.

Changes in probing depth

There were a total of seven literature reports on the PD of rhPDGF-BB combined with grafting materials in periodontal intrabony defect management. According to the findings, there was a statistically significant difference ($p < 0.01$, SMD: 0.75; 95% CI: 0.39, 1.11) (Figure 4) [15–21]. This showed that individuals receiving rhPDGF-BB in addition to grafting materials had greater PD than those in the control group.

Bone fill

There were a total of four literature reports on the BF of rhPDGF-BB combined with grafting materials in periodontal intrabony defect management. The outcomes revealed a statistically significant difference ($p < 0.01$, SMD: 22.33; 95% CI: 15.71, 28.95) (Figure 5) [15–17, 23]. This indicated that the BF of patients receiving rhPDGF-BB, pooled with grafting materials, was greater compared to the control group.

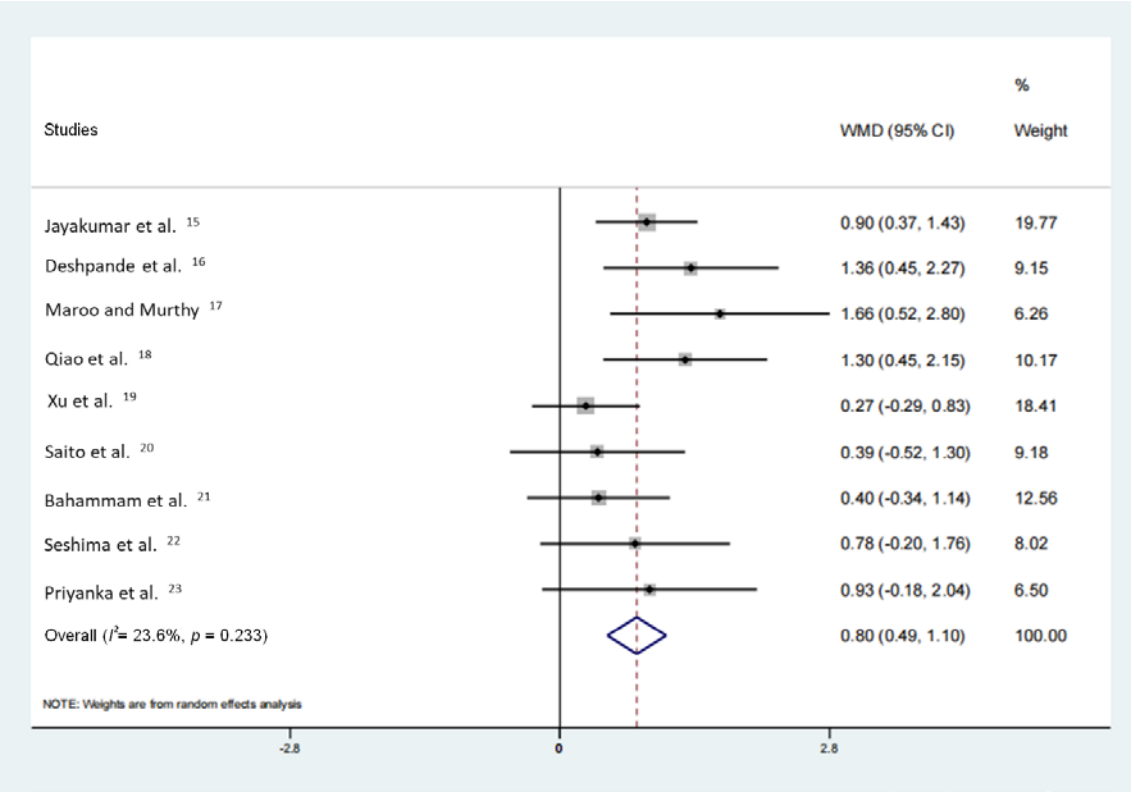


Fig. 2 – Forest plot of clinical attachment level of growth factor combined and grafting materials in the treatment of periodontal intrabony defects. WMD – weighted mean difference.

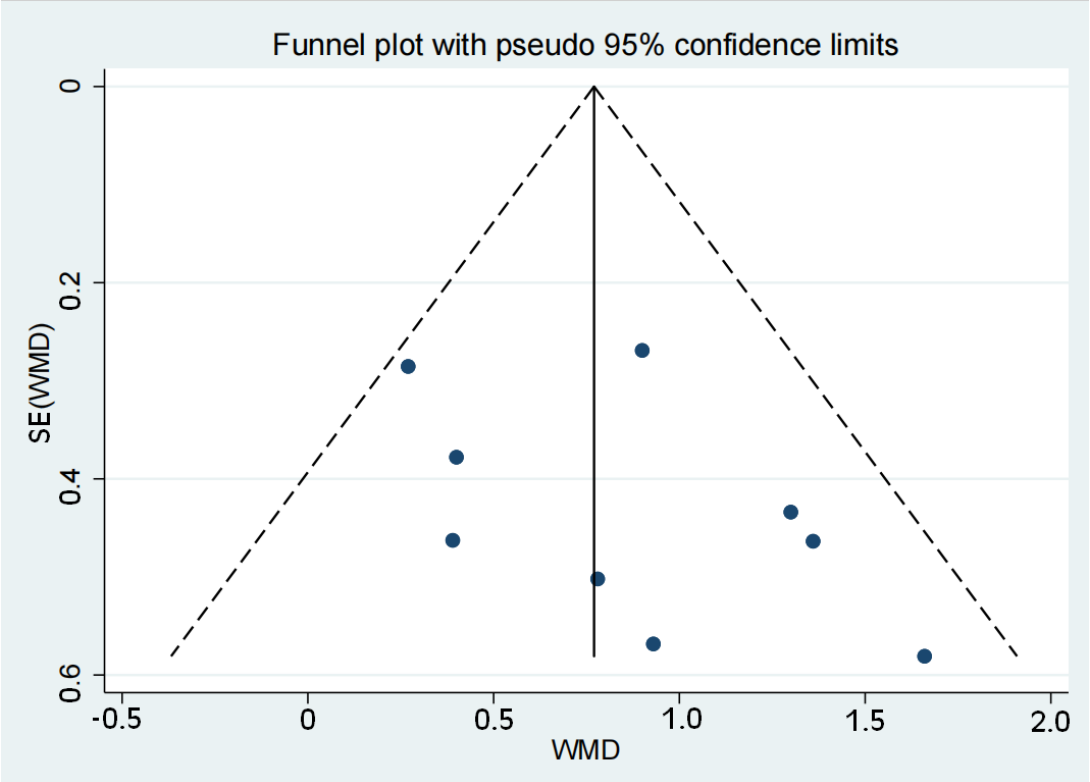


Fig. 3 – Published bias funnel plot of clinical attachment level of growth factor combined and grafting materials in the treatment of periodontal intrabony defects. SE – standard error; WMD – weighted mean difference.

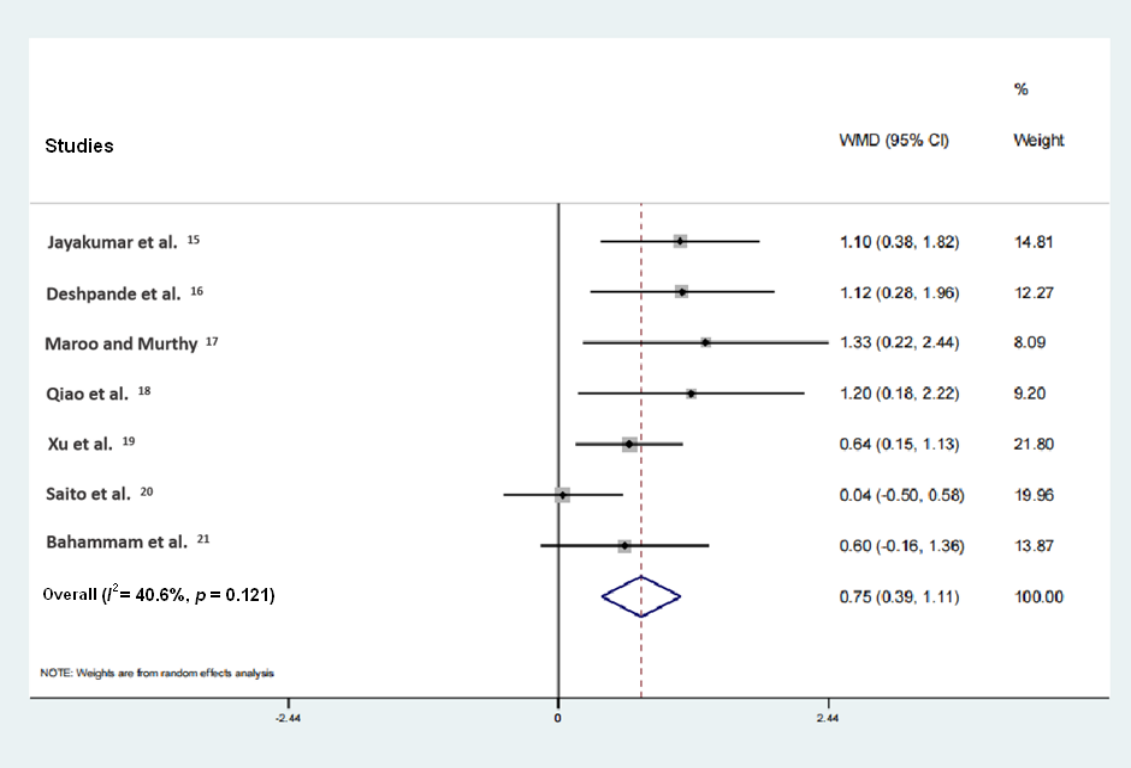


Fig. 4 – Forest plot of changes in probing depth of growth factor combined and grafting materials in the treatment of periodontal intrabony defects. WMD – weighted mean difference.

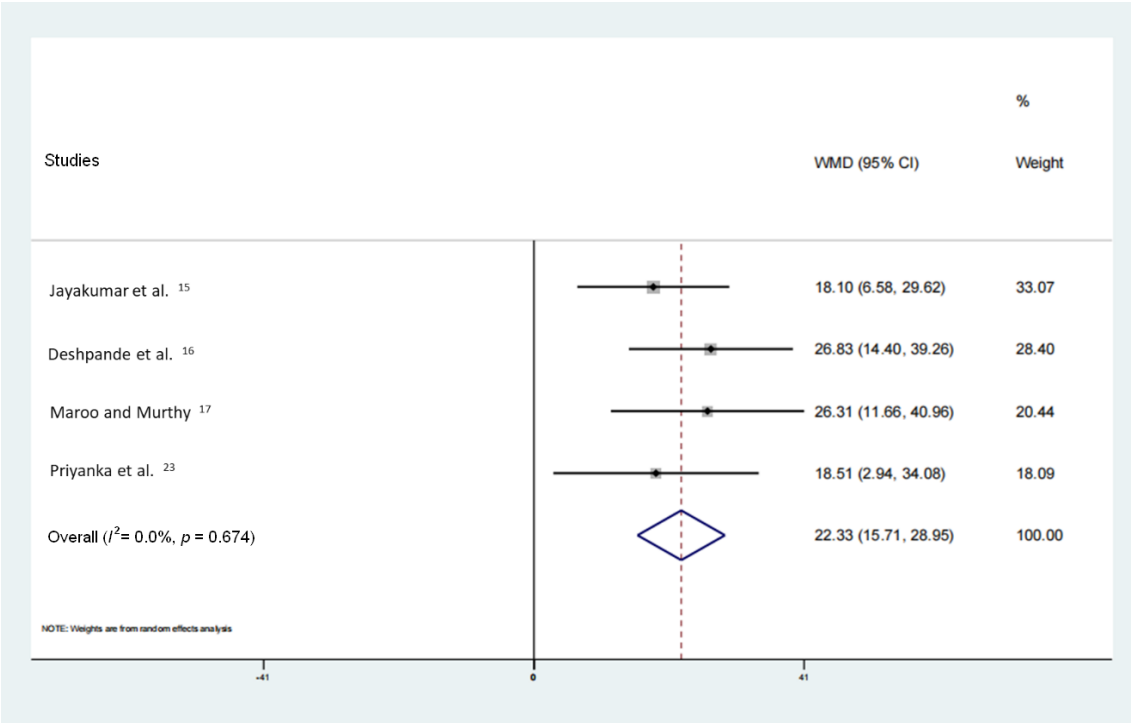


Fig. 5 – Forest plot of bone fill of growth factor combined and grafting materials in the treatment of periodontal intrabony defects. WMD – weighted mean difference.

Gingival recession

There are a total of four literature reports on the GR of rhPDGF-BB added to the grafting materials in periodontal intrabony defect management. The outcomes revealed a statistically significant difference ($p < 0.05$, SMD: -0.27; 95% CI: -0.51, -0.03) (Figure 6 [16–18, 20]). This indicated that the GR of patients receiving rhPDGF-BB added to grafting materials was lower compared to the control group.

Linear bone growth

There were a total of three literature reports on LBG of rhPDGF-BB added to the grafting materials in periodontal intrabony defect management. The outcomes revealed a statistically significant difference ($p < 0.01$, SMD: 0.87; 95% CI: 0.43, 1.30) (Figure 7) [15, 16, 23]. This indicated that the LBG of patients receiving rhPDGF-BB, pooled with grafting materials, was greater compared to the control group.

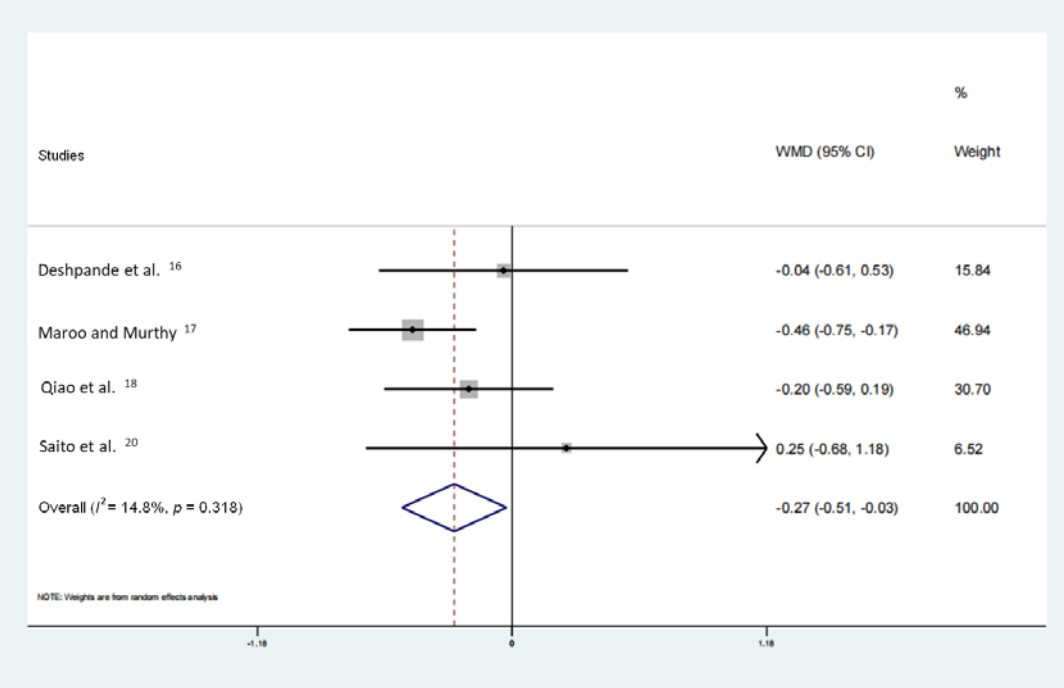


Fig. 6 – Forest plot of gingival recession of growth factor combined and grafting materials in the treatment of periodontal intrabony defects. WMD – weighted mean difference.

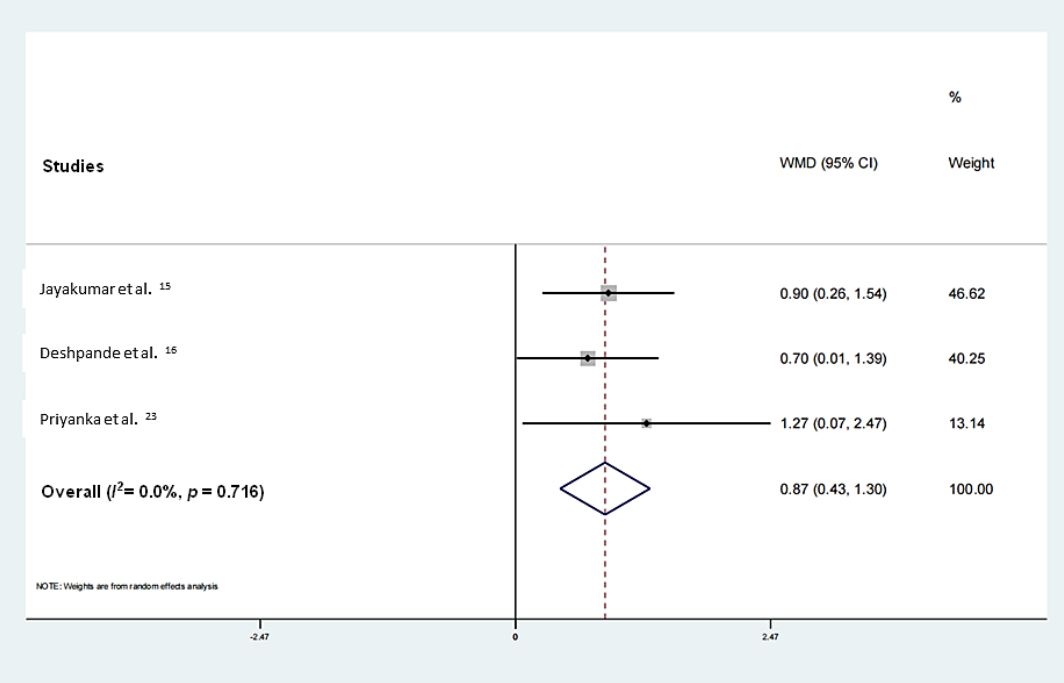


Fig. 7 – Forest plot of linear bone growth of growth factor combined and grafting materials in the treatment of periodontal intrabony defects. WMD – weighted mean difference.

Discussion

After screening, 9 studies were examined. There were 9 literature reports [15–23] on the CAL of patients with periodontal intrabony defects, and the integration outcomes presented substantial heterogeneity. The difference was statistically significant ($p < 0.01$, SMD: 0.80; 95% CI: 0.49, 1.10). This result indicates that the combination of rhPDGF-BB and grafting materials effectively enhances tissue integration and periodontal regeneration. The low heterogeneity ($I^2 = 23.6\%$) suggests consistency across the studies. This is particularly noteworthy as improved CAL is a cornerstone of successful periodontal defect management, reflecting the biological integration of new tissue with the existing periodontal structure. There were seven literature reports [15–21] on the PD of patients with periodontal intrabony defects. The difference was statistically significant ($p < 0.01$, SMD: 0.75; 95% CI: 0.39, 1.11). This indicates that the changes in PD of periodontal intrabony defects after a combination therapy of rhPDGF-BB and grafting materials were more prominent in the experimental than in the control group. Meanwhile, this parameter highlights the restoration of periodontal health, with reduced PD indicative of pocket resolution and reduced inflammation. The positive outcomes can be attributed to the bioactive properties of rhPDGF-BB, which promote cellular proliferation and migration critical for tissue repair. There were four literature reports [15–17, 23] on the BF of patients with periodontal intrabony defects. The transformation was statistically significant ($p < 0.01$, SMD: 22.33; 95% CI: 15.71, 28.95), which indicates that the BF of patients with periodontal intrabony defects after rhPDGF-BB therapy combined with grafting materials was higher than that of the control group. This substantial improvement also underscores the osteogenic potential of rhPDGF-BB, which facilitates mineralized matrix deposition. BF is a direct measure of defect resolution, representing the structural regeneration of periodontal architecture. There were four literature reports [16–18, 20] on the GR of patients with periodontal intrabony defects. The transformation was statistically significant ($p < 0.05$, SMD: -0.27; 95% CI: -0.51, -0.03), which indicates that the GR of patients with periodontal intrabony defects after rhPDGF-BB treatment combined with grafting materials was lower than in the control group. This finding is critical as excessive GR can compromise aesthetics and sensitivity, despite other successful regenerative outcomes. The combination therapy not only promotes bone and tissue regeneration but also mitigates soft tissue recession, contributing to overall periodontal stability. There were three literature reports [15, 16, 23] on the LBG of patients with periodontal intrabony defects. The difference was statistically significant ($p < 0.01$, SMD: 0.87; 95% CI: 0.43, 1.30), indicating that LBG of patients with periodontal intrabony defects after rhPDGF-BB, along with grafting materials, was greater than in the control group. This metric reflects the vertical regeneration of alveolar bone, essential for restoring periodontal support. The bioactivity of rhPDGF-BB in stimulating angiogenesis and osteoblast activity appears to play a pivotal role in this enhancement.

When dental bone transplant materials were first studied in the 1960s, researchers discovered that demineralized dentin matrix has osteoinductive properties and can induce ectopic osteogenesis [24]. The use of demineralized dentin matrix as a material for bone grafting has been validated by further research. Scholars have also studied methods such as boiling and calcination to prepare dentin matrix, which also has osteogenic ability [24–27]. The autologous dental bone powder is developed based on research on dentin matrix and is a bone graft content prepared from a patient's self-unusable teeth [25–27]. The first instance of autologous dental bone powder successfully used for maxillary sinus elevation surgery in a clinical setting was documented by researchers in 2003 [25]. A teeth grinder was created in 2007 by Japanese researchers, which simplified the process of making autologous tooth bone powder [26]. In 2009, the Korea Tooth Bank was founded to gather, preserve, and prepare teeth for use as bone transplant materials. The research on autologous tooth bone powder has developed rapidly in recent years, confirming its safety and effectiveness. However, there is relatively little comparative research with other bone transplant materials. The Bio-Oss bone powder is a deproteinized bovine bone matrix proven to be a safe and effective bone transplant material, having several different clinical uses [27].

Commonly used GFs comprise platelet-rich plasma (PRP), platelet-rich fibrin (PRF), basic fibroblast growth factor (bFGF), BMP-2, rhPDGF-BB, etc. After a growth factor is recognized, it is attached to a receptor on the cell membrane, which thereby triggers intracellular signaling pathways that govern cell growth and several cellular functions [28]. Among them, rhPDGF-BB has the strongest osteogenic activity, achieved by upregulating angiogenesis. However, due to the extremely low content of rhPDGF-BB in human bones, it is difficult to meet the needs of various complex bone defect repairs, and the high cost of separation and purification seriously limits its application.

Such GFs can be used alone with many biomaterials or in combination. Özveri Koyuncu et al. [29] applied concentrated GF to the alveolar fossa of impacted mandibular third molars, and the results showed that rhPDGF-BB can effectively alleviate postoperative pain in patients and have a positive effect in eliminating swelling. A prior meta-analysis indicated that rhPDGF-BB when used in conjunction with grafting materials for the treatment of periodontal intrabony defects, had superior efficacy compared to the application of grafting materials alone [30]. The use of rhPDGF-BB for growth in conjunction with bone replacement materials in the bone defect repair field has gradually gained the favor of clinical physicians.

Conclusion

According to the findings, the combination of growth factor and grafting materials has the potential to enhance the clinical attachment ratio, changes in probing depth, gingival recession, bone fill, and linear bone growth in periodontal intrabony defects.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

The authors appreciate the amenities provided by the institution.

R E F E R E N C E S

1. *Eke PI, Wei L, Borgnakke WS, Thornton-Evans G, Zhang X, Lu H,* et al. Periodontitis prevalence in adults ≥ 65 years of age, in the USA. Periodontol 2000 2016; 72(1): 76−95.
2. *Hajishengallis G, Chavakis T.* Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat Rev Immunol 2021; 21(7): 426−40.
3. *Loi F, Córdova LA, Pajarinen J, Lin TH, Yao Z, Goodman SB.* Inflammation, fracture and bone repair. Bone 2016; 86: 119−30.
4. *Chen MX, Zhong YJ, Dong QQ, Wong HM, Wen YF.* Global, regional, and national burden of severe periodontitis, 1990-2019: An analysis of the Global Burden of Disease Study 2019. J Clin Periodontol 2021; 48(9): 1165−88.
5. *Tan B, Tang Q, Zhong Y, Wei Y, He L, Wu Y,* et al. Biomaterial-based strategies for maxillofacial tumour therapy and bone defect regeneration. Int J Oral Sci 2021; 13(1): 9.
6. *Zhao R, Yang R, Cooper PR, Khurshid Z, Shavandi A, Ratnayake J.* Bone Grafts and Substitutes in Dentistry: A Review of Current Trends and Developments. Molecules 2021; 26(10): 3007.
7. *Shaikh MS, Zafar MS, Alnazzawi A.* Comparing Nanohydroxyapatite Graft and Other Bone Grafts in the Repair of Periodontal Infrabony Lesions: A Systematic Review and Meta-Analysis. Int J Mol Sci 2021; 22(21): 12021.
8. *Solakoğlu Ö, Ofluoğlu D, Schwarzenbach H, Heydecke G, Reißmann D, Ergun S,* et al. A 3-year prospective randomized clinical trial of alveolar bone crest response and clinical parameters through 1, 2, and 3 years of clinical function of implants placed 4 months after alveolar ridge preservation using two different allogeneic bone-grafting materials. Int J Implant Dent 2022; 8(1): 5.
9. *Li Z, Du T, Ruan C, Niu X.* Bioinspired mineralized collagen scaffolds for bone tissue engineering. Bioact Mater 2020; 6(5): 1491−511.
10. *Du T, Niu X, Hou S, Xu M, Li Z, Li P,* et al. Highly aligned hierarchical intrafibrillar mineralization of collagen induced by periodic fluid shear stress. J Mater Chem B 2020; 8(13): 2562−72.
11. *Fernandez-Yague MA, Abbah SA, McNamara L, Zeugolis DI, Pandit A, Biggs MJ.* Biomimetic approaches in bone tissue engineering: Integrating biological and physicomechanical strategies. Adv Drug Deliv Rev 2015; 84: 1−29.
12. *Buettmann EG, McKenzie JA, Migotsky N, Sykes DA, Hu P, Yoneda S,* et al. VEGFA From Early Osteoblast Lineage Cells (Osterix+) Is Required in Mice for Fracture Healing. J Bone Miner Res 2019; 34(9): 1690−706.
13. *Lee J, Lee S, Ahmad T, Madhurakkat Perikamana SK, Lee J, Kim EM,* et al. Human adipose-derived stem cell spheroids incorporating platelet-derived growth factor (PDGF) and biominerals for vascularized bone tissue engineering. Biomaterials 2020; 255: 120192.
14. *Ozaki Y, Nishimura M, Sekiya K, Suehiro F, Kanawa M, Nikawa H,* et al. Comprehensive analysis of chemotactic factors for bone marrow mesenchymal stem cells. Stem Cells Dev 2007; 16(1): 119−29.
15. *Jayakumar A, Rajababu P, Rohini S, Butchibabu K, Naveen A, Reddy PK,* et al. Multi-centre, randomized clinical trial on the efficacy and safety of recombinant human platelet-derived growth factor with β-tricalcium phosphate in human intra-osseous periodontal defects. J Clin Periodontol 2011; 38(2): 163−72.
16. *Deshpande A, Koudale SB, Bhongade ML.* A comparative evaluation of rhPDGF-BB + β-TCP and subepithelial connective tissue graft for the treatment of multiple gingival recession defects in humans. Int J Periodontics Restorative Dent 2014; 34(2): 241−9.
17. *Maroo S, Murthy KR.* Treatment of periodontal intrabony defects using β-TCP alone or in combination with rhPDGF-BB: a randomized controlled clinical and radiographic study. Int J Periodontics Restorative Dent 2014; 34(6): 841−7.
18. *Qiao J, Duan J, Zhang Y, Chu Y, Sun C.* The effect of concentrated growth factors in the treatment of periodontal intrabony defects. Future Sci OA 2016; 2(4): FS136.
19. *Xu Y, Qiu J, Sun Q, Yan S, Wang W, Yang P,* et al. One-Year Results Evaluating the Effects of Concentrated Growth factors on the Healing of Intrabony Defects Treated with or without Bone Substitute in Chronic Periodontitis. Med Sci Monit 2019; 25: 4384−9.
20. *Saito A, Bizenjima T, Takeuchi T, Suzuki E, Sato M, Yoshikawa K,* et al. Treatment of intrabony periodontal defects using rhFGF-2 in combination with deproteinized bovine bone mineral or rhFGF-2 alone: A 6-month randomized controlled trial. J Clin Periodontol 2019; 46(3): 332−41.
21. *Bahammam MA, Attia MS.* Expression of Vascular Endothelial Growth factor Using Platelet Rich Fibrin (PRF) and Nanohydroxyapatite (nano-HA) in Treatment of Periodontal Intra-Bony Defects – A Randomized Controlled Trial. Saudi J Biol Sci 2021; 28(1): 870−8.
22. *Seshima F, Bizenjima T, Aoki H, Imamura K, Kita D, Irokawa D,* et al. Periodontal Regenerative Therapy Using rhFGF-2 and Deproteinized Bovine Bone Mineral versus rhFGF-2 Alone: 4-Year Extended Follow-Up of a Randomized Controlled Trial. Biomolecules 2022; 12(11): 1682.
23. *Priyanka M, Reddy K, Pradeep K.* Efficacy of rh-PDGF-BB and Emdogain with or without DFDBA using M-MIST in the treatment of intrabony defects. Niger J Clin Pract 2023; 26(1): 116−24.
24. *Urist MR.* Bone: formation by autoinduction. 1965. Clin Orthop Relat Res 2002; (395): 4−10.
25. *Um IW, Lee JK, Kim JY, Kim YM, Bakhshalian N, Jeong YK,* et al. Allogeneic Dentin Graft: A Review on Its Osteoinductivity and Antigenicity. Materials (Basel) 2021; 14(7): 1713.
26. *Murata M, Kabir MA, Hirose Y, Ochi M, Okubo N, Akazawa T,* et al. Histological Evidences of Autograft of Dentin/Cementum Granules into Unhealed Socket at 5 Months after Tooth Extraction for Implant Placement. J Funct Biomater 2022; 13(2): 66.
27. *Grawish ME, Grawish LM, Grawish HM, Grawish MM, Holiel AA, Sultan N,* et al. Demineralized Dentin Matrix for Dental and Alveolar Bone Tissues Regeneration: An Innovative Scope Review. Tissue Eng Regen Med 2022; 19(4): 687−701. Erratum in: Tissue Eng Regen Med 2022; 19(4): 887−9.
28. *Park DJ, Yoon C, Thomas N, Ku GY, Janjigian YY, Kelsen DP,* et al. Prognostic significance of targetable angiogenic and growth factors in patients undergoing resection for gastric and gastroesophageal junction cancers. Ann Surg Oncol 2014; 21(4): 1130−7.
29. *Özveri Koyuncu B, Işık G, Özden Yüce M, Günbay S, Günbay T.* Effect of concentrated growth factor (CGF) on short-term clinical outcomes after partially impacted mandibular third molar surgery: A split-mouth randomized clinical study. J Stomatol Oral Maxillofac Surg 2020; 121(2): 118−23.
30. *Yao M, Hu J, Jiang L, Guo R, Wang X.* Efficacy of concentrated growth factor combined with grafting materials *vs.* grafting materials alone for the treatment of periodontal intrabony defects: a systematic review and meta-analysis. Ann Transl Med 2023; 11(4): 184.

Received on October 29, 2024
Revised on December 11, 2024
Revised on January 3, 2025
Accepted on January 15, 2025
Online First April 2025

CASE REPORTS

UDC: 616.72-001.6-07-089
DOI: https://doi.org/10.2298/VSP240206017V

Successful operative treatment of neglected pure Chopart joint dislocation: two case reports and literature review

Uspešno operativno lečenje previđene čiste luksacije Šopartovog zgloba: prikaz dva slučaja i pregled literature

Draško Vasović*, Lazar Mičeta*†, Filip Čučaković*, Aleksandar Jevtić*†, Danilo Jeremić*†, Nemanja Slavković*†

***Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia**

Abstract

Introduction. Chopart joint dislocation (CJD) represents a rare injury that is often initially unrecognized. Because of this, but also because of the increased morbidity it leads to, and due to poor treatment outcomes, this injury represents a significant clinical problem. **Case report.** We present two patients with overlooked CJD admitted to our institution within one year. The first case was a 63-year-old male, who suffered an injury after falling down the stairs, while the second case was a 33-year-old female injured in a traffic accident. Both patients were initially treated under the diagnosis of foot and ankle sprain. Upon their admission and subsequent computed tomography diagnostics, overlooked pure CJD was diagnosed in both patients. They both underwent open reduction and internal fixation of the Chopart joint using K-wires. Six weeks after the surgery, the K-wires were removed, a below-knee orthosis was applied for walking, and partial weight-bearing was allowed with a gradual increase to full weight-bearing over the next six weeks. Physical therapy was initiated. After the six-month follow-up, both patients were successfully rehabilitated, with a final American Orthopaedic Foot and Ankle Society score of 76 out of 100 for the female patient and a score of 84 out of 100 for the male patient. **Conclusion.** Despite the delayed diagnosis and postponed operative treatment, the functional outcomes of pure CJD treated by open reduction and percutaneous K-wiring can be satisfactory. Additional studies are required to gain a better insight into the prevalence and causative factors of the possible complications in such a treatment approach to the mentioned injury.

Key words:
joint dislocations; orthopedic procedures; tarsal joints; treatment delay; treatment outcome.

Apstrakt

Uvod. Dislokacija Šopartovog zgloba (DŠZ) predstavlja retku povredu, inicijalno često neprepoznatu. Najpre zbog toga, ali i zbog povišenog morbiditeta do koga dovodi, kao i zbog loših ishoda lečenja, ta povreda predstavlja značajan klinički problem. **Prikaz bolesnika**. Prikazana su dva slučaja previđene DŠZ kod bolesnika primljenih u našu ustanovu tokom jedne godine. Prvi slučaj je bio muškarac star 63 godine, povređen prilikom pada niz stepenice, dok je drugi slučaj bila žena stara 33 godine, povređena u saobraćajnom udesu. Oba bolesnika su u početku lečena pod dijagnozom distorzije stopala i skočnog zgloba. Po prijemu u ustanovu, nakon sprovedene dijagnostike kompjuterizovanom tomografijom, kod oba bolesnika konstatovano je postojanje previđene čiste DŠZ. Oba bolesnika su hirurški lečena otvorenom repozicijom i unutrašnjom fiksacijom Šopartovog zgloba pomoću K-igala. Šest nedelja nakon operacije, K-igle su uklonjene, primenjena je potkolena ortoza za hodanje, uz dopušten delimičan oslonac na operisanu nogu i sa postepenim povećanjem do punog oslanjanja u narednih šest nedelja. Započeta je fizikalna terapija. Nakon šestomesečnog praćenja, oba bolesnika su bila uspešno rehabilitovana, dostigavši konačni *American Orthopaedic Foot and Ankle Society* skor od 76/100 za bolesnicu i 84/100 za bolesnika. **Zaključak.** Uprkos kasnom postavljanju dijagnoze i odloženom operativnom lečenju, funkcionalni ishodi bolesnika sa čistim DŠZ, lečenih otvorenom repozicijom i perkutanom fiksacijom pomoću K-igala, mogu biti zadovoljavajući. Potrebne su dodatne studije da bi se stekao bolji uvid u prevalenciju i uzročne faktore mogućih komplikacija ovakvog pristupa lečenju navedene povrede.

Ključne reči:
zglob, iščašenja; ortopedske procedure; tarzalni zglob; lečenje, odlaganje; lečenje, ishod.

Correspondence to: Draško Vasović, Institute for Orthopedic Surgery "Banjica", Mihaila Avramovića 28, 11 000 Belgrade, Serbia.
E-mail: draskovasovic@yahoo.com

Introduction

The Chopart joint (CJ), composed of the talonavicular and calcaneal-cuboidal joints, plays a crucial role in foot mechanics and stability. According to Ponkilainen et al. [1], CJ dislocations (CJD) are rare injuries that account for 18.2% of midfoot traumas (56 out of 307) or 5.9% of foot and ankle injuries (out of a total of 953) shown using computed tomography (CT) scan analysis during the 5-year period. Neglected CJD are rare but pose significant challenges in diagnosis and management [2]. CJD typically result from high-energy trauma and can involve the entire Chopart complex or isolated joints within it. Prompt reduction and restoration of anatomical alignment are essential for successful outcomes, but literature shows that up to 41% of these injuries are unrecognized on the first patient-doctor encounter [3]. Due to this reason, such injury poses a significant clinical problem, leading to increased morbidity and poor treatment outcomes.

Nonoperative treatment is possible in some cases by performing closed reduction and cast immobilization, but in many acute cases, closed reduction is unsuccessful [4]. In chronic dislocations, there is no place for closed reduction, and surgical treatment is necessary. Surgical treatment aims to reconstruct the talonavicular joint and address associated fractures (if they exist), while fusion may be considered for severe calcaneal-cuboidal joint damage or in unsuccessful reduction of the joint [4, 5]. Operative management is focused on joint alignment and congruence restoration, which are essential for favorable outcomes.

This paper presents two cases of neglected CJD treated surgically and followed by favorable medium-term outcomes.

Case reports

Case 1

A 63-year-old male sustained an injury from falling down the stairs. Initially evaluated at a regional trauma center, he was diagnosed with a left ankle sprain. Treatment consisted of bandaging, non-steroidal anti-inflammatory medications, and rest. While pain and discomfort during weight-bearing decreased after the first week of the treatment, they persisted at a lower level afterward. The patient was examined at our institution eight weeks after the injury, presenting a visible midfoot deformity with an altered shape of the medial arch.

Imaging diagnostics were performed, and although the initial biplanar X-rays did not show definitive radiological signs of an injury (Figure 1 a, b), subsequent computed tomography (CT) diagnostics revealed a CJD (Figure 1 c, d). The patient underwent surgical treatment involving an open reduction of the CJ and transfixion using four K-wires (Figure 1 e, f). Postoperatively, the patient was immobilized with a below-knee orthosis for six weeks without weight-bearing. After the removal of the K-wires, physical therapy commenced, gradually progressing from partial

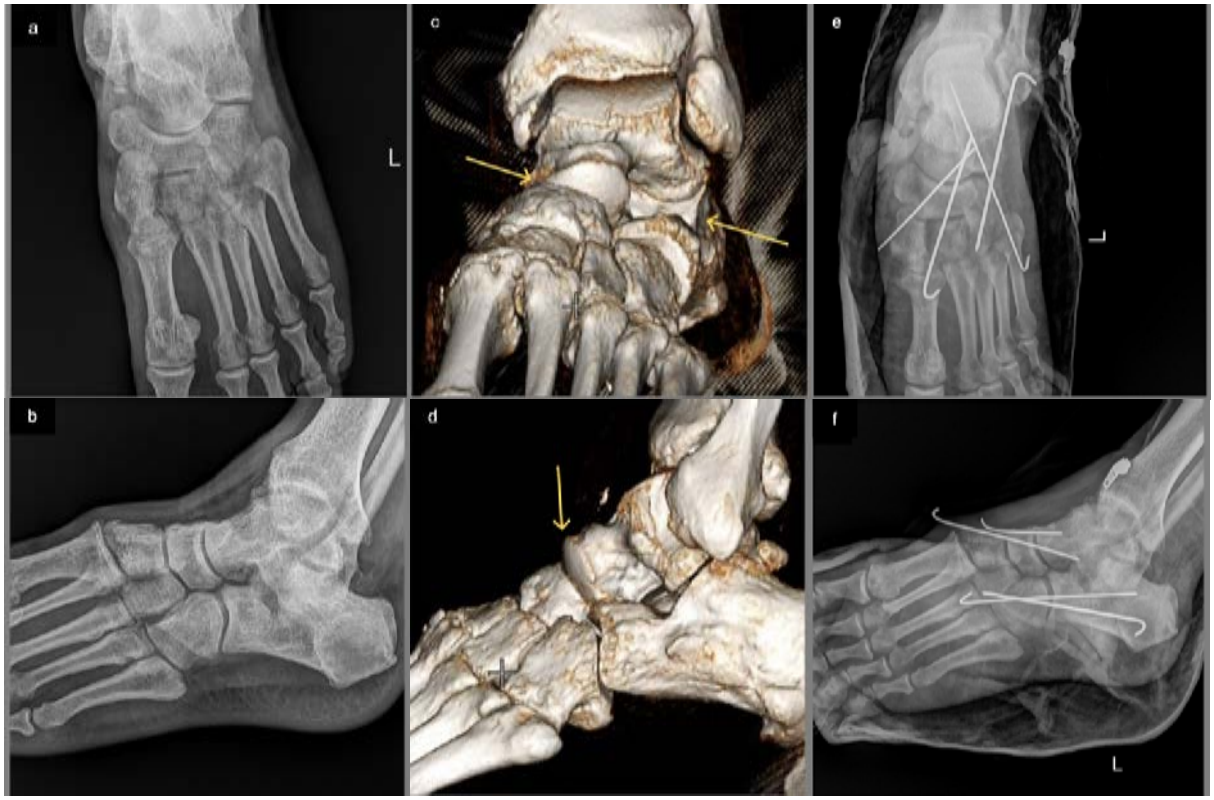


Fig. 1 – Imaging diagnostics of Case 1 patient: plain X-rays did not show definitive radiological signs of injury (a, b); three-dimensional computed tomography showed dislocation in the Chopart joint (yellow arrows) (c, d); postoperative X-rays, with transfixion of talonavicular and calcaneocuboid joints, show the congruence of these joints (white lines indicate K-wires) (e, f).

weight-bearing with the orthosis to full weight-bearing at twelve weeks after surgery (Figure 2 a, b).

The patient returned to normal daily activities without pain or deformity. During the follow-up period of eighteen months, the patient achieved an American Orthopaedic Foot and Ankle Society (AOFAS) score of 84 out of 100.

Case 2

A 33-year-old female was injured in a traffic accident. Initially managed according to the trauma protocol at a large emergency center, she received a diagnosis of a right ankle sprain without any other major injuries. Initially, there were no significant symptoms apart from diffuse ankle-foot pain. Due to prolonged pain, the patient presented to our institution ten months after the initial trauma.

Follow-up X-rays of the foot did not confirm clear radiological signs of injury. However, subsequent CT scans revealed a Chopart dislocation of the right foot (Figure 3 a, b). Similar to Case 1, surgical treatment involved an open reduction of the CJ and transfixion using four K-wires (Figure 3 c, d), followed by an identical postoperative protocol (Figure 2 c, d).

The final follow-up of this patient was conducted two years after surgery. The patient exhibited no deformity and reported no pain but experienced a reduction in foot movement, resulting in an AOFAS score of 76 out of 100.

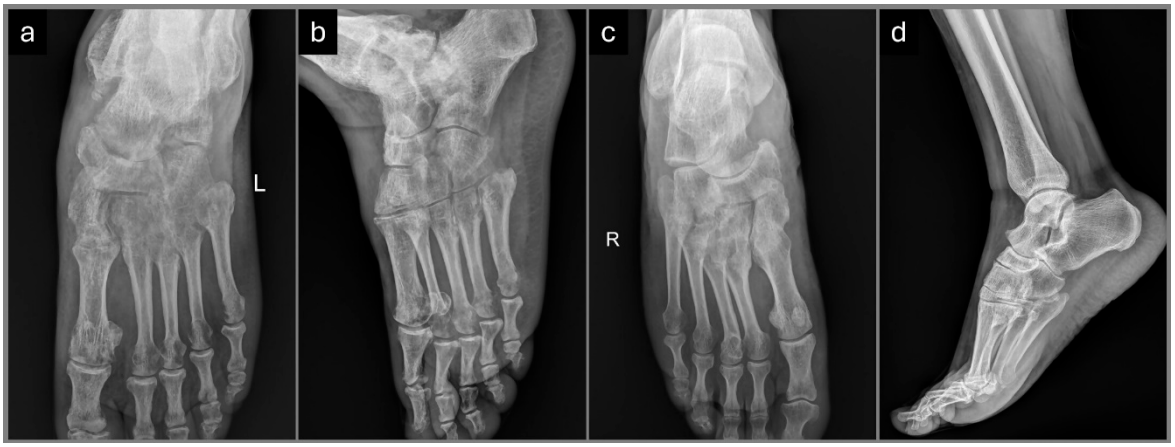


Fig. 2 – X-rays at the end of follow-up: in both cases, Case 1 (a, b) and Case 2 (c, d), the normal foot anatomy with congruent Chopart joint was confirmed.

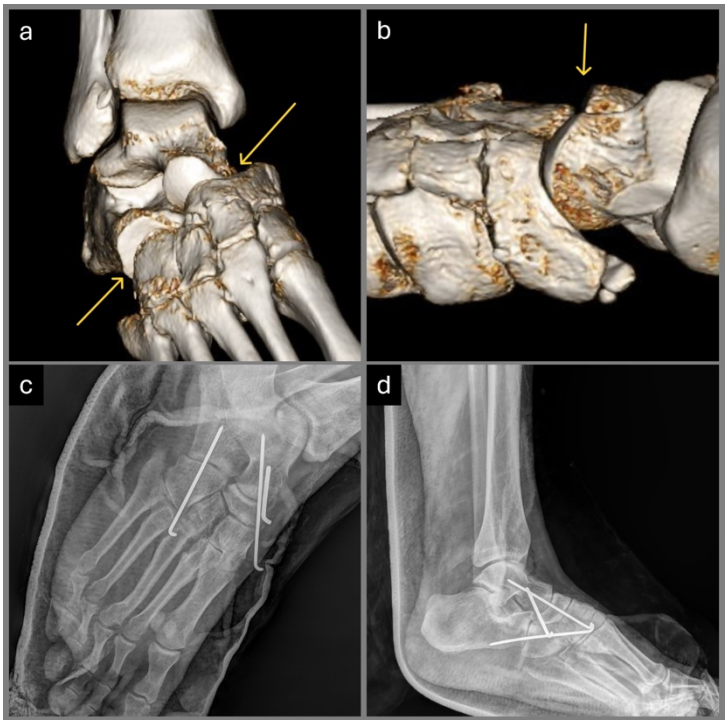


Fig. 3 – Imaging diagnostics of Case 2 patient: three-dimensional computed tomography shows plantar dislocation of the Chopart joint (yellow arrows) (a, b); postoperative X-rays (white lines indicate K-wires) (c, d).

Discussion

The CJ complex, comprised of the talonavicular and calcaneocuboid joints, represents a low-mobile but essential anatomical construct that enables proper mechanics of the foot. It is critical to normal gait and weight-bearing and is intrinsically involved in the movements of inversion and eversion [6, 7]. The normal function of the CJ requires strong ligamentous support. Ligaments stabilizing the joint include the dorsal talonavicular ligament, bifurcate ligament, dorsal calcaneocuboid ligament, short and long plantar ligaments, and spring ligament [8].

Trauma in this region may cause fractures and/or dislocations. Chopart dislocations include both pure-dislocations (exclusively capsulo-ligament injuries) and fracture-dislocations, being mainly caused by motor vehicle accidents and fall from height [3, 9]. Main and Jowett [10] classified these injuries into the following five types according to the direction of the deforming force and the resulting displacement: medial, longitudinal, lateral, plantar, and crush. The frequency is by far the highest for the medial and plantar dislocations [3, 9, 10].

Purely transligamentous dislocations are quite rare (4%) and generally missed or misdiagnosed. The reason for misdiagnosis, in addition to the low prevalence, could be the lack of familiarity with the entity and the absence of obvious radiological signs on standard radiographs (anteroposterior, lateral, and 45° oblique projections). The CJ appears as a harmonic double wave ("lazy S-shape"), the so-called "cyma line", when viewed laterally. Incongruity at the CJ needs to be suspected if this line is disrupted. Furthermore, stress radiographs with forced abduction and adduction carried out under sufficient local anesthesia can be useful [11].

Many authors suggest CT scanning because it allows reconstructive modeling to determine the presence and degree of the dislocation. Delays in diagnosis are common and may adversely affect the long-term prognosis, with the potential for developing instability, foot deformity, and eventual osteoarthritis of the CJ [2].

So far, no consensus has been made on the treatment choice of Chopart pure dislocations (CPD). Different procedures are imposed as a method of selection, and they imply closed reduction with or without percutaneous fixation, open reduction with or without internal fixation, and primary fusion of CJ.

Restoring the proper length of the medial and lateral columns, as well as the proper alignment of the foot axes, are the primary objectives of the treatment. Compared to closed reduction before internal fixation, initial open reduction with internal fixation generates noticeably better results for CPD [9]. According to Richter et al. [9], 43% of pure dislocations need internal fixation after closed reduction. The authors concluded that maintaining the anatomical integrity of a pure dislocation is necessary so that closed reduction can be successfully treated. Furthermore, repeated attempts at closed reduction often result in more damage to the soft tissues [12].

Rammelt and Missbach [11] found over a 10-year follow-up that open reduction and internal fixation (with K-wires) generate better results than closed reduction and percutaneous fixation. However, the pure dislocations had the worst prognosis. Generally, K-wire (1.6–2.0 mm) fixation allows adequate joint congruence and ligamentous healing [13].

Contaminated or infected soft tissues, advanced peripheral vascular disease, chronic venous insufficiency with skin ulceration, poor patient compliance, diabetic neuro-osteoarthropathy (Charcot foot), evident immunodeficiency, and a critical state of the patient's overall health are all contraindications to a formal open reduction [13]. Primary fusion of the CJ should be reserved for cases of severe instability and destruction of the articular surface because of the pivotal role of these joints in global foot function [14].

Although CJ injuries are rare, complications of such injuries are common. Metcalfe et al. [2] analyzed 10 cases with follow-up between six and 76 months, and results indicated that 50% of them had significant long-term complications. Complications of nonoperative treatment included deformity, instability, and posttraumatic arthritis. The most frequent operative complications were wound healing problems and nerve injuries. Wound healing complications are related to operative timing (too early) and technique (presence of undermining wound edges and flaps after suturing the surgical wound). In this context, early operative timing refers to the condition of the soft tissues. High-energy injuries often result in extensive soft tissue damage, characterized by significant swelling, hematomas, and the formation of skin blisters. Performing surgery too early, before the soft tissue damage has sufficiently stabilized, can compromise wound healing, as the tissues may not yet be capable of tolerating the stress of surgical intervention. Peroneal and sural nerve injury can be caused by inadequate surgical approach or aggressive retraction. Other complications like deep venous thrombosis, compartment syndrome, and complex regional pain syndrome (especially after initially overlooked Chopart injuries) have been rarely reported [2, 3, 9, 13–15]. It should be emphasized that intraoperative relative shortening of the medial column leads to *cavus* deformity, whereas relative shortening of the lateral column leads to a posttraumatic flatfoot [12].

Both of our CPD cases were successfully treated with open reduction and K-wire transfixation without postoperative complications. After the physical rehabilitation, both patients had a satisfactory AOFAS score result and did not indicate the existence of any clinically significant complaints. After six months of follow-up, radiographic images indicated a maintained congruence and anatomical position of the CJ. Only two cases were presented here. However, considering that there is no data in the literature about the protocol and recommendations for the treatment of CPD patients who were not timely diagnosed, these cases can serve as an incentive for some future research.

Conclusion

Despite the delayed diagnosis and postponed operative treatment, the functional outcomes of pure Chopart dislocation treated by open reduction and percutaneous K-wiring can be satisfactory. Additional studies are required for a better insight into the prevalence and causative factors of the possible complications in such treatment.

R E F E R E N C E S

1. *Ponkilainen VT, Laine HJ, Mäenpää HM, Mattila VM, Haapasalo HH.* Incidence and Characteristics of Midfoot Injuries. Foot Ankle Int 2019; 40(1): 105–12.
2. *Metcalfe TSN, Aamir J, Mason LW.* Chopart dislocations: a review of diagnosis, treatment and outcomes. Arch Orthop Trauma Surg 2024; 144(1): 131–47.
3. *Van Dorp KB, de Vries MR, van der Elst M, Schepers T.* Chopart joint injury: a study of outcome and morbidity. J Foot Ankle Surg 2010; 49(6): 541–5.
4. *Viegas G.* Midtarsal joint dislocations: acute and chronic management with review of the literature and case presentation. The Foot 2000; 10(4): 198–206.
5. *Klaue K.* Chopart fractures. Injury 2004; 35 Suppl 2: SB64–70.
6. *Elftman H.* The transverse tarsal joint and its control. Clin Orthop 1960; 16: 41–6.
7. *Blackwood CB, Yuen TJ, Sangeorzan BJ, Ledoux WR.* The midtarsal joint locking mechanism. Foot Ankle Int 2005; 26(12): 1074–80.
8. *Walter WR, Hirschmann A, Alaia EF, Tafur M, Rosenberg ZS.* Normal anatomy and traumatic injury of the midtarsal (chopart) joint complex: An imaging primer. Radiographics 2019; 39(1): 136–52.
9. *Richter M, Thermann H, Huefner T, Schmidt U, Goesling T, Krettek C.* Chopart joint fracture-dislocation: initial open reduction provides better outcome than closed reduction. Foot Ankle Int 2004; 25(5): 340–8.
10. *Main BJ, Jowett RL.* Injuries of the midtarsal joint. J Bone Joint Surg Br 1975; 57(1): 89–97.
11. *Rammelt S, Missbach T.* Chopart joint injuries: Assessment, treatment, and 10-year results. J Orthop Trauma 2023; 37(1): e14–21.
12. *Honeycutt MW, Perry MD.* The Chopart variant dislocation: Plantar dislocation of the cuboid and navicular. Foot Ankle Orthop 2019; 4(3): 2473011419876262.
13. *Rammelt S, Schepers T.* Chopart injuries: When to fix and when to fuse? Foot Ankle Clin 2017; 22(1): 163–80.
14. *Rammelt S.* Chopart and Lisfranc Fracture-Dislocations. In: *Bentley G,* editor. European Surgical Orthopaedics and Traumatology. Berlin, Heidelberg: Springer; 2014. p. 3835–57.
15. *Kotter A, Wieberneit J, Braun W, Rüter A.* The Chopart dislocation: results of a frequently underestimated injury and its sequelae. A clinical study. Unfallchirurg 1997; 100(9): 737–41. (German)

Received on February 6, 2024
Revised on November 8, 2024
Revised on December 10, 2024
Revised on December 26, 2024
Accepted on January 15, 2025
Online First March 2025

CASE REPORT

UDC: 616.728.3 -089-06: 616.147.3-001
DOI: https://doi.org/10.2298/VSP241005021B

Anatomical variations of the popliteal artery as a risk factor for its laceration during total knee arthroplasty: controversies with illustrative case report

Anatomske varijacije poplitealne arterije kao faktor rizika za njenu laceraciju prilikom totalne artroplastike kolena: kontroverze uz ilustrativni prikaz slučaja

Jelena Boljanović*, Miodrag Glišić†, Nenad Ilijevski‡, Matija Bećirović†, Smiljana Stojanović§, Ivana Pavković‖, Katarina Djurdjević*, Valentina Blagojević*

University of Belgrade, Faculty of Medicine, *Institute of Anatomy "Niko Miljanić", §Laboratory for Vascular Anatomy, Belgrade, Serbia; †Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia; ‡Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia; ‖Institute for the Improvement of Life Activities, Belgrade, Serbia

Abstract

Introduction. Popliteal artery (PA) injury during knee replacement surgery is a rare but extremely serious complication. Most vascular complications during knee surgery can be prevented by a careful preoperative assessment of the patient. **Case report.** We present the case of a 51-year-old woman who was admitted to the hospital to undergo routine total knee arthroplasty (TKA) surgery due to rheumatoid arthritis. The patient underwent surgery *via* standard medial parapatellar approach under spinal anesthesia. A fixed-bearing implant was used. The operation was successfully performed, but after removing the tourniquet, excessive bleeding was encountered, indicating a possible injury to the PA, hence the tourniquet was placed again. Severed PA was noted after exploration of the popliteal region, while the posterior capsule was not damaged. Revascularization with a Dacron vascular graft was performed immediately. On the third day following surgery, the patient had complaints that caused a suspicion of graft occlusion, and she was transferred to the Clinic for Cardiovascular Surgery. Multidetector computed tomography angiography confirmed total occlusion of the popliteal Dacron graft, and surgery was performed. The occluded graft was removed, and a popliteal-tibioperoneal trunk bypass was performed using an autologous great saphenous vein graft. Lateral and posterior fasciotomies were performed as well. At the three-month follow-up examination, the patient remained asymptomatic, with improvement in ankle dorsiflexion function. **Conclusion.** Preoperative assessment can help identify patients who are at the highest risk of complications of PA injury during their TKA. If vascular complications occur, early recognition and immediate intervention by a vascular surgeon are essential for a positive treatment outcome.

Key words:
arthritis, rheumatoid; arthroplasty, replacement, knee; computed tomography angiography; intraoperative complications; popliteal artery; risk factors; treatment outcome.

Apstrakt

Uvod. Povreda poplitealne arterije (PA) tokom operacije zamene zgloba kolena je retka ali izuzetno ozbiljna komplikacija. Većina vaskularnih komplikacija prilikom operacija kolena može se sprečiti pažljivim preoperativnim pregledom bolesnika. **Prikaz bolesnika.** Prikazana je 51-godišnja žena koja je primljena u bolnicu na rutinsku operaciju totalne artroplastike kolena (TAK) zbog reumatoidnog artritisa. Bolesnica je operisana standardnim medijalnim parapatelarnim pristupom u spinalnoj anesteziji. Korišćen je implant sa fiksnim ležajem. Operacija je uspešno obavljena, ali je nakon otpuštanja poveske došlo do prekomernog krvarenja, što je ukazalo na moguću povredu PA, te je poveska ponovo postavljena. Povreda PA je uočena nakon eksploracije poplitealne regije, dok zadnja kapsula nije bila oštećena. Revaskularizacija Dacron vaskularnim graftom je odmah urađena. Trećeg dana nakon operacije, bolesnica je imala tegobe koje su izazvale sumnju na okluziju grafta te je prebačena na Kliniku za kardiovaskularnu hirurgiju. Angiografija multidetetorskom kompjuterizovanom tomografijom je potvrdila totalnu okluziju poplitealnog Dacron grafta i urađena je operacija. Okludirani graft je uklonjen, a poplitealno-tibioperonealni bajpas je urađen korišćenjem autolognog grafta vene safene magne.

Correspondence to: Jelena Boljanović, University of Belgrade, Faculty of Medicine, Institute of Anatomy "Niko Miljanić", Dr Subotića 4, 11 000, Belgrade, Serbia. E-mail: jelena.boljanovic145@gmail.com

Urađene su i bočne i zadnje fasciotomije. Na tromesečnom kontrolnom pregledu bolesnica je i dalje bila bez simptoma, uz poboljšanje dorzalne fleksije skočnog zgloba. **Zaključak**. Preoperativna ispitivanja mogu pomoći u identifikaciji bolesnika koji imaju najveći rizik od komplikacija povrede PA tokom TAK. Ako dođe do vaskularnih komplikacija, rano prepoznavanje i hitna intervencija vaskularnog hirurga su esencijalni za pozitivan ishod lečenja.

Ključne reči:
artritis, reumatoidni; artroplastika kolena; angiografija, tomografska, kompjuterizovana; intraoperativne komplikacije; a. poplitea; faktori rizika; lečenje, ishod.

Introduction

The incidence of popliteal artery (PA) injury (PAI) during total knee arthroplasty (TKA) is relatively low, from 0.03% to 0.2%. The consequences of the PAI might be very serious, including limb loss or even death [1, 2]. The most commonly reported vascular complications following TKA were a variety of injuries such as laceration or transection, (false) aneurysm formation, arteriovenous fistula, and thrombosis. PAIs are present in several forms, ranging from acute bleeding to discrete swelling, occurring sometimes several months following TKA. The literature on this topic comprises individual case reports, case series, and surveys that provide information concerning surgeons' experiences of PAI following TKA. A review of the literature has shown multiple risk factors that predispose patients to arterial complications after TKA [3–8]. It is not surprising that the highest risks of PAI are associated with revision arthroplasty or previous injuries of the knee joint affecting the posterior capsule [2, 3, 9, 10]. Revision surgery or surgery near the knee joint compromises the anatomic relationships in the popliteal *fossa,* which may also increase the risk of vascular complications [2, 10–14].

Morphological variations and branching patterns of the PA, as well as the anatomical positions of other structures, such as the popliteal vein, tibial nerve, and common peroneal nerve, are considered risk factors during TKA [8, 15]. In order to minimize the risk of lesions on the PA during TKA, most knee surgeries are performed with the knee at 90° of flexion, as it is believed to be a safe position for the PA. On the other hand, it has been reported that the PA moved closer to the tibia or was not removed when the knee was flexed [16]. The popliteal vessels are found to be at major risk at 90° of knee flexion, as confirmed by other authors [17, 18].

Arthroscopy and TKA are two orthopaedic procedures in which instrumentation is routinely placed near the posterior capsule of the knee. Closer proximity of the PA and the posterior horn of the lateral meniscus was noted as opposed to the medial meniscus, and with respect to the clinically relevant tibial plateau as it crosses the joint line [19–21].

We present a case of the PA lesion caused by indirect injury during TKA, thus highlighting the anatomic relationships as a respectable risk factor. We clarify the patient factors that influence the TKA surgery, such as the PA position to the tibial plateau, the movement of the PA during knee flexion, the influence of the variable branching pattern of the PA, as well as the importance of meticulous surgery, especially in cases in which anatomical relationships of the major vessels to the tibial surface could be affected by comorbidities.

Case report

A 51-year-old female was admitted to the hospital to undergo routine TKA for rheumatoid arthritis (RA). She had symptoms of marked pain, including left leg weakness, limited motion, and left knee instability. She had been suffering from RA for 10 years and from hypothyroidism for 5 years, during which time she had been properly medicated. The patient's medical history included right total hip arthroplasty (5 years ago). She denied lower extremity claudication, vascular disease, or chest pain.

A physical examination prior to primary TKA revealed a moderately obese female (165 cm, 70 kg) with left knee pain, knee deformity (hypertrophic synovial membrane and severe varus), and laxity of the lateral collateral ligament. The range of motion of the knee was from 15° lack of full extension to 90° of flexion. She had no signs of chronic ischemia or thrombosis in the leg. Preoperative radiographs revealed marked depression on the medial tibial plateau and destruction on the medial femoral condyle (probably necrosis). There were osteophytes on the posterior tibia. Preoperative radiographs confirmed the 18° of femorotibial anatomic varus.

The patient underwent surgery under spinal anesthesia, which lasted 100 minutes. Surgery had been performed *via* standard medial parapatellar approach with a tourniquet in place. A SIGMA PS fixed-bearing implant (De Puy – Johnson & Johnson, Warsaw, Indiana, USA) had been used.

The knee position during the operation was between 90° and 100° of flexion. The articular surface of the femur was resected, and the trial implant was inserted. The tibia was pushed forward and held in a position similar to the knee dislocation during the resection of the articular surface. The distal femur was held posteriorly, compressing the posterior capsule. The trial tibial component was inserted to check the position of the knee. The same maneuver was performed during the insertion and fixation of the cemented prosthesis. Bearing in mind that the physical examination revealed severe limitation of the knee movement in RA and a significant varus deformity, it could be assumed that the posterior capsule was thickened, shortened, and lacked elasticity. Damage to the PA might have been caused by the force used to position the tibia for inserting the implant.

The tourniquet was released after 87 minutes, prior to implantation of the bearing component to ensure hemostasis. Excessive bleeding was encountered, indicating injury to the PA. The bleeding was stopped by repositioning the tourniquet. Due to this, the general surgeon was immediately alerted, surgery was completed, and the patient was introduced to

general anesthesia and placed into the prone position. The patient underwent immediate exploration without prior investigation. The popliteal region was explored *via* the straight posterior incision. Intraoperatively, the general surgeon noted the extremely thin and fragile PA, which was completely severed and retracted. The posterior capsule was not damaged. Revascularization with a Dacron vascular graft was performed using systemic heparinization. The tourniquet was released after 150 minutes (encountering the prior intervention). The regular thromboembolic arthroplasty prevention protocol, which included subcutaneous injection of fraxiparine twice daily for 5 days (determined based on the patient's body weight), was administered.

Postoperatively, the peripheral arterial pulse on the *dorsalis pedis* artery was palpable, and the temperature and coloration of the lower leg were normal. The orthopedic surgeon examined the patient daily and did not notice anything that could indicate any circulatory problem in the left leg and foot. The patient experienced severely decreased sensation in the right foot and calf, along with no significant weakness of ankle dorsiflexion, which was related to the tourniquet utilization time. On the third day following surgery, the patient began experiencing the absence of palpable peripheral pulses (pedal and posterior tibial) and coolness of the left lower limb, with complaints of numbness and tingling below the knee and rest pain in the calf. Based on medical history, the patient was diagnosed with graft occlusion and was immediately transferred to the clinic for cardiovascular surgery. Vascular examination revealed a livid left foot, very cold on palpation, without dorsal flexion movement ability. Multidetector computed tomography angiography revealed total occlusion of the popliteal Dacron graft, and emergency reconstruction was indicated (Figures 1 and 2). The occluded graft was resected, and a popliteal-tibioperoneal trunk bypass

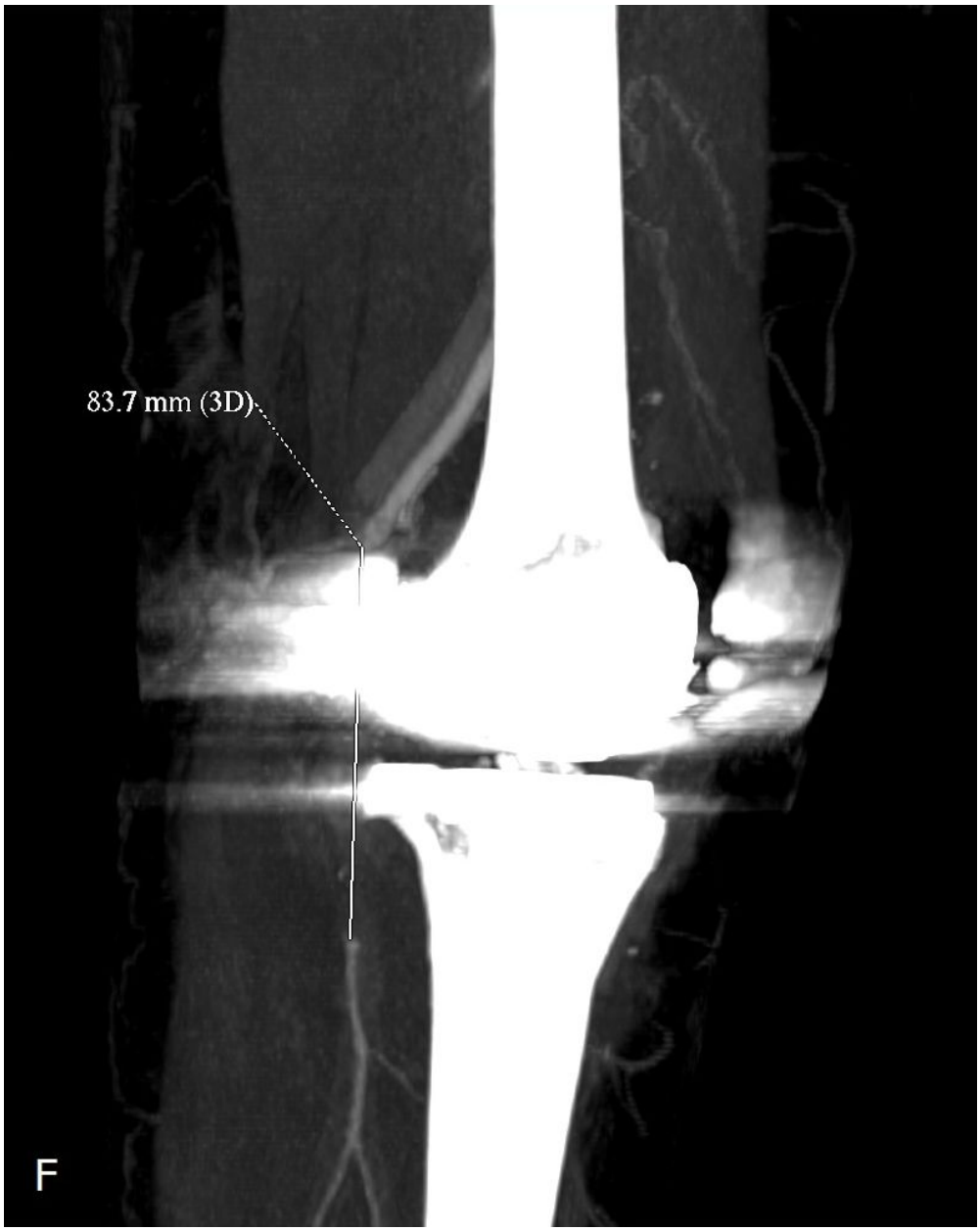


Fig. 1 – Multidetector computed tomography angiography – lateral view of the occluded popliteal Dacron graft.

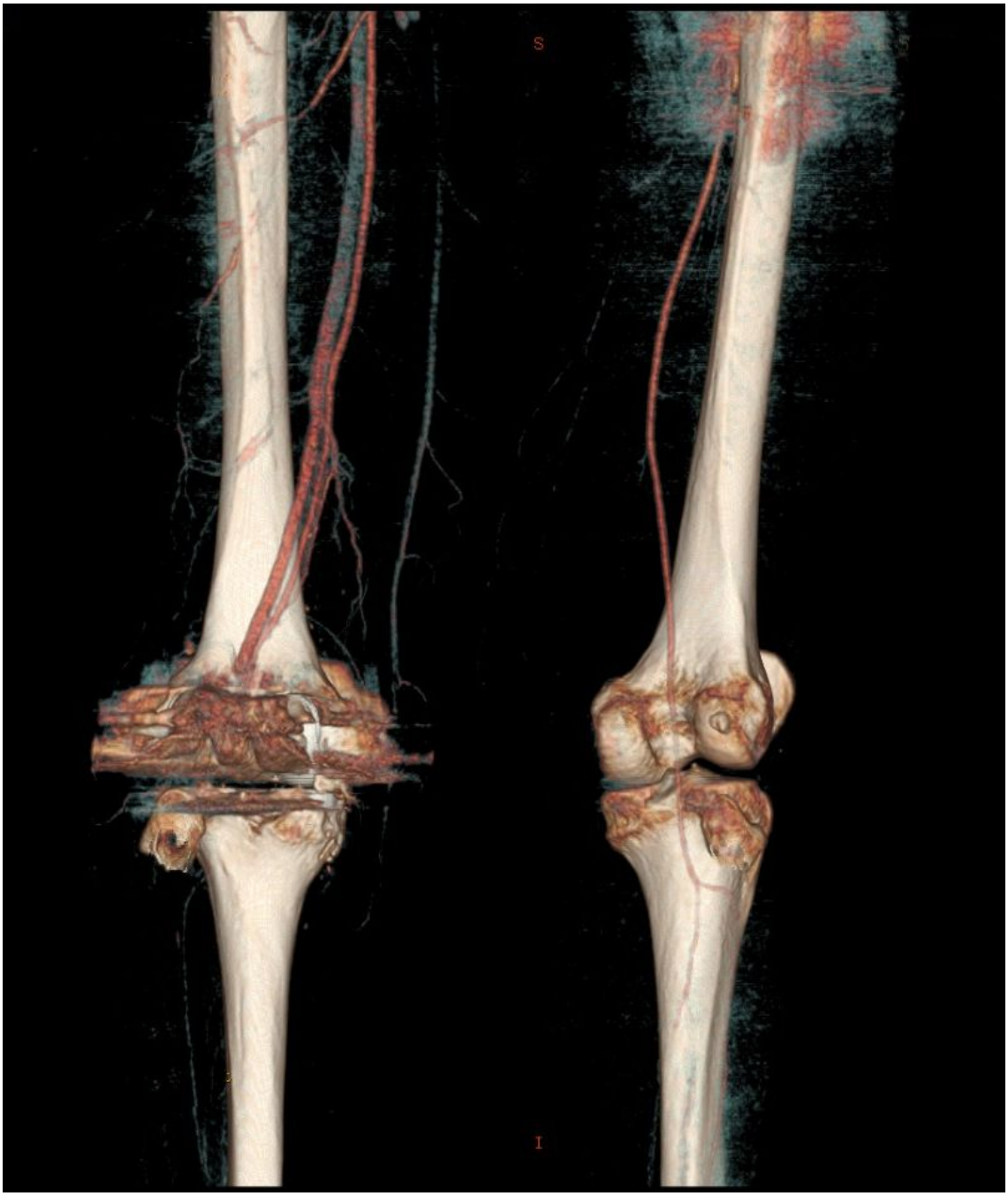


Fig. 2 – Multidetector computed tomography angiography – posterior view of the occluded popliteal Dacron graft.

was performed using an autologous great saphenous vein graft. Both anastomoses were performed in the terminal-lateral method using a 6–0 polypropylene continuous suture. Both lateral and posterior fasciotomies were performed as well.

Necrectomy of the wound, secondary seam, and cover of the skin defect with Thiersch's grafts were performed in the later postoperative course. The wound on the front side healed primarily. There were no signs of infection, the peroneal nerve was partially recovered, and the patient was able to walk with a cane. At a three-month follow-up examination, the patient remained asymptomatic without pain or swelling. The examination indicated evidence of improved function of ankle dorsiflexion.

Discussion

TKA and high tibial osteotomy are common orthopaedic operations with rare vascular complications. The incidence of reported complications is 0.03–0.5%, but they probably occur more commonly than is suggested by the literature [4, 5, 22, 23]. The PA is especially at risk during the following situations: the removal of osteophytes from the proximal aspect of the posterior femoral condyles, the release of

the posterior capsule, and the resection of the proximal tibia with an oscillating saw [24].

Analyzing national data over 14 years in the USA, Dua et al. [5] noted that 93% of patients underwent TKA that was complicated by PAI and had osteoarthritis as their primary diagnosis. In addition, 65% were female and the average age was 61.7 ± 12.3 years. Tearing of the PA during standard surgical manoeuvres might be related to peripheral vasculopathy caused by hypothyroidism in older patients [25, 26].

PAI has two mechanisms – direct and indirect. Direct trauma may cause intraoperative hemorrhage, pseudoaneurysm, or arteriovenous fistula, while indirect trauma may cause intimal damage, atheromatous plaque disruption, or both [5, 9, 13, 14]. A review of the literature demonstrated that direct trauma is the most common mechanism in up to 78% [9, 22, 23, 27]. Pal et al. [9] presented nine cases of PAI following TKA. Seven (78%) were due to direct injury (three arterial lacerations, two pseudoaneurysms, and one arteriovenous fistula), and two (22%) were due to indirect trauma (thrombosis). Identical results were published by Bernhoff et al. [23] as a nationwide study of PAI during knee arthroplasty in Sweden (78% were due to penetration and 22% due to blunt trauma). They identified three different presentations of injury: bleeding in 44%, ischaemia in 21%, and false aneurysm formation in 35%. Compared to what can be found in the literature, the mechanism of the PAI in our case was common – during the removal of osteophytes and release of the posterolateral capsule. We could not exactly discover the obvious reasons for damage, but direct laceration with an oscillating saw or some other instrument was excluded because the posterior capsule remained intact. The fact that the joint capsule remained intact presumes an indirect trauma. The injury presented with excessive bleeding after deflating the tourniquet, which was the most common clinical presentation in the literature.

Our patient was a young female with no risk factors for vascular impairment reported in the literature, such as smoking, hypercholesterolemia, or hypertension. She had been suffering from RA for 10 years and from hypothyroidism for 5 years, during which time she had been properly medicated. During the preoperative physical examination, she had good palpable pulses of the *dorsalis pedis* artery and a good capillary refill of the foot. Given that adequate care was taken throughout the procedure, including the manipulation of the knee, the use of the oscillating saw, and the placement of the posterior blunt retractor, it appears that an external factor caused this vascular complication. We present the anatomical description of the PA variant location to help explain this case.

PA descends obliquely as a continuation of the femoral artery from the opening of the adductor magnus medially to the crural interosseous space laterally, where it is divided into anterior and posterior tibial arteries. As the artery traverses the popliteal *fossa*, inclining obliquely from the medial condyle of the femur to the distal border of the popliteus muscle, it crosses the knee joint line, closely opposed to the posterior capsule of the knee, placing it at risk of injury during the knee surgery [27]. The PA has variant patterns in the number of terminal branches and in the length of the tibioperoneal trunk. The widely used PA variant classification was created by Kim et al. [19], distinguishing three types and ten subtypes. The main categories were formed by the location at which the anterior tibial artery (ATA), posterior tibial artery, and fibular artery branched from the PA. ATA lesions are more frequent due to the type II-B anatomic variant, in which the ATA is in direct contact with the posterior tibial cortex and may be damaged by the saw or the retractors. Our patient has the I-B variant pattern of the PA (Figure 3). The incidence of that variant is the second most frequent in most studies and varies from 2% to 5.46% [8, 19].

Avoiding the vascular injury of the PA has included numerous variations in its positioning to the joint line and its relation to the posterior tibial cortex, posterior capsule, and other neurovascular structures. Most authors have concluded that the PA is the most lateral structure at the level of the tibial plateau, in 94.3–95% of cases [20, 28]. Keser et al. [20] reported the PA localization on the central axis in 5.7% of cases, stressing that such a position could increase the risk of PAI. They did not report PA on the medial side of the central axis of the knee joint. Rubash et al. [17] performed a cadaver study to propose a clock system to be used to guide the surgeon in selecting a safe location during the screw placement. They determined the anatomical relationships of major vessels to the tibial surface, which was compared to a clock face. The PA and ATA were found to be at risk in a zone between the twelve o'clock and two o'clock positions, which is the central and lateral area. Standard medial parapatellar approach, avoiding the "danger zone", was performed on our patient.

An anatomical description of the PA usually refers only to the extended knee. The general position seems to be when the knee flexion releases tension on the PA and displaces it posteriorly, away from the joint capsule. Bartlett et al. [29] conducted a study on injuries of the popliteal vessels and evaluated their incidence, anatomical factors, and the influence of surgery. They concluded that PA came close to the tibia as the knee was flexed, being at risk of PAI. Cadaver studies confirmed that the flexed knee does not always guarantee safety [15, 18]. A larger cut, removing more bone, will further decrease the distance from the cut surface of the tibial plateau to these vulnerable structures. It seems that anatomic variation in the relative depths of the vital structures exists.

Early detection and repair of arterial injuries during TKA often improve outcomes [4, 9, 23, 27, 30]. In their nationwide study, Bernhoff et al. [23] reported that six of seven patients with complete recovery had an early diagnosis of PAI and immediate treatment. The urgency of vascular treatment varies in the literature, from four hours for good outcomes [30] to five days for limb salvage [2]. Our patient achieved a complete recovery, which could be associated with the fact that PAI was intraoperatively recognized and repaired.

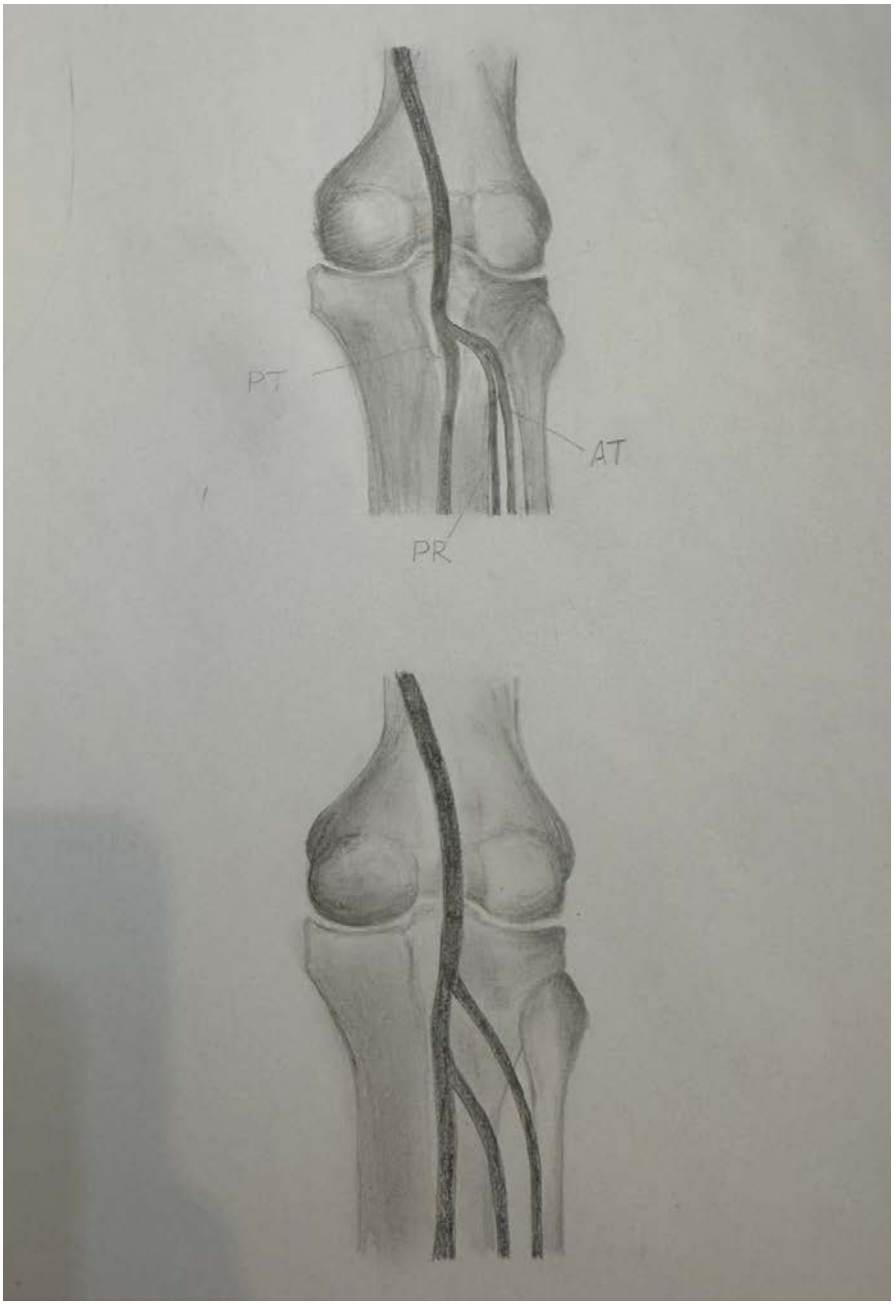


Fig. 3 – Anatomic classification of popliteal artery by Kim et al. [19]: I-B variant. PT –posterior tibial artery; PR – peroneal artery; AT – anterior tibial artery.

Conclusion

Anatomical variation of the popliteal artery should be considered to identify patients at the highest risk of arterial complications during their total knee arthroplasty. Comorbidities such as rheumatoid arthritis or revision surgery could fix the popliteal artery against the posterior capsule by firm connective tissue septa, compromising its movement during the knee flexion or extension.

If an acute vascular complication occurs, early recognition of ischemic arterial complications and rapid diagnosis decreases the period of ischemia. In cases of iatrogenic popliteal artery injury, initial interventions are commonly performed because of faster reperfusion time.

However, the vascular surgeon is the only one able to perform the autologous venous graft/bypass surgery to improve the functional outcome and prevent serious consequences.

Acknowledgment

The study was financed by the Ministry of Science, Technology, and Development, the Republic of Serbia (grant No. 451-03-66/2024-03/200110).

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Sierra RJ, Trousdale RT, Pagnano MW.* Above-the-knee amputation after a total knee replacement: prevalence, etiology, and functional outcome. J Bone Joint Surg Am 2003; 85(6): 1000–4.
2. *Abularrage CJ, Weiswasser JM, Dezee KJ, Slidell MB, Henderson WG, Sidawy AN.* Predictors of lower extremity arterial injury after total knee or total hip arthroplasty. J Vasc Surg 2008; 47(4): 803–7.
3. *Ng VY, Lustenberger D, Hoang K, Urchek R, Beal M, Calhoun JH,* et al. Preoperative risk stratification and risk reduction for total joint reconstruction: AAOS exhibit selection. J Bone Joint Surg Am 2013; 95(4): e191–15.
4. *Langkamer VG.* Local vascular complications after knee replacement: a review with illustrative case reports. Knee 2001; 8(4): 259–64.
5. *Dua A, Zepeda R, Hernanez FC, Igbadumhe AA, Desai SS.* The national incidence of iatrogenic popliteal artery injury during total knee replacement. Vascular 2015; 23(5): 455–8. Erratum in: Vascular 2021; 29(5): 797.
6. *Park IH, Lee SC, Park IS, Nam CH, Ahn HS, Park HY,* et al. Asymptomatic peripheral vascular disease in total knee arthroplasty: preoperative prevalence and risk factors. J Orthop Traumatol 2015; 16(1): 23–6.
7. *Ishii Y, Noguchi H, Sato J, Takahashi I, Ishii H, Ishii R,* et al. Patient factors impacting localization of popliteal artery before total knee arthroplasty. Arch Orthop Trauma Surg 2023; 143(10): 6353–60.
8. *Tarasiuk A, Tubbs RS, Zielinska N, Karauda P, Gonera B, Olewnik Ł.* Variations of the popliteal artery: A review. Ann Anat 2023; 249: 152100.
9. *Pal A, Clarke JM, Cameron AE.* Case series and literature review: popliteal artery injury following total knee replacement. Int J Surg 2010; 8(6): 430–5.
10. *Ko LJ, DeHart ML, Yoo JU, Huff TW.* Popliteal artery injury associated with total knee arthroplasty: trends, costs and risk factors. J Arthroplasty 2014; 29(6): 1181–4.
11. *Tunggal JA, Higgins GA, Waddell JP.* Complications of closing wedge high tibial osteotomy. Int Orthop 2010; 34(2): 255–61.
12. *Smith DE, McGraw RW, Taylor DC, Masri BA.* Arterial complications and total knee arthroplasty. J Am Acad Orthop Surg 2001; 9(4): 253–7.
13. *Calligaro KD, Dougherty MJ, Ryan S, Booth RE.* Acute arterial complications associated with total hip and knee arthroplasty. J Vasc Surg 2003; 38(6): 1170–7. Erratum in. J Vasc Surg 2004; 39(3): 628.
14. *Kerens B, Boonen B, Schotanus MG, Kort NP.* Popliteal lesion due to traction during unicompartmental knee revision surgery. J Orthop 2013; 10(1): 38–40.
15. *Shetty AA, Tindall AJ, Qureshi F, Divekar M, Fernando KW.* The effect of knee flexion on the popliteal artery and its surgical significance. J Bone Joint Surg Br 2003; 85(2): 218–22.
16. *Zaidi SHA, Cobb AG, Bentley.* Danger to the popliteal artery in high tibial osteotomy. J Bone Joint Surg Br 1995; 77(3): 384–6.
17. *Rubash HE, Berger RA, Britton CA, Nettrour WS, Seel MJ.* Avoiding neurologic and vascular injuries with screw fixation of the tibial component in total knee arthroplasty. Clin Orthop Relat Res 1993; (286): 56–63.
18. *Darnis A, Villa V, Debette C, Lustig S, Servien E, Neyret P.* Vascular injuries during closing-wedge high tibial osteotomy: A cadaveric angiographic study. Orthop Traumatol Surg Res 2014; 100(8): 891–4.
19. *Kim D, Orron DE, Skillman JJ.* Surgical significance of popliteal arterial variants. A unified angiographic classification. Ann Surg 1989; 210(6): 776–81.
20. *Keser S, Savranlar A, Bayar A, Ulukent SC, Ozer T, Tuncay I.* Anatomic localization of the popliteal artery at the level of the knee joint: a magnetic resonance imaging study. Arthroscopy 2006; 22(6): 656–9.
21. *Farrington WJ, Charnley GJ, Harries SR, Fox BM, Sharp R, Hughes PM.* The position of the popliteal artery in the arthritic knee. J Arthroplasty 1999; 14(7): 800–2.
22. *Bernhoff K, Björck M.* Iatrogenic popliteal artery injury in non arthroplasty knee surgery. Bone Joint J 2015; 97–B(2): 192–6.
23. *Bernhoff K, Rudström H, Gedeborg R, Björck M.* Popliteal artery injury during knee replacement: a population-based nationwide study. Bone Joint J 2013; 95–B(12): 1645–9.
24. *Shin YS, Hwang YG, Savale AP, Han SB.* Popliteal artery pseudoaneurysm following primary total knee arthroplasty. Knee Surg Relat Res 2014; 26(2): 117–20.
25. *González Rodríguez JC, Jordan Sales M, Aguilera Roig X, Monllau García JC, Celaya Ibañez F.* Popliteal pseudoaneurysm as a complication in total knee replacement. Presentation of a case and an updated literature review. Rev Esp Cir Ortop Traumatol 2012; 56(3): 205–9. (Spanish)
26. *Mya MM, Aronow WS.* Increased prevalence of peripheral arterial disease in older men and women with subclinical hypothyroidism. J Gerontol A Biol Sci Med Sci 2003; 58(1): 68–9.
27. *Rudstrom Hakan.* Iatrogenic vascular injuries [Ph.D.]. Uppsala: Acta Universitatis Upsaliens; 2013. p. 64.
28. *Ninomiya JT, Dean JC, Goldberg VM.* Injury to the popliteal artery and its anatomic location in total knee arthroplasty. J Arthroplasty 1999; 14(7): 803–9.
29. *Bartlett RJ, Roberts A, Wong J.* Risk to popliteal vessels in major knee surgery, an anatomical study and survey of vascular surgeons. Orthop Proc 2004; 86(4): 468.
30. *Saleh KJ, Hoeffel DP, Kassim RA, Burstein G.* Complications after revision total knee arthroplasty. J Bone Joint Surg Am 2003; 85–A Suppl 1: S71–4.

Received on October 5, 2024
Revised on November 11, 2024
Revised on December 22, 2024
Accepted on January 15, 2025
Online First April 2025

INSTRUCTIONS TO THE AUTHORS

The VSP publishes only papers not published before, nor submitted to any other journals, in the order determined by the Editorial Board. Any attempted plagiarism or self-plagiarism will be punished. When submitting a paper to the VSP electronic editing system **(http://aseestant.ceon.rs/index.php)**, the following should be enclosed: a statement on meeting any technical requirements, a statement signed by all the authors that the paper on the whole and/or partly has not been submitted nor accepted for publication elsewhere, a statement specifying the actual contribution of each author, no conflict of interest statement that make them responsible for meeting any requirements set. What follows subsequently is the acceptance of a paper for further editing procedure. The manuscripts submitted to the VSP pass in-house and external peer review. All authors pay "Article Processing Charge" for coverage all editing and publishing expenses. Domestic authors pay 5,000 RSD, and those from aboard 150 euros. The editing and publishing fee is required for substantive editing, facts and references validations, copy editing, and publishing online and in print by editorial staff of the Journal. No additional fees, other than stated above, are required even if an author who already paid the fee would have more articles accepted for publishing in the year when fee was paid. All authors who pay this fee may, if want, receive printed version of the Journal in year when fee is payed. Please note that the payment of this charge does not guarantee acceptance of the manuscript for publication and does not influence the outcome of the review procedure. The requirement about paying "Article Processing Charge" does not apply to reviewers, members of the Editorial Board and the Publisher's Council of the Journal, young researchers and students, as well as any of the subscribers of the Journal.

The VSP publishes: **editorials, original articles**, **short communications**, **reviews**/**meta-analyses, case reports**, **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, and other. Original articles, short communications, meta-analyses and case reports are published with abstracts in both English and Serbian.

General review papers will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided as reserved for subtitles. Original articles, reviews, meta-analyses and articles from medical history should not exceed 16 pages; current topics 10; case reports 6; short communications 5; letters to the editor and comments 3, and reports on scientific meetings and book reviews 2.

All measurements should be reported in the metric system of the International System of Units (SI), and the standard internationally accepted terms (except for mmHg and °C).

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office** (**Excel**, **Word Graph**)**.** The use of colors and shading in graphs should be avoided.

Papers should be prepared in accordance with the **Vancouver Convention**.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

1. ***Title page***

a) The title should be concise but informative, while subheadings should be avoided;

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

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

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

e) Data on the corresponding author.

2. ***Abstract and key words***

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

3. ***Text***

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

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

Methods. The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethnics Committee for the tests in humans and animals.

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

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

References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al*. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

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

Tables

Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper left corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

Illustrations

Any forms of graphic enclosures are considered to bi figures and should be submitted as additional databases in the System of Assistent. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure (**Figure 1**, **Figure 2** and so on). If a figure has been published, state the original source.

Legends for illustrations are typed on a separate page, with Arabic numbers corresponding to the illustrations. If used to identify parts of the illustrations, the symbols, arrows, numbers, or letters should be identified and explained clearly in the legend. Explain the method of staining in photomicrographs.

Abbreviations and acronyms

Authors are encouraged to use abbreviations and acronyms in the manuscript in the following manner: abbreviations and acronyms must be defined the first time they are used in the text consistently throughout the whole manuscript, tables, and graphics; abbreviations should be used only for terms that appear more than three times in text; abbreviations should be sparingly used.

An alphabetical list of all abbreviations used in the paper, followed by their full definitions, should be provided on submission.

Detailed Instructions are available at the web site:

www.vsp.mod.gov.rs

UPUTSTVO AUTORIMA

VSP objavljuje radove koji nisu ranije nigde objavljivani, niti predati za objavljivanje redosledom koji određuje uređivački odbor. Svaki pokušaj plagijarizma ili autoplagijarizma kažnjava se. Prilikom prijave rada u sistem elektronskog uređivanja „Vojnosanitetskog pregleda“**(http://aseestant.ceon.rs/index.php)** neophodno je priložiti izjavu da su ispunjeni svi postavljeni tehnički zahtevi uključujući i izjavu koju potpisuju svi autori da rad nije ranije ni u celini, niti delimično objavljen niti prihvaćen za štampanje u drugom časopisu. Izjavu o pojedinačnom doprinosu svakog od autora rada potpisanu od svih autora, treba skenirati i poslati uz rad kao dopunsku datoteku. Takođe, autori su obavezni da dostave i potpisanu izjavu o nepostojanju sukoba interesa čime postaju odgovorni za ispunjavanje svih postavljenih uslova. Ovome sledi odluka o prihvatanju za dalji uređivački postupak. Rukopisi pristigli u Redakciju časopisa podležu internoj i eksternoj recenziji. Svi autori dužni su da plate “Article Processing Charge” za pokriće troškova jezičke, stručne i tehničke obrade rukopisa, kao i njegovog objavljivanja. Domaći autori plaćaju iznos od 5 000 dinara, a inostrani 150 eura. Dodatna plaćanja nisu predviđena čak i u slučaju da autor koji je već prethodno platio traženi iznos, ima više prihvaćenih radova za objavljivanje u godini u kojoj je izvršio uplatu. Svi autori koji su platili “Article Processing Charge” mogu, ukoliko žele, dobijati štampanu verziju časopisa tokom godine u kojoj je izvršena uplata. Plaćanje ovog iznosa ne garantuje prihvatanje rukopisa za objavljivanje i ne utiče na ishod recenzije. Od obaveze plaćanja pokrića navedenih troškova oslobođeni su recenzenti, članovi Uređivačkog odbora i Izdavačkog saveta VSP, studenti i mladi istraživači, kao i pretplatnici časopisa.

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme, metaanalize**, **kazuistika, seminar praktičnog lekara,** članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Pre objave, rad se upućuje autoru određenom za korespodenciju na konačnu saglasnost.

Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi **– Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

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

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

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

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

Literatura

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

Primeri referenci:

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

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

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

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

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

Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vsp.mod.gov.rs