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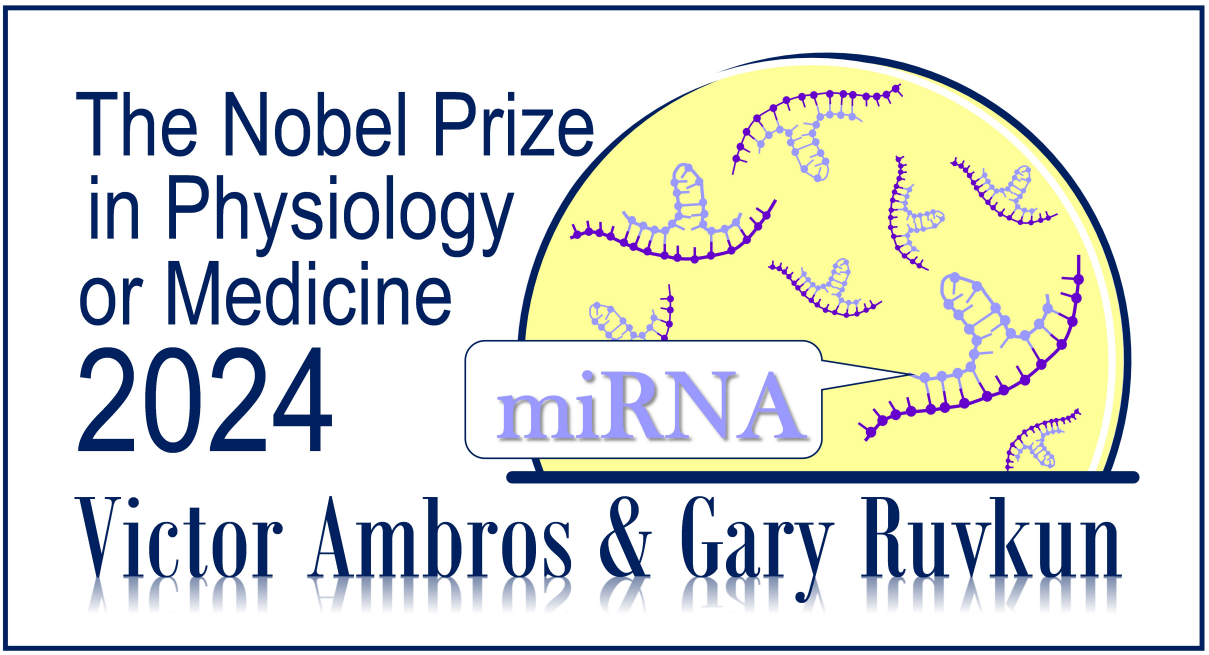

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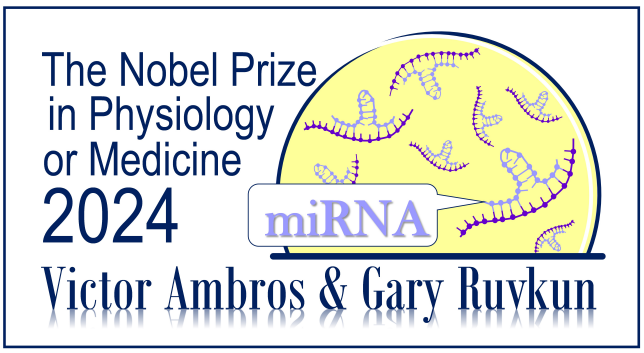

This year's winners of the Nobel Prize in Physiology or Medicine are Victor Ambros (born in 1953 in Hanover, USA) and Gary Ruvkun (born in 1952 in Berkeley, USA). Two American scientists were awarded for the discovery of microRNA, a new class of tiny RNA molecules, and its role in post-transcriptional gene regulation. This revolutionary discovery demonstrated a completely new principle of gene regulation, essential for multicellular organisms, including humans.

Ovogodišnji dobitnici Nobelove nagrade za fiziologiju ili medicinu su Viktor Ambros (rođen 1953. godine u Hanoveru, SAD) i Gari Ruvkun (rođen 1952. godine u Berkliju, SAD). Dvojica američkih naučnika nagrađeni su za otkriće mikroRNK, nove klase sićušnih RNK molekula i njenu ulogu u post-transkripcionoj regulaciji gena. Ovim revolucionarnim otkrićem pokazan je potpuno novi princip regulacije gena, od suštinskog značaja za višećelijske organizme, uključujući ljude.

ORIGINAL ARTICLES

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Acute intracranial hemorrhage in 76 COVID-19 patients during the first and second pandemic waves

Akutno intrakranijalno krvarenje kod 76 bolesnika obolelih od COVID-19 u prvom i drugom talasu pandemije

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Abstract

Background/Aim. There is limited data on the frequency of intracranial hemorrhage (ICrH) in the first wave [beta variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)] and second wave (delta variant of SARS-CoV-2) coronavirus disease 2019 (COVID-19) pandemic. The aim of this study was to analyze the appearance of ICrH in COVID-19 patients (CP). **Methods.** Among 505 CP treated at the Special Hospital for Cerebrovascular Diseases "Sveti Sava" intermittently during the 2020–2021 period, ICrH was diagnosed in 76 (15.1%) patients. The available information from the medical records regarding clinical, demographic, as well as radiological data (multislice computed tomography examination of the endocranium) was collected and analyzed. **Results.** In the first wave, out of 308 CP, 63 (20.5%) were diagnosed with ICrH. In the second wave, out of 197 CP, ICrH was diagnosed in 13 (6.6%) patients, which was a statistically significant difference ($p < 0.002$). There was no statistically significant difference for the presence of hypertension ($p = 0.271$), diabetes mellitus ($p = 0.558$), and chronic obstructive pulmonary disease ($p = 0.794$) among CP with ICrH comparing the two waves of the pandemic. However, a statistically significant difference was proven in the frequency of patients with atrial fibrillation and anticoagulant drug therapy ($p = 0.021$ each). There was no statistically significant difference in the frequency of patients with fever ($p = 0.637$), fatigue ($p = 0.587$), hemiparesis ($p = 0.831$), respiratory symptoms ($p = 0.289$), and loss of consciousness ($p = 0.247$). D-dimer values in the second pandemic wave were statistically significantly lower ($p = 0.003$). The combination of ischemic stroke and ICrH was six times more common in the second wave ($p = 0.003$). However, cerebral parenchymal hemorrhage was two times less frequent ($p = 0.001$) in the second wave but with statistically higher frequencies of multifocal (23.1%) and diffuse type (36.4%) of ICrH ($p = 0.007$). Combined hemorrhages, as well as subarachnoid and subdural subtypes, were more common in the second wave (23.1% each). Fatal outcomes occurred in 38.1% of patients in the first wave compared to 69.2% in the second wave ($p = 0.039$). **Conclusion.** In the first pandemic wave of COVID-19, ICrH in CP was significantly more frequent and D-dimer was singled out in laboratory analyses, the values of which were statistically significantly higher in comparison with second wave. In the second wave of COVID-19, parenchymal ICrH was less frequent and multifocal and diffuse ICrH were more common in CP with ICrH. The mortality rate was very high in the second wave.

Key words:
covid-19; fibrin fragment d; intracranial hemorrhage; mortality; risk factors; serbia; tomography, x-ray computed.

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Apstrakt

Uvod/Cilj. Nedovoljno je podataka o učestalosti intrakranijalnih krvarenja (IKK) u prvom talasu (beta varijanta virusa *severe acute respiratory syndrome coronavirus 2* – SARS-CoV-2) i drugom talasu (delta varijanta virusa SARS-CoV-2) pandemije izazvane koronavirusom 2019 (*coronavirus disease 2019* - COVID-19). Cilj rada bio je da se analizira pojava IKK kod obolelih od COVID-19 (CB). **Metode.** Od 505 CB koji su lečeni u Specijalnoj bolnici za cerebrovaskularne bolesti „Sveti Sava" tokom 2020–2021, IKK je dijagnostikovana kod 76 (15,1%) bolesnika. Prikupljeni su i analizirani dostupni podaci iz medicinske dokumentacije: klinički, demografski i radiološki (multislajsna kompjuterizovana tomografija endokranijuma). **Rezultati.** U prvom talasu je od 308 CB bilo njih 63 (20,5%) sa IKK. U drugom talasu je od 197 CB kod 13 (6,6%) bolesnika dijagnostikovana IKK, što je bila statistički značajna razlika ($p < 0,002$). Poređenjem dva talasa pandemije utvrđeno je da nije bilo statistički značajne razlike u postojanju hipertenzije ($p = 0,271$), dijabetesa melitusa ($p = 0,558$) i hronične opstruktivne bolesti pluća ($p = 0,794$) kod CB sa IKK. Međutim, pokazana je statistički značajna razlika u učestalosti bolesnika sa atrijalnom fibrilacijom i bolesnika koji su lečeni antikoagulantnim lekovima ($p = 0,021$ za obe učestalosti). Nije bilo statistički značajne razlike u učestalosti bolesnika sa temperaturom ($p = 0,637$), umorom ($p = 0,587$), neurološkim deficitom ($p = 0,831$), respiratornim simptomima ($p = 0,289$) i gubitkom svesti ($p = 0,247$). Vrednosti D-dimera u drugom talasu pandemije bile su statistički značajno niže ($p = 0,003$). Kombinacija ishemijskog moždanog udara i IKK bila je šest puta češća u drugom talasu ($p = 0,003$). Međutim, cerebralno parenhimsko krvarenje bilo je dva puta ređe ($p = 0,001$) u drugom talasu, ali sa statistički višom učestalošću multifokalnog (23,1%) i difuznog (36,4%) IKK ($p = 0,007$). Kombinovane hemoragije, kao i podtipovi subarahnoidna i subduralna hemoragijia, bili su češći u drugom talasu (23,1% svaki podtip). U prvom talasu, bilo je 38,1% fatalnih ishoda, u odnosu na 69,2% tokom drugog talasa pandemije ($p = 0,039$). **Zaključak.** U prvom pandemijskom talasu COVID-19, u poređenju sa drugim talasom, IKK su bile znatno češće kod CB, a u laboratorijskim analizama se izdvajao D-dimer, čije su vrednosti bile statistički značajno više. U drugom talasu COVID-19, kod CB sa IKK, parenhimalna IKK je bila manje učestala a multifokalne i difuzne IKK bile su češće. Stopa mortaliteta bila je veoma visoka u drugom talasu pandemije.

Ključne reči:
covid-19; d-dimer; krvarenje, intrakranijalno; mortalitet; faktori rizika; srbija; tomografija, kompjuterizovana, rendgenska.

Introduction

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), manifesting as coronavirus disease 2019 (COVID-19), often causes rapidly progressive interstitial pneumonia [1]. The virus exerts its pathogenic effect mainly by binding its spike glycoprotein to the angiotensin-converting enzyme II (ACE 2) surface receptors to enter target host cells [1–3]. Since these receptors are distributed throughout the body, COVID-19 is, in fact, a multiorgan disease.

The central nervous system can also be affected due to the virus neurotropism and neuroinvasive capability based, among others, on the presence of the ACE 2 receptors on cerebrovascular endothelial cells, arterial smooth muscle cells, neurons, and glial cells [1–7]. In this case, there is a leakage of the blood-brain barrier, i.e., an increase of vascular permeability and extravasation of the blood cells and plasma [7]. Those cerebral vessel abnormalities can lead to parenchymal hemorrhage (PH), subarachnoid hemorrhage (SAH), epidural, subdural, intraventricular hemorrhage, or a combination of these types. Consequently, intracranial hemorrhage (ICrH) can cause severe morbidity and mortality [1, 6–11].

The hemorrhagic stroke was mostly diagnosed in less than 1% of the COVID-19 patients [6, 12–14]. Although infrequent, ICrH is clinically very important due to a high morbidity and mortality rate [11]. In addition, there are less than 40 reports [7], with a corresponding statistical analysis, regarding ICrH in COVID-19 patients, and an even smaller number of articles about ICrH in various epidemic waves [3, 4, 9, 14–19].

Accordingly, the aim of this study was to determine the frequency and characteristics of ICrHs in COVID-19 patients in the first and second epidemic waves.

Methods

The study was designed as a single-center cross-sectional study. Namely, the research included patients from two time periods who were hospitalized at the Special Hospital for Cerebrovascular Diseases "Sveti Sava" due to neurological symptoms and COVID-19 infection. The research was conducted according to the valid permission received by the Ethics Committee of the Special Hospital for Cerebrovascular Diseases "Sveti Sava" (No. 03/3116-3, from June 30, 2021). Written consents were provided by all patients or their relatives.

Some other centers transferred their patients with cerebrovascular diseases to our institution. All these patients tested positive for SARS-CoV-2 following a nasopharyngeal swab examination by reverse transcription polymerase chain reaction, and most of them had positive pulmonary signs in radiograms or computerized tomography (CT) scans.

Patient enrollment and selection

The first wave (wave 1) of epidemics in our country occurred from November 2020 to February 2021 (dominant beta variant SARS-Cov-2), while the second wave (wave 2) happened from August until December 2021 (dominant delta variant SARS-Cov-2). In total, 505 patients were hospitalized in our COVID care center. Among them,

76 patients with ICrH were enrolled. The following spontaneous hemorrhages were noted: PH, SAH, subdural hematoma (SDH), and intraventricular hemorrhage (IVH), as well as their various combinations.

Patient examination

Demographic data (age, gender), clinical data (risk factors: hypertension, diabetes mellitus, chronic obstructive pulmonary diseases), disease symptoms (fever, headache, fatigue, hemiparesis, respiratory symptoms, atrial fibrillation, anticoagulant therapy, consciousness disorders), and laboratory analyses [leukocytes, lymphocytes, C-reactive protein (CRP), D-dimer] were analyzed. Clinical severity was quantified using the National Institutes of Health Stroke Scale (NIHSS) on admission and at discharge. The patient's functional status was assessed by applying a modified Rankin scale.

Computerized tomography imaging

First, each patient underwent a radiography or CT chest examination to confirm the clinical COVID-19 diagnosis. For stroke imaging, we have used a 160-slice CT machine, Toshiba Aquiline Prime, by applying field of view (FOV) 240 and a slice thickness of 2.0 mm. The type, number, and location of the hemorrhages in the multislice CT scans were determined in each patient of both waves.

Statistical analysis

Statistical analyses were performed using the statistical software SPSS v22.0. Statistical tests are 2-sided, and $p < 0.05$ was considered statistically significant. Continuous variables are shown using mean (standard deviation) and median (interquartile range) and compared by applying the nonparametric Mann-Whitney *U* test for all non-normally distributed data. Categorical variables were reported as frequency rates and percentages and compared by Chi-square (χ^2) tests.

Results

Among the total of 505 patients (308 were hospitalized in wave 1, and 197 in wave 2), 76 had ICrH (15.1%). In total, patients were aged 72.9 ± 7.8 (53–89) years, with a median of 73 years. The average interval between the onset of symptoms of COVID-19 infection and diagnosis of ICrH was a median of three days: patients developed ICrH 0–45 days after infection.

Patients in the first wave

Out of the 308 COVID-19 patients in the first wave, 63 (20.5%) experienced ICrH, and all of them were nonvaccinated since the national vaccination program was at its beginning in the general population**.** They were 73.1 ± 8.1 (53–89) years old, with a median of 73 years, without gender differences in frequency. Hypertension (93.7%), diabetes (30.2%), and chronic obstructive pulmonary disease (12.7%) were among the most frequent risk factors. Atrial fibrillation was present in 19.0% of patients, and all of them were taking anticoagulant treatment. Fever (81.0%), fatigue (88.9%), and hemiparesis (85.7%) were the most frequent symptoms and signs, in addition to respiratory disorders (58.7%) and consciousness alterations (57.1%). There were no statistical differences in the mentioned parameters between patients in different COVID-19 waves (Table 1).

Table 1

Clinical and demographic characteristics of the coronavirus disease 2019 patients with intracranial hemorrhage in the first (n = 308) and second (n = 197) waves

Patient characteristics	First wave (n = 308)	Second wave (n = 197)	*p*-value
Patients with ICrH	63 (20.4)	13 (6.6)	< **0.001**
Age, years	73.1 ± 8.1	71.7 ± 6.4	0.551
Male/female, %	50.8/49.2	69.2/30.8	0.225
Hypertension	59 (93.7)	11 (84.6)	0.271
Diabetes mellitus	19 (30.2)	5 (38.5)	0.558
COPD	8 (12.7)	2 (15.4)	0.794
Fever	51 (81.0)	9 (75.0)	0.637
Headache	35 (55.6)	4 (33.3)	0.158
Fatigue	56 (88.9)	10 (83.3)	0.587
Hemiparesis	54 (85.7)	10 (76.9)	0.831
Respiratory symptoms	37 (58.7)	9 (75.0)	0.289
Atrial fibrillation	12 (19.0)	6 (50.0)	0.021
Anticoagulant therapy	12 (19.0)	6 (50.0)	0.021
Consciousness disorder	36 (57.1)	9 (75.0)	0.247
Leukocytes × 10^9/L (RR 3.4–9.7)	11.7 ± 9.1	11.1 ± 5.1	0.854
Lymphocytes × 10^9/L (RR 1.2–3.4)	1.4 ± 0.9	1 ± 0.4	0.242
CRP, mg/L (RR 0.0–5.0)	41.1 ± 67.2 (13.4)	71.6 ± 69.2 (67.4)	0.155
D-dimer, ng/mL (RR 0–570)	1,646.9 ± 269.0	730.3 ± 125.8	**0.003**

Table 1 (continued)

Patient characteristics	First wave (n = 308)	Second wave (n = 197)	*p*-value
Isolated ICrH	95.9	69.2	**0.003**
ICrH + ischemic stroke	4.8	30.8	
NIHSS score on admission	14.8 ± 9.2	14.8 ± 8.4	0.778
NIHSS score at discharge	23.3 ± 15.8	31.7 ± 16.5	0.086
Mortality rate, %	38.1	69.2	0.039

ICrH – intracranial hemorrhage; COPD – chronic obstructive pulmonary disease; RR – reference range; CRP – C-reactive protein; NIHSS – National Institute of Health Stroke Scale.
Values are given as numbers (percentages), mean ± standard deviation, or mean ± standard deviation (median) unless otherwise indicated.

The CRP values were high (49.9 ± 12.2 mg/L), as well as D-dimer (1,646.9 ± 269.0 ng/L). Mainly spontaneous, pure ICrHs were registered, while the association of ICrH and ischemic stroke (IS) was very rare (4.8%, $p < 0.001$) (Table 1).

PH was more frequent (77.8%) among the ICrH findings. It usually was of a lobar, subcortical location (46.0%), with various radiologic signs: a swirl sign, a satellite bleeding, or a blend sign (Figure 1). The isolated lobar PH can occur in any of the cerebral regions, but the posterior lobar was the most frequent one (40.3%), while the corpus callosum hemorrhage was rare (1.8%) (Figure 2). Occasionally, multifocal (9.5%) or both lobar and multifocal PH appeared (25.5%, $p < 0.001$). A diffuse multifocal type involving several hemispheric lobes was less frequent (8.8%) (Figure 3).

Deep intrahemispheric PH type occurred in 21.1%, and it mainly involved the basal ganglia or the thalamus (Figure 4). The brain stem and cerebellum were also rarely affected (the latter in 5.3%). In any case, the supratentorial location was more often present (88.2%) than the infratentorial one (11.8%) (Figure 5).

Other ICrH subtypes, e.g., SDH and SAH, were less frequent, i.e., only 1.6% and 11.1%, respectively. A former hemorrhage was present either in the infratentorial compartment or the supratentorial space, while SAH always had a supratentorial position (Figure 6). The IVH was associated with other subtypes of the hemorrhage, e.g., with SAH, PH, and SDH (Figures 6 and 7).

Finally, the NIHSS score of the patients was 23.3 on average at discharge, and the mortality rate reached 38.1% (Table 1).

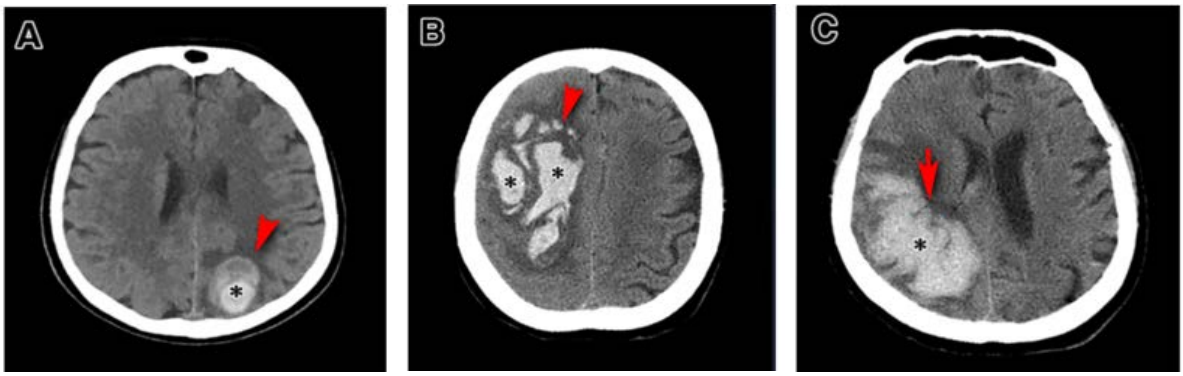

Fig. 1 – Various appearances of parenchymal hemorrhages (asterisk) in axial computed tomography scans: with a blend sign (A) (red arrowhead), a satellite bleeding (B) (red arrowhead), and swirl sign (C) (red arrow).

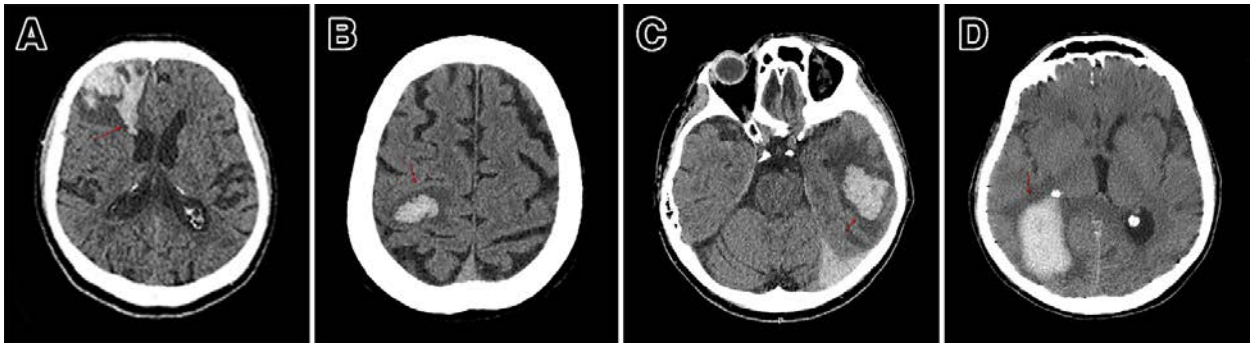

Fig. 2 – Lobar types of intracerebral hemorrhages (red arrows) with various locations in axial computed tomography scans: frontal (A), parietal (B), temporal (C), and temporooccipital (D).

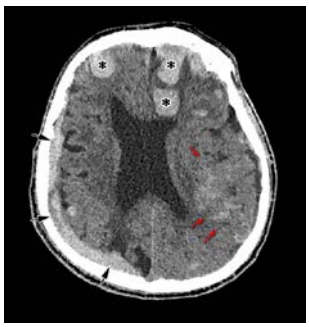

Fig. 3 – Multifocal hemorrhages, the largest being located bilaterally in the frontal lobes (asterisks). A right subdural hematoma (black arrows) was also present, and a left frontoparietal subarachnoid hemorrhage (red arrows) in an axial computed tomography scan.

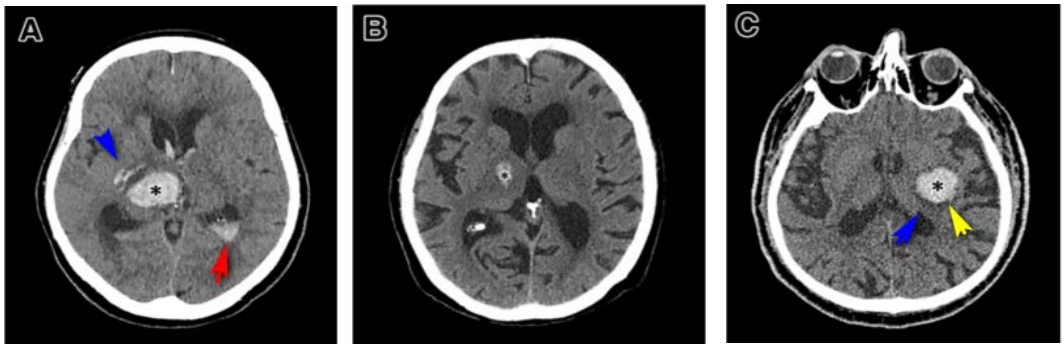

Fig. 4 – Deep hemispheric hemorrhages in axial computed tomography scans. Note entire thalamus (asterisk) affecting the posterior limb of the internal capsule (blue arrowhead) with intraventricular hemorrhage (red arrow) (A); hemorrhage involving the anterior part of the thalamus – asterisk (B); basal ganglia hemorrhage (asterisk) affecting the posterior limb of the internal capsule (blue arrowhead) and the posterior part of the left putamen (yellow arrowhead) (C).

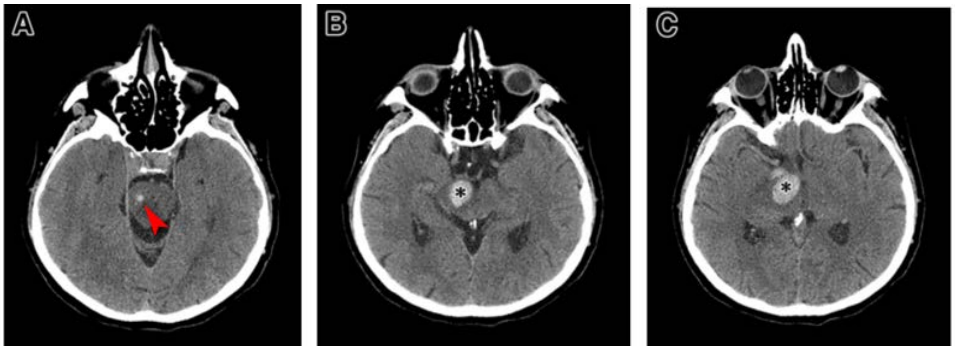

Fig. 5 – A brain stem hemorrhage in the pons (red arrowhead) (A) and midbrain (asterisk) (B), as an extension of a right thalamic hemorrhage (asterisk) (C) in axial computed tomography scans.

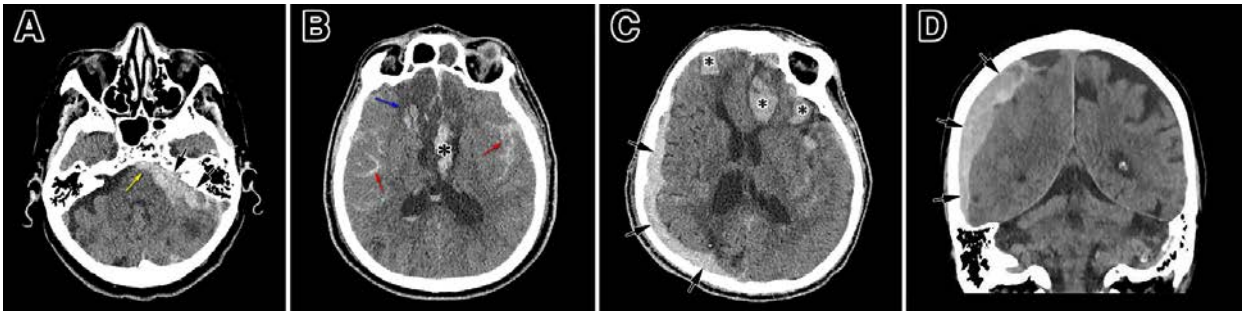

Fig. 6 – Infratentorial subarachnoid hemorrhage (SAH) in the basal cisterns (yellow arrow) and subdural hemorrhage on the left (black arrows) (A) in an axial computed tomography scan. Note a supratentorial SAH (red arrows) associated with a frontal parenchymal hemorrhage (PH) (blue arrow) and the intraventricular hemorrhage (IVH) (asterisk) (B), as well as a subdural hematoma (SDH) (black arrows) in the axial plane (C), and a right SDH (arrows) in the coronal plane (D), along with multifocal PH (D).

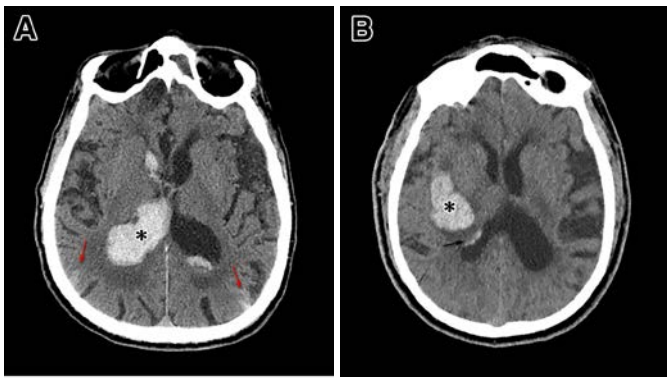

Fig. 7 – Bilateral intraventricular hemorrhage (asterisk) and subarachnoid hemorrhage (red arrows) (A), as well as a small intraventricular hemorrhage (black arrow) associated with a right parenchymal hemorrhage (asterisk) (B) in axial computed tomography scans.

Patients in the second wave

A smaller number of this group of patients with ICrH was revealed, i.e., 13 cases of the total 197 (6.6%), which is significantly less frequent than in wave 1 ($p < 0.001$).

They were aged 71.7 ± 6.4 (57–82) years, with a median of 70 years, i.e., without statistically significant difference to the patient age in the first wave ($p = 0.551$). Males predominated without a statistically significant difference to the patient gender in the first wave ($p = 0.225$). The risk factor frequencies were somewhat lower for hypertension (84.6%, $p = 0.271$) but higher for diabetes (38.5%, $p = 0.558$) in comparison with the first wave. Symptoms like fever (75.0%), headache (33.3%), fatigue (83.3%), and hemiparesis (76.9%) were less expressed, except the respiratory ($p = 0.289$) and the consciousness disorders ($p = 0.247$) which were more frequent (75.0% each) in the second wave. Atrial fibrillation and anticoagulant therapy were present in 50.0% patients each. However, the hemiparesis incidence was somewhat lower (76.9%) in wave 2 ($p = 0.831$), although without a statistical difference (Table 1).

D-dimer values were also smaller (mean 730 ng/L, $p = 0.003$), with a statistical difference, but CRP values were higher than in the first wave (mean 71.6 mg/L, $p = 0.155$) without a statistical difference. Lymphocytopenia was registered in these patients. The association of ICrH and IS was more frequent in wave 2, i.e., 30.8%, compared to only 4.8% in wave 1, and this difference was statistically significant ($p = 0.003$) (Table 1).

As for hemorrhage subtypes, PH was more than 2-fold less frequent in wave 2 ($p = 0.001$), as was a lobar PH. However, multifocal hemorrhages (23.1%) and their combination with isolated lobar ones (46.1%) were more frequent ($p = 0.007$), as was a diffuse type (36.4% compared to only 8.8% in wave 1). A posterior lobar PH was much more rare (18.2%) than in the first wave (40.3%), but cerebellar hemorrhages appeared more often (18.2% compared to 5.3%).

A *corpus callosum* hemorrhage was not present in wave 2. On the other hand, there was a much higher incidence of SAH and SDH (23.1% each) and of combined bleeding (23.1% compared to 9.5%, $p = 0.112$).

The NIHSS mean score was higher at discharge than in the first wave (mean 31.7, compared to 23.3) ($p = 0.086$). The mortality rate was significantly higher in wave 2 (69.2%, $p = 0.039$) (Table 1).

Discussion

In our hospital, ICrH occurred in 76 (15.1%) patients. This percentage was high because the hospital became a COVID center for patients with cerebrovascular disorders for Belgrade and the wider area. It was our specificity, which is difficult to compare with other centers.

The authors of some systematic reviews found acute stroke in 1.4% on average of COVID-19 patients, with a usual range of 0.5–8.1% [6, 7, 9, 16]. Acute stroke incidence is higher within certain ethnic and racial groups [10–13].

The ICrH incidence in other groups of COVID-19 patients usually ranged from 0.1% to 3.3% (mean 0.6–0.7%) [6, 7, 10–12]. If compared to the total number of stroke patients with COVID-19, the ICrH frequency can reach 30.0%. Different SARS-CoV-2 strains and variants causing infection most likely influenced the event incidence [3, 18, 19].

Combinations of ICrH and IS, or cerebral venous thrombosis, were reported in some patients [12]. PH was associated with IS in 1.0–39.2% [12], which is, in general, more frequent than in our patients from the first wave (4.8%), but our result from the second wave (30.8%) is within this range.

Of all the COVID-19 ICrH subtypes, PH occurred more often compared to SAH, SDH, epidural hematoma (EDH), and IVH, which were much less frequent [11, 17–23]. Several authors reported only PH in their studies [6, 9, 24–26]. A pure PH incidence among the ICrH patients ranged from 45.8% to 92.6% (mean 65.9%) [10, 11, 20, 21, 23, 27–31], which is in agreement with the incidence in wave 1 (77.8%), but not regarding wave 2 of our patients (30.1%).

Some PHs were associated with other hemorrhagic subtypes. We noted certain associations of PH with SAH and SDH or IVH, which is somewhat more frequent than in certain reports [12, 27].

There were drastic differences regarding a pure SAH incidence, observed in 5.8–71.0% (mean 22.0%) of the ICrH COVID-19 cases [11–13, 20, 21, 23, 29–32]. Our study showed almost the same average frequency in wave 2. SDH was mentioned in 10.9–48.6% (mean 24.4%) of ICrHs [7, 27], similar to our patients in wave 2.

EDH was reported only by several authors, who mentioned it as being about 1% of all ICrHs [7, 27]. This hemorrhage subtype was not registered in our study.

Intracranial hemorrhage in COVID-19 patients in the first wave

In the first wave, hemorrhage incidence commonly was less than 1.0% of all COVID-19 patients, i.e., between 0.1% and 0.9%, and rarely up to 2.0% [10, 26, 29, 32–35]. Hemorrhages were much more frequent in our patients (20.5%) for the mentioned reason.

Patients with ICrH usually had severe COVID-19, including pneumonia [6, 10, 36, 37]. According to certain reports, COVID-19 patients with stroke were younger on average (mean 52.6–65.0 years) than those only with stroke [6, 36, 38, 39], while some others reported the opposite results [12]. The mean age of our patients was 73.1 years in the first wave. Stroke was the main reason for hospital admission in 37.7% of COVID-19 patients, while 77.0% experienced ICrH after COVID-19 symptoms occurrence [6].

Typical vascular risk factors and comorbid diseases were present in the ICrH patients, especially hypertension (52.6–81.8%), diabetes mellitus (20.5–49.4%), dyslipidemia (16.0–50.6%), obesity (11.0%), smoking (6.5–20.8%), alcohol abuse (8.4%), previous cerebrovascular events (4.3–21.8%, including PH in 18.2% on average), heart disease (20.0–31.1%), chronic renal failure (10.3–19.1%) or acute renal failure (48.7%), liver disorder (15.8%), pulmonary disease (8.3–18.7%), malignancy (17.9%), and rarely amyloid angiopathy, epilepsy, dementia, hypothyroidism, rheumatoid arthritis, and acquired immunodeficiency syndrome [10, 12, 29, 36, 37, 39]. We noted hypertension as high as 93.7%, diabetes mellitus at 30.2%, and atrial fibrillation at 19.0% within our group during wave 1.

In addition, there are also certain other risk and predisposing factors, such as serious COVID-19 illness [16], with an NIHSS score of 11–13 on average, compared to a mean value of 14.8 in our patients. The Glasgow Coma Scale mostly ranged from 8–15, and the Rankin score was 3–6 [28, 39]. According to the literature, other important factors are also endotracheal intubation, prolonged mechanical ventilation (in 70.0–77.0%), extracorporeal membrane oxygenation, immobility, prolonged general hypoxemia, coagulopathy, renin-angiotensin axis disorder, metabolic and enzyme disorder, septic shock, post-viral bacterial superinfection, and multiple organ damage [39–41].

Furthermore, there is occasionally an important role of immune overexpression and hyperinflammation (high CRP levels, cytokine storm, proteases activation), enhanced catecholamine secretion due to stress, changes of blood pressure, loss of brain autoregulation, and anticoagulation treatment (up to 71.0%) [4, 6, 10–12, 27, 30, 37, 42]. The level of D-dimer was commonly higher in the PH patients (mean 3,387 ng/L) [16], but almost 2-fold lower in our study (1,647 ng/L).

ICrH was usually diagnosed in 2–25 days following admission [29]. PH alone may occur on admission or commonly during the first 9 to 14 days on average following the onset of COVID-19 [37]. In general, it may develop 1–32 days after infection [11, 28, 36], and in our study, patients developed ICrH 0–45 days after infection.

The most common symptoms in ICrH patients were impairment or loss of consciousness (32.1%), aphasia (42.3%), motor deficit (9.0–43.5%), severe headache (15.4%), syncope (10.3%), seizures (9.0%), and a changed mental status (16.7%), including signs of encephalitis and meningitis in some cases [4, 31, 39]. In our patients in the first wave, consciousness disorder was more frequent (57.1%) than in other reports, as were hemiparesis (85.7%) and headache (55.6%).

The mortality rate was usually 33.0–59.0% [10, 11, 21, 36]. Our results are within this range (38.1%). According to some reports, the male gender (up to 73.0%), old age (over 65 years on average), smoking, mechanical ventilation, ischemic heart disease, and lower leukocyte and platelet count were predictors of a death outcome [16, 23]. Gender frequencies were almost equal in our patients in the first wave.

PH was the most frequent event compared to other subtypes of ICrH since it occurred in almost 77.8% of our ICrH patients. There were most often single PHs (44.1–67.0%, and 46.0% in our patients) and less frequently multifocal ones (32.0–36.0%, but somewhat lower in our cases) [6, 10, 29].

The isolated PHs were most frequent (44.1–93.5%), particularly in the cerebral white matter (lobar position). A location in the deep hemispheric region occurred rarely (4.9–6.6%), sometimes bilaterally [43], but it was seen in as much as 21.1% of our patients. An atypical callosal hemorrhage was rare [15]. PH was also infrequent in the infratentorial compartment (9.1%), i.e., in the brain stem or the cerebellum (1.1%) [10, 11, 29, 36, 44]. We noted a cerebellar hemorrhage in 5.3% of patients.

The presence of both lobar and deep hematomas was rare in the mentioned reports (4.0–6.0%) [22, 29, 39, 44]. Some of them can enter the ventricular system [22, 36]. Hematomas had an average volume of 45.9 cm^3 [36].

In our study, SAH occurred in 11.1% of patients. In the literature, the presence of SAH is very variable.

Some authors presented only SAH, which is in general less frequent than PH (0.1–0.7%, mean 0.2%) in COVID-19 patients [13]. It was in the range of 5.8–71.0% (mean 22.0%) among the ICrH patients with COVID-19. Various numbers of the SAH individuals were presented in different reports [13, 14, 17, 45–47].

These patients were aged 35 to 54 years (mean 47.0) [48]. The risk and predisposing factors, as well as comorbidity and complications, were similar to patients with PH, but hypertension predominated (68.6%). A previous SAH was registered in 14.0% of the patients [13]. There was a rare combination of SAH with IVH or IS [12], but more often, including PH [11–13, 20, 23, 28–31].

SAH appeared nine days on average following COVID-19 onset. The Rankin scale usually showed 4 points. The mortality rate was 31.4%, which is much higher than in non-COVID-19 patients with SAH (12.2%) [13].

SDH was noticed in 1.6% of patients, with low frequencies compared to other studies [20, 27].

In the literature, the incidence of the non-traumatic SDH was 0.2% on average (range 0.1–0.4%) in COVID-19 patients and 10.9–48.6% (mean 24.4%) within the ICrH group [20, 27, 29]. A combination of SDH, SAH, and PH was rarely reported.

EDH was not observed in our patients. EDH is formed between the *dura mater* and the inner table of the skull. It is extremely rare in COVID-19 patients, i.e., less than 0.1% on average in this group and less than 2.0% within the ICrH groups [27].

An isolated IVH was not seen in our patients, only in combinations with other subtypes. IVH is commonly secondary, as a consequence of the blood entering the ventricular system from PH or rarely from SAH [23, 29]. It accounts for only 0.1–1.4% of COVID-19 patients and less than 2.8% within the ICrH groups [10, 29]. Blood in the ventricles can cause obstruction of the ventricular system and the consecutive hydrocephalus [36].

Intracranial hemorrhage in COVID-19 patients in the second wave

There is limited literature regarding acute stroke in wave 2 compared to the first one [32–35, 45, 46]. In general, stroke admission was 18.4% lower in the second wave, and the acute stroke decline was usually 1.8–2.5%, and rarely up to 8.0% in some reports [32, 35, 45, 46]. As for ICrH in certain countries, a 3.9% decline was observed, but a slight increase was observed in some others [33]. Hemorrhages usually varied from 0.1% to 3.3% [33]. The frequency of ICrH in our patients is much higher (6.6%) for the mentioned reason.

As for ICrH subtypes in wave 2, as much as a 47.0% decline was noted in our study regarding the PH incidence, including its lobar type (23.1% compared to 46.0%). However, there was an increase in multifocal (23.1% vs. 9.5%), combined multifocal and lobar (46.1% vs. 25.5%), diffuse (36.4% vs. 8.8%), and cerebellar PH (18.2% vs. 5.3%), as well as an absence of callosal hemorrhages.

Some authors reported only patients with SAH in their articles [13, 14, 47]. Some others noted a 4.6–8.0% decline of SAH in the second wave [46]. However, SAH in our patients was observed 2-fold more frequently in wave 2 than in wave 1.

SDH was slightly more often present in the second wave (0.9%) [45] but less frequent in some other reports [23, 46]. In our patients, SDH incidence was 23.1% in wave 2, compared to only 1.6% in wave 1. EDH and isolated IVH was not present in our patients. Combinations of various ICrH subtypes were more frequent in the second wave (23.1%) than in the first one (9.5%).

The mean age of patients in the second wave was two years lower than in the first wave, or five years higher in some reports [8], but over 70 years on average in our patients. The type and frequency of the risk factors were reported to be similar in both waves [32] but not in our patients. As regards the symptoms and laboratory findings in wave 2, hypertension, fever, headache, hemiparesis, and the D-dimer value were lower. On the other hand, diabetes, respiratory symptoms, anticoagulant therapy, consciousness disorder, and CRP values were more frequent or higher. Our results are similar to those in another report [18].

According to some authors, less stroke severity was observed in the second wave, i.e., 1–13 NIHSS score compared to 2–16 in the first wave [32]. However, the mean score was 14.8 on admission but 31.7 at discharge in our study. Mortality was also higher in the second wave, e.g., 15.9%, compared to 9.9% in the first wave [49]. According to some authors, there was a 31.0% increased risk of mortality in the second wave [33]. The mortality rate in our study was much higher (69.2%) in wave 2 than in wave 1 (38.1%) and even higher in another report [7].

First of all, the different values of some parameters in wave 2 can be explained by vaccination and better medical treatment [2, 46, 50], but probably by some ethnic characteristics as well [12, 13]. Some others are mainly based on the biological features of new viral lineages and variants, especially regarding the evasion of the previous specific antiviral immunity and increased viral pathogenicity [7, 18, 33].

Conclusion

Intracranial hemorrhage in COVID-19 patients was more frequent in wave 1 with statistical significance. COVID-19 patients with intracranial hemorrhage in wave 2 showed a lower parenchymal hemorrhage incidence but with a higher multifocal and diffuse type, more frequent subarachnoid hemorrhage, subdural hematoma, and cerebellar parenchymal hemorrhage, but an absence of a corpus callosum hemorrhage. Hypertension, fever, and headache were less frequent, while diabetes, respiratory and consciousness disorders, anticoagulant therapy, and D-dimer values were less lower in wave 2. The association of intracranial hemorrhage with ischemic stroke was more often present, as well as combined hemorrhages in wave 2. NIHSS score and the mortality rate were very high in this wave.

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Clinical and laboratory status in Parkinson's disease patients with and without polyneuropathy

Klinička i laboratorijska slika obolelih od Parkinsonove bolesti sa i bez polineuropatije

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Abstract

Background/Aim. The etiology of polyneuropathy (PNP) in patients with Parkinson's disease (PD) is unclear, and there is a possible association between levodopa therapy, hyperhomocysteinemia, and PNP development due to methylation processes involving vitamin B12 and folic acid. The aim of this study was to analyze the difference in clinical presentation and disease severity between PD patients with and without PNP and to evaluate blood levels of vitamin B12, homocysteine, and folic acid in these patients. **Methods.** This cross-sectional study included 200 consecutive patients diagnosed with PD, divided into two groups: those with PNP and those without PNP. Diagnosis of PNP was confirmed by electromyoneurography. The first group consisted of 50 patients with PD with confirmed PNP, and the second 50 patients with PD without PNP. All patients were receiving levodopa therapy. Laboratory tests analyzed vitamin B12, folic acid, and homocysteine levels. **Results.** Patients with PNP were older when PNP was diagnosed (71 vs. 66 years, $p < 0.0001$), without differences in duration of levodopa therapy ($p = 0.359$) or daily dose ($p = 0.442$), and with significant motor impairment according to Unified Parkinson's Disease Rating Scale III ($p = 0.017$). No difference was found between groups for vitamin B12 ($p = 1.0$), folic acid ($p = 0.124$), and homocysteine ($p = 0.313$) serum levels. **Conclusion.** PD patients with PNP have a more pronounced motor deficit, while differences in vitamin B12, homocysteine, and folic acid values compared to the group without PNP were not registered.

Key words:
age factors; levodopa; parkinson's disease; polyneuropathies; severity of illness index; vitamin b 12.

Apstrakt

Uvod/Cilj. Etiologija polineuropatije (PNP) kod Parkinsonove bolesti (PB) je nejasna, a moguća je povezanost između terapije levodopom, hiperhomocisteinemije i razvoja PNP zbog procesa metilacije, koji uključuje vitamin B12 i folnu kiselinu. Cilj rada bio je da se utvrdi razlika u kliničkoj prezentaciji i težini bolesti između obolelih od PB sa i bez PNP, kao i da se proceni nivo vitamina B12, homocisteina i folne kiseline u krvi ovih bolesnika. **Metode.** Studijom preseka analizirano je 200 konsekutivnih bolesnika sa dijagnozom PB, podeljenih u dve grupe: bolesnici sa PNP i oni bez PNP. Dijagnoza PNP potvrđena je elektromioneurografijom. Prvu grupu činilo je 50 bolesnika obolelih od PB sa potvrđenom PNP, a drugu 50 bolesnika obolelih od PB bez PNP. Svi bolesnici bili su na terapiji levodopom. Laboratorijskim testovima analizirani su nivoi vitamina B12, folne kiseline i homocisteina. **Rezultati.** Bolesnici sa PNP bili su stariji u momentu postavljanja dijagnoze PNP (71 vs. 66 godina, $p < 0{,}0001$), bez statistički značajnih razlika u dužini uzimanja ($p = 0{,}359$) ili dnevnim dozama levodope ($p = 0{,}442$) i sa statistički značajno izraženijim motornim deficitom prema *Unified Parkinson's Disease Rating Scale III* ($p = 0{,}017$). Nisu nađene razlike između grupa za nivoe vitamina B12 ($p = 1{,}0$), folne kiseline ($p = 0{,}124$) i homocisteina ($p = 0{,}313$) u serumu. **Zaključak.** Bolesnici oboleli od PB sa PNP imaju izraženiji motorni deficit, dok razlike u vrednostima vitamina B12, homocisteina i folne kiseline u odnosu na grupu bez PNP nisu registrovane.

Ključne reči:
životno doba, faktor; levodopa; parkinsonova bolest; polineuropatije; bolest, indeks težine; vitamin b12.

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Introduction

Parkinson's disease (PD) is a significant cause of disability in the elderly population with a trend of increasing incidence and mortality [1]. Considering the aging of the population as a global phenomenon, it is predicted that by the year 2040, the number of people suffering from PD will exceed 17 million [2]. According to studies covering the last three decades, the incidence of PD is related to age and increases proportionally in both sexes over the age of 65. A higher incidence was recorded in males, and a significant growth trend in both genders was observed in patients over 80 years of age [3, 4].

PD primarily affects parts of the central nervous system (CNS), leading to motor symptoms such as tremor, rigidity, bradykinesia, and postural instability [5]. In addition to motor symptoms, PD is also characterized by non-motor symptoms that include cognitive impairment, mood disorders, sleep disturbances, autonomic dysfunction, and sensory deficits [6]. While PD primarily involves the CNS, some reports and studies suggest that patients with PD may also present with peripheral neuropathies [7–9]. Polyneuropathy (PNP) refers to damage or dysfunction of peripheral nerves that can affect sensation, movement, and organ function. Common symptoms of PNP include numbness, tingling, weakness, pain in the hands and feet, and postural instability [10]. However, the exact relationship between PD and PNP is not fully understood and may involve multiple factors like medication side effects and age-related factors [8, 11].

PNP is an underestimated and often underdiagnosed comorbidity in patients with PD. The prevalence of PNP in patients with PD varies significantly and depends largely on the diagnostic protocols. In one of the earlier studies, Toth et al. [7] reported an incidence of PNP up to 55%. In a study published in 2008 [8], PNP was found in 69% of PD patients, while a significantly lower prevalence of 9.53% was recorded in an Indian cohort [12]. In these studies, the investigated PNP was found to be idiopathic.

The debate about the etiopathogenesis of PNP in PD markedly took place during the last decade. Several authors emphasized evidence of the possible neurotoxicity of levodopa, which leads to the occurrence of PNP in patients suffering from PD [8].

Patients on long-term levodopa therapy may exhibit increased plasma homocysteine and reduced serum vitamin B12 levels. This elevation is primarily due to the metabolism of levodopa by the enzyme catechol-O-methyltransferase (COMT) [13]. COMT activity necessitates the presence of certain vitamins, particularly B12, B6, and folic acid, which act as essential cofactors. During the breakdown of levodopa, methyl groups, which are integral to numerous biological processes, are depleted, leading to an accumulation of homocysteine. Under normal physiological conditions, homocysteine is recycled into methionine, a crucial amino acid, through a process requiring vitamin B12 and folate. Vitamin B6 plays a pivotal role in converting homocysteine into other non-toxic metabolites. A deficiency in these vitamins can thus impair the conversion process, resulting in elevated homocysteine levels [14]. This mechanism is also confirmed by the analysis of PD patients who used COMT inhibitors [15]. These drugs stop the methylation of levodopa, and thus, the toxic effects of hyperhomocysteinemia on peripheral nerve fibers and the development of PNP are absent. Certain authors also pointed to the correlation between the duration of exposure to levodopa, the daily dose of levodopa, and the risk of developing PNP [16].

Additionally, alpha-synuclein, as the main pathoanatomical substrate in PD, was detected in the peripheral nerve fibers of the skin, submandibular salivary glands, and the enteric nervous system [17, 18]. A study conducted by Finnish authors confirmed the presence of alpha-synuclein in the peripheral nerve fibers of the skin and sensory PNP in 50% of subjects. The authors were not able to confirm the association between alpha-synuclein and sensory PNP with a cumulative dose of levodopa and vitamin B12 hypovitaminosis, which would suggest that the development of PNP in PD is caused by neurodegeneration *per se* and not toxic effects of levodopa [18].

Neurological status in PD patients with and without PNP demonstrates significant heterogeneity. Various studies have suggested that the presence of PNP in patients with PD is associated with worse motor symptoms [11, 19]. However, other studies have not found a statistically significant difference in motor symptom severity between PD patients with and without PNP [9, 20, 21].

The aim of this study was to determine differences in the neurological status of PD patients with and without PNP, as well as to analyze the potential association of vitamin B12, folic acid, and homocysteine levels in PD patients with and without PNP.

Methods

This cross-sectional study was conducted in a five-year period, from January 1, 2018, until December 31, 2023, including two hundred patients with PD (aged 60–80 years) who were consecutively included during hospitalization and outpatient visits at the Neurology Clinic and Specialist Polyclinic, University Clinical Center of Vojvodina, Serbia. The research was approved by the Ethics Committee of the University Clinical Center of Vojvodina (No. 00-246/2022).

The study patients were diagnosed with PD according to the United Kingdom PD Society Brain Bank diagnostic criteria [22].

Clinical research included a collection of sociodemographic indicators, an assessment of the clinical characteristics of PD, a scale for assessing the neurological and functional status of patients with PD [Movement Disorders Society (MDS) Unified PD Rating Scale (UPDRS) – MDS-UPDRS] [23, 24, 25] symptoms and signs of PNP and electromyoneurography (EMNG). A five-channel device (Natus Neurology Inc., USA, 2014) was used for EMNG analysis. The electroneurography of the sensory nerves included the examination of the *nervus suralis*, *nervus peroneus superficialis*, *nervus ulnaris*, and *nervus radialis*. The Hoehn and Yahr scale, part of MDS-UPDRS, was used to evaluate the disease stage [25].

Exclusion criteria included a history of significant psychiatric diseases and dementia.

In the presence of symptoms and signs of PNP, EMNG was performed. The diagnosis of PNP was subsequently confirmed by EMNG. All patients were receiving levodopa therapy. The presence of other therapies for PD was not an exclusion criterion.

The included patients underwent laboratory tests to rule out other known causes of PNP. Blood samples were analyzed at the Center for Laboratory Testing, University Clinical Center of Vojvodina. Laboratory analyses included: complete blood cell count, renal functional test, liver function tests, fasting blood glucose and hemoglobin A1c, serum protein electrophoresis, test for inflammation (c-reactive protein) and autoimmunity (antinuclear antibody, rheumatoid factor, anti-neutrophil cytoplasmic antibody), thyroid function test (thyroxine, triiodothyronine, thyroid-stimulating hormone), and test for infectious disease screening (human immunodeficiency virus – HIV, hepatitis B, hepatitis C, Lyme disease, syphilis serology). Patients with previously known hereditary, metabolic (including diabetes mellitus), toxic, inflammatory, and/or autoimmune PNPs were also not included, nor were patients with confirmed severe radiculopathies and plexopathies.

Based on clinical findings, medical history, and also EMNG findings, all patients were categorized into two groups: those with PD and PNP (50 patients) and those with PD without PNP (50 patients).

In all PD patients with and without PNP, laboratory analyses also included the levels of vitamin B12, folic acid, and homocysteine. The normal serum values of homocysteine, vitamin B12, and folic acid were 5.1–15.4 μmol/L, 138.0–652.0 pmol/L, and 7.0 to 45.3 nmol/L, respectively.

Finally, this study included 100 patients diagnosed with PD according to the United Kingdom PD Society Brain Bank diagnostic criteria [22], who were able to understand the text of the informed consent and sign it voluntarily.

The data collected were entered into the database using the Excel for Windows. The descriptive statistical method, including arithmetic mean, standard deviation, and parameters indicating the shape of the distribution, was applied to describe the relevant values of continuous measures. The frequency method was applied to describe the categorical variants, which are relevant for describing the sample of patients and answering the research hypothesis. Pearson's correlation coefficient was used to examine the connection between continuous variants.

Pearson's Chi-squared test was applied when the difference in the frequency of responses to two categorical variables was examined, while the Mann-Whitney *U* and *t*-tests were used for continuous measures for non-dependent samples. The Mann-Whitney *U* test was used when the dependent variable was continuous and deviated from the normal distribution or when the measurement level of the dependent variable was ordinal. The *t*-test for dependent samples was applied when the dependent variable belonged to an interval measurement with normal distribution.

Results

The clinical and demographic characteristics of the examined groups are presented in Table 1. Most of the patients in both groups were male, but the differences between the groups of patients with PNP and without PNP did not reach statistical significance. There was no difference between the group's stages of the disease and disease severity, daily doses of levodopa, as well as the duration of levodopa administration. The group of subjects with associated PNP included older subjects (during the clinical examination of the patient when PNP was diagnosed), more

Table 1

Descriptive statistical indicators for Parkinson's disease patients in relation to the presence or absence of polyneuropathy (PNP)

Parameter	With PNP	Without PNP	*p*-value
Gender, n (%)			
male	41 (57.7)	20 (69.0)	0.297
female	30 (42.3)	9 (31.0)	
Age, mean (SD)	71.77 (5.05)	66.41 (6.13)	0.000*
[1]HY, n (%)			
I	4 (5.6)	2 (6.9)	0.082
II	30 (42.3)	20 (69.0)	
III	33 (46.5)	6 (20.7)	
IV	4 (5.6)	1 (3.4)	
Levodopa (month), mean (SD)	38.58 (28.58)	44.76 (34.76)	0.359
Levodopa daily dose, mean (SD)	360.56 (160.56)	393.10 (251.68)	0.442
Levodopa monotherapy, n (%)	53 (74.6)	13 (44.8)	0.004*
Levodopa + dopa agonist, n (%)	18 (25.4)	16 (55.2)	0.046*
MDS-UPDRS, mean (SD)			
I	9.90 (5.4)	9.55 (3.61)	0.749
II	13.30 (6.46)	13.14 (4.63)	0.905
III	35.39 (12.68)	29.45 (10.94)	0.017*
main score	58.68 (21.6)	52.52 (12.93)	0.155

HY – Hoehn and Yahr rating scale; MDS-UPDRS – Movement Disorder Society Unified Parkinson's Disease Rating Scale; SD – standard deviation; n – number. ****p* **– value reaches statistical significance.

[1]Note: For details on the scale used, see reference 22.

Table 2

Vitamin B12, homocysteine, and folic acid levels and the presence of polyneuropathy (PNP) in patients with Parkinson's disease

Parameter	PNP		*p*-value (χ^2 test)
	no	yes	
Folic acid			
normal	48	44	
high	0	4	0.124
low	2	2	
Vitamin B_{12}			
normal	49	49	
high	0	0	1.000
low	1	1	
Homocysteine			
normal	32	27	
high	17	23	0.313
low	1	0	

Results are given as number of patients.

frequent on levodopa monotherapy (74.6%), and they had more pronounced motor symptoms of PD (motor part, MDS-UPDRS III score).

Considering the relationship between the levels of folic acid, vitamin B12, and homocysteine, no significant differences were registered between the studied groups (Table 2).

Discussion

In our study, patients with PD who also had PNP did not differ significantly from those without PNP in terms of gender, stage of disease, and daily doses of levodopa, as well as the duration of levodopa administration. However, they exhibited significant differences in the motor component of the MDS-UPDRS. Patients with PD and PNP were frequently receiving levodopa therapy. Among the cohort of patients with both PD and PNP, male gender was more prevalent.

The authors of several recent studies similar to our sample report a male prevalence of up to 62% [26 - 28]. There are several hypotheses to explain this gender difference in patients with PNP. A higher incidence of PD in men has been recognized in all age groups [4, 5]. The influence of social and behavioral factors and different exposure to potential harmful noxes, i.e., higher exposure of the male sex to toxins, trauma, etc., are also mentioned [29]. It is presumed that the higher incidence of PNP in men is both because of biological/hormonal differences and different environmental influences [30]. Thus, in a recent study, Cardinez et al. [31] confirmed a higher frequency of PNP in men, while women more often reported symptoms of neuropathic pain.

By analyzing the subjects who have PD with associated PNP in our research, it can be concluded that these were older patients at the moment of examination. PD patients used levodopa monotherapy more frequently, and there is statistical significance between the groups. PD patients had higher motor test scores, i.e., worse neurological motor deficits. A more pronounced neurological deficit in patients with PD and PNP (when the study was conducted), a higher average age of these subjects as well as a later stage of the disease were also reported in other studies [19, 32]. The structure of the studied groups also indicates that the incidence of PNP increases with age [33].

The etiopathogenesis of PNP in PD is still unknown. Numerous studies have provided evidence of the toxic effects of levodopa [7, 8]. In recent decades, the role of alpha-synuclein and neurodegeneration in the etiopathogenesis of PNP in PD has been increasingly reported [18].

In this study, no significant statistical association was confirmed between the levels of vitamin B12, homocysteine, and folic acid and the presence of PD. However, in the available literature, there was no study with significant homogeneity of the obtained results.

The first significant study on the relationship between the cumulative daily dose of levodopa, the duration of exposure to levodopa, and the toxic effect of homocysteine was published in 2008 by Toth et al. [8]. The results of the study showed an increase in the value of homocysteine, which correlated with the cumulative dose of levodopa in subjects with PD associated with PNP. It was also concluded that there was no significant association between the values of vitamin B12 and PNP. Compared to our research, subjects included in the study by Toth et al. [8] were older, with a longer duration of PD and a higher cumulative dose of levodopa. Contrary to this study, a group of researchers from India found a low but still positive statistical association between the duration of the disease and hyperhomocysteinemia and a low but negative association between the reduction of B12 levels and the duration of the disease [12]. There was no statistically significant association with cumulative levodopa doses. In their sample, only 7.23% of subjects suffering from PD were diagnosed with PNP, and a non-significant association between PNP and hypovitaminosis B12, low folate level, and hyperhomocysteinemia was verified.

Ceravolo et al. [11] proved a statistically significant association between the duration of exposure to levodopa and the cumulative dose of levodopa and the occurrence of PNP, while a similar association could not be confirmed for the duration of PD. They also showed a positive statistical association with low levels of B12 and elevated homocysteine in

PD with PNP. Andréasson et al. [34] did not find an association between the cumulative daily dose of levodopa and PNP in PD. However, a strong positive association was identified between the PNP assessment scale score and hyperhomocysteinemia.

The authors of the meta-analysis published in 2023 point out that patients with PD may have elevated homocysteine values and decreased vitamin B12 values compared to healthy subjects and that the mechanism of PNP in PD is probably multifactorial [35]. In contrast to our results, the authors indicate that cumulative doses of levodopa and the duration of exposure to levodopa may contribute to the development of PNP, that hypovitaminosis B12 may be in a negative statistical relationship with the duration of PD and the stage of the disease, and that hyperhomocysteinemia can be correlated with the frequency of PNP in PD. From all that has been said, it can be concluded that PNP in PD is the result of complex mechanisms, both external and internal, which require additional research.

In our study, significantly higher values of the total MDS-UPDRS III score were recorded in patients with PNP. In contrast, in research published by Corrà et al. [9], differences between PD patients with and without PNP were not registered. The research of Schindlbeck et al. [36] was conducted on 39 newly diagnosed patients with PD who had not previously received levodopa therapy (levodopa naive patients) and also did not have a significantly higher MDS-UPDRS III score.

A group of American authors got similar results by analyzing gait in patients with PD with and without PNP, grouped according to disease duration and Hoehn and Yahr stage. Significant differences in the total MDS-UPDRS III score between groups were not recorded, although significantly slower gait, shorter step length, and greater variability of the step length were registered in patients with PNP [21].

In a recent study, Kühn et al. [19] analyzed motor and non-motor symptoms of PD in patients with PNP using both EMNG and high-resolution ultrasound in addition to a neuropathy questionnaire. Compared to our study, these patients were younger and in a later stage of the disease, and the MDS-UPDRS III score was lower for patients with PNP. Despite these differences, a positive correlation was found between the severity of symptoms of PNP and the values of the total MDS-UPDRS III score.

Our study has some limitations. First, a larger sample would be necessary for a complex or causal interpretation of the obtained results. The research was conducted as a cross-sectional study, which included a selected sample of PD patients with PNP. However, the analyzed subjects do not represent an objective sample from the tertiary health center. Second, other methods, such as skin biopsy, are necessary to achieve reasonably high sensitivity in diagnosing PNP, especially small-fiber PNP in PD patients. Yet, due to its invasive approach and high requirements, EMNG used in this study is still considered the most widely used method.

Conclusion

Our study shows that polyneuropathy in Parkinson's disease is more often diagnosed in older patients and is associated with the worse motor status established by the MDS-UPDRS III score. Low levels of serum vitamin B12, folic acid, and hyperhomocysteinemia were not associated with the presence of polyneuropathy in patients with Parkinson's disease.

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The effect of vitamin D serum levels on the values of C-reactive protein and fecal calprotectin in patients with ulcerative colitis in clinical remission

Uticaj nivoa vitamina D u serumu na vrednosti C-reaktivnog proteina i fekalnog kalprotektina kod obolelih od ulceroznog kolitisa u kliničkoj remisiji

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Abstract

Background/Aim. Vitamin D plays a critical role in digestive calcium absorption and, thanks to its immunomodulatory properties, affects intestinal barrier integrity, gut microbiota, and immune system functionality. The aim of the study was to examine 25-hydroxyvitamin D [25(OH)D] levels in patients with ulcerative colitis (UC) in clinical remission, as well as its effects on the values of fecal calprotectin (FC) and C-reactive protein (CRP). **Methods.** The research, conducted as a cross-sectional study, included 62 patients with UC in clinical remission. Serum levels of 25(OH)D and CRP were determined from venous blood specimens, while FC levels were assessed from stool samples. Endoscopic activity was evaluated through colonoscopy and was expressed by the Mayo Endoscopic Score (MES). **Results.** Out of the 62 participants with UC in clinical remission, 38 (61.3%) were males, and 24 (38.7%) were females. The average 25(OH)D level in those patients was 49.87 ± 23.5 nmol/L. Among the patients with UC, six (9.7%) participants had sufficient vitamin D levels (> 75 nmol/L), whereas insufficiency (< 50 nmol/L) and deficiency (50–75 nmol/L) were established in 32 (51.6%) and 24 (38.7%) participants, respectively. In the analyzed sample, 25(OH)D serum levels did not significantly correlate either with FC (r = 0.077, p = 0.551), CRP (r = –0.111, p = 0.392), or MES (r = 0.02, p = 0.787). **Conclusion.** In our investigation, the 25(OH)D serum level did not significantly influence the values of the MES nor the biomarkers of inflammation – FC and CRP.

Key words:
biomarkers; c-reactive protein; colitis, ulcerative; feces; remission induction; vitamin d.

Apstrakt

Uvod/Cilj. Vitamin D igra ključnu ulogu u apsorpciji kalcijuma iz digestivnog trakta i, zahvaljujući svojim imunomodulacijskim svojstvima, utiče na integritet intestinalne barijere, mikrobiotu creva i funkcionalnost imunskog sistema. Cilj rada bio je da se ispita nivo 25-hidroksivitamina D [25(OH)D] u serumu obolelih od ulceroznog kolitisa (UK) u stanju kliničke remisije, kao i njegov uticaj na vrednosti fekalnog kalprotektina (FK) i C-reaktivnog proteina (CRP). **Metode.** Istraživanje, sprovedeno kao studija preseka, obuhvatilo je 62 ispitanika obolelih od UK u stanju kliničke remisije. Nivoi 25(OH)D i CRP u serumu određivani su iz uzoraka venske krvi, dok su nivoi FK određivani iz uzoraka stolice. Endoskopska aktivnost procenjivana je kolonoskopijom i izražena je Mayo endoskopskim skorom (MES). **Rezultati.** Od ukupno 62 obolelih od UK u stanju kliničke remisije, 38 (61,3%) je bilo muškog, a 24 (38,7%) ženskog pola. Prosečna vrednost 25(OH)D kod bolesnika iznosila je 49,87 ± 23,5 nmol/L. Među obolelima od UK, šest (9,7%) učesnika imalo je dovoljan nivo vitamina D (> 75 nmol/L), dok su insuficijencija (< 50 nmol/L) i deficit (50–75 nmol/L) utvrđeni kod 32 (51,6%) i 24 (38,7%) učesnika, redom. U ispitivanom uzorku, nivoi 25(OH)D u serumu nisu značajno korelisali ni sa FK (r = 0,077, p = 0,551), ni sa CRP (r = 0,111, p = 0,392), kao ni sa MES (r = 0,02, p = 0,787). **Zaključak.** U našem ispitivanju, nivo 25(OH)D u serumu nije značajno uticao na vrednosti MES, niti na vrednosti biomarkera inflamacije – FK i CRP.

Ključne reči:
biološki pokazatelji; c-reaktivni protein; kolitis, ulcerativni; stolica; remisija, indukcija; vitamin d.

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Introduction

Ulcerative colitis (UC) and Crohn's disease are both conditions that belong to a group of chronic inflammatory diseases of the digestive system. The etiology of UC is still unknown, and the condition develops due to various factors affecting genetically predisposed individuals and manifests as an inflammatory process that affects the large intestine. The mechanism of the disease has still not been fully elucidated. However, it is well established that it is an immune-mediated inflammatory reaction and disruption of the colonic mucosa. The disease takes a chronic course, including periods of exacerbations and remissions, and the diagnosis is commonly established between 30 and 40 years of age [1]. The effects of vitamin D on the gastrointestinal system, apart from calcium absorption and metabolism, rely on its immunomodulatory properties and are reflected in its effects on intestinal barrier integrity, gut microbiota, and immune system functionality. All these mechanisms are mediated by the vitamin D receptors (VDRs).

In patients with inflammatory bowel disease (IBD), the gut microbiota alters in terms of decreasing the number of butyrate-producing bacteria. During inflammation, VDR signaling is increased and regulates the antibacterial function of natural killer (NK) cells and Paneth cells. Higher 25-hydroxyvitamin D [25(OH)D] levels are related to increased serum cathelicidin that manifests anti-inflammatory and antimicrobial properties [2]. Moreover, it inhibits the maturation of dendritic cells as the most potent antigen-presenting cells, reduces T-cell proliferation, and redirects T-cell differentiation from Th1 and Th17 pathways towards Th2 and Treg [3, 4]. Vitamin D metabolism and signaling play an important role in intestinal homeostasis and barrier integrity. VDR is a transcription factor regulating tight junction proteins such as claudin-2, -5, -12, and claudin-15 epithelial cells. Vitamin D deficiency can lead to a reduced synthesis of these proteins and, consequently, increased permeability and inflammation [5].

It is still doubtful whether vitamin D deficiency is the cause or consequence of the disease activity, but the correlation is clearly negative. Many research papers revealed that patients with higher 25(OH)D levels manifest with lower disease activity, lower risk for clinical relapse, and improved therapeutic response to biologic treatment [6–9]. The optimal concentration of 25(OH)D is based mainly on its effect on the musculoskeletal system and is defined as: adequate [> 75 nmol/L (30 ng/mL)], inadequate or insufficient (50–75 nmol/L), and deficient [< 50 nmol/L (20 ng/mL)] [10]. Even though clear clinical guidelines are unavailable, monitoring of 25(OH)D level is strongly recommended in patients with UC, and it should be maintained at a level above 75 nmol/L. This can be achieved by sufficient exposure to sunlight, adequate dietary intake, or supplementation [11].

Given the controversial results of previous studies, which mostly involved sample populations with varying clinical disease activity, and considering the insufficient data on vitamin D levels in patients with UC in clinical remission in our geographic area, our study aimed to investigate the association between vitamin D levels and inflammatory biomarkers in UC patients in clinical remission.

Methods

The study, designed as a cross-sectional study, included 62 participants with UC in clinical remission, defined as a Simple Clinical Colitis Activity Index (SCCAI) score of 2 or less. The research was approved by the Ethics Committee of the University Clinical Center of Vojvodina (UCCV), Serbia (No. 00-108, from February 27, 2020). The study population consisted of 38 male and 24 female patients. All patients were older than 18 years. The patients did not take corticosteroid therapy during the past six months or vitamin D supplements during the past three months, and they all signed the informed consent to participate in the study. Individuals with primary bone disease (such as Paget's disease), myeloma multiplex, primary hyperparathyroidism, malignancy, and secondary deposits in the bones, as well as patients with chronic kidney disease, pregnant and breastfeeding women, were excluded from the study. After evaluating the previous medical documentation, a comprehensive history of underlying and associated diseases, conditions, and dietary habits was taken. Clinical examination and determination of body weight and body mass index (BMI) were performed. Upon conclusion of the fulfillment of the criteria for the involvement in the study, written consent was obtained from all participants. The evaluation of the clinical disease activity was performed based on the relevant questionnaire (SCCAI), while endoscopic activity was expressed by the Mayo Endoscopic Score (MES) after colonoscopy.

The concentration of serum 25(OH)D and C-reactive protein (CRP) was determined by obtaining venous blood samples, and fecal calprotectin (FC) was determined from fecal samples. All measurements were performed in the Center for Laboratory Diagnostics of the UCCV. The concentration of serum 25(OH)D was determined by the method of chemiluminescence immunoassay – CLIA using Liason XL (Diasorin Inc.) analyzer. The value is expressed in nmol/L. CRP value was measured by an immunoturbidimetric method on an Architect c8000 analyzer (Abbott). The value was expressed in mg/L. The FC value was measured using enzyme-linked immunosorbent assay – ELISA on a Chorus Trio (Diesse) device. The value was expressed in µg/g. A colonoscopy with biopsies was performed in the Endoscopy department of the UCCV on Olympus Evis Exera III endoscope.

Statistical data analysis was performed using the software package JASP 0.17.2. The descriptive statistics parameters and Pearson's correlation coefficient between the variables were calculated.

Power considerations

Statistical power analyses were conducted using GPower statistical software v.3.1.9.7. Regarding Pearson and Spearman correlation coefficients, the results showed that to

perform two-tailed statistical tests for the effect size r = 0.30, with a statistical power of 0.80 and an alpha level of 0.05, sample size N = 84 is sufficient, while for the effect size r = 0.40 (alpha level 0.05, statistical power 0.80), sample size N = 46 is sufficient. Given the results, one may assume that the sample is sufficiently powered to correctly estimate medium-sized correlations (effects) as statistically significant at alpha level 0.05. Provisional power calculations for the Kruskal-Wallis test were calculated using the MultNonParam package in the R statistical computation environment. The results suggest that, for the design containing a three-group factor with ten participants in each group and an alpha level of 0.05 under the assumption of underlying normal distribution, estimated statistical power would approximate 0.88.

Results

The study included 62 participants with UC in clinical remission. The study population consisted of 38 (61.3%) male and 24 (38.7%) female patients with an average age of 44.9 ± 14.1 (22–73) years, average body weight of 78.21 ± 15.5 (48–120) kg, and average BMI of 25.08 ± 4.58 (20.2–40.0) kg/m^2. From the moment of establishing the diagnosis to the moment of being involved in the study, the average duration of the disease was 10.08 ± 7.81 years. As related to the disease extent, 46 (74.2%) participants presented with extensive colitis, while 14 (22.59%) and 2 (3.22%) patients had left-sided colitis and proctitis, respectively. The average vitamin D level was 49.87 ± 23.5 nmol/L.

According to classification, the established vitamin D levels in patients with UC were as follows: 6 (9.7%) patients had sufficient levels (> 75 nmol/L), whereas insufficiency (< 50 nmol/L) and deficiency (50–75 nmol/L) were established in 32 (51.6%) and 24 (38.7%) participants, respectively. The results according to age, duration of the disease, weight, and BMI are presented in Table 1.

The results of the Kruskal-Wallis tests at the level of the entire study group did not reveal any significant differences between participants with different 25(OH)D levels regarding any of the investigated variables.

The average value of FC in the studied sample was 82.69 ± 139.91 μg/g, for CRP, it was 4.598 ± 5.527 mg/L, and for MES, it was 0.855 ± 0.698. Kurtosis values of the variables suggest the absence of any substantial deviations from the values characterizing normal distribution, except for somewhat more pronounced kurtosis for the variables FC and CRP (Table 2).

Calculation of Pearson's correlation coefficient for the variables mentioned above revealed that serum 25(OH)D levels in the analyzed sample did not significantly correlate either with FC [$r = 0.077$, $p = 0.551$, Spearman's rho (ρ) = –0.160, $p = 0.213$] (Figure 1), CRP ($r = –0.111$, $p = 0.392$, $\rho = –0.107$, $p = 0.407$) (Figure 2), or MES ($r = 0.02$, $p = 0.787$, $\rho = 0.021$, $p = 0.874$) (Figure 3).

Table 1

Vitamin D levels in patients (n = 62) with UC in relation to age, duration of UC, BW, and BMI

Parameter	Median	AM	SD	Skewness	Kurtosis	*p-value
Age (years)						
SL	41.813	15.489	0.65	-0.606	22	
IL	47.708	12.063	0.062	-0.587	26	0.136
DL	50.5	12.818	0.754	-1.82	39	
Disease period (years)						
SL	8.625	8.454	3.57	15.479	2	
IL	12.292	7.428	0.338	-1.595	3	0.07
DL	9	3.162	-0.797	-1.868	5	
Weight (kg)						
SL	79.625	18.502	0.506	-0.057	51	
IL	75.609	11.835	-0.346	-0.396	48	0.7
DL	80.667	9.73	-0.757	-0.25	65	
BMI (kg/m^2)						
SL	25.441	5.979	-1.255	7.524	2.2	
IL	24.578	2.473	0.288	2.249	18.5	0.772
DL	25.133	1.183	0.04	-2.446	23.7	

UC – ulcerative colitis; BW – body weight; BMI – body mass index; AM – arithmetic mean; SD – standard deviation. *Kruskal-Wallis test.

Note: Patient groups according to the vitamin D level were as follows: 6 patients were in the group with sufficient level (SL) > 75 nmol/L, 32 patients were in the group with insufficient level (IL) < 50 nmol/L, and 24 patients were in the group with deficient level (DL) from 50–75 nmol/L.

Table 2

Values of FC, vitamin D, CRP, and MES in patients (n = 62) with ulcerative colitis

Parameter	Median	AM	SD	Skewness	Kurtosis	Shapiro-Wilk
FC (μg/g)	17.0	82.7	139.9	2.621	6.896	0.582
25(OH)D (nmol/L)	49.0	49.9	23.5	0.518	0.190	0.967
CRP (mg/L)	2.4	4.6	5.5	2.171	4.219	0.690
MES	1.0	0.855	0.698	0.206	-0.893	0.8

FC – fecal calprotectin; 25(OH)D – 25-hydroxyvitamin D; CRP – C-reactive protein; MES – Mayo Endoscopic Score. For other abbreviations, see Table 1.

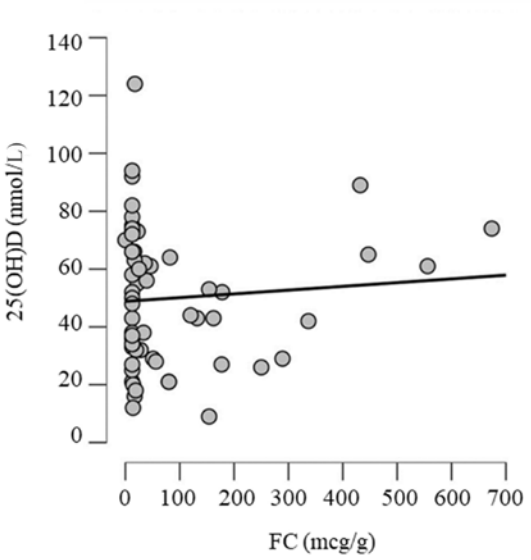

Fig. 1 – Fecal calprotectin (FC) and 25-hydroxyvitamin D [25(OH)D].

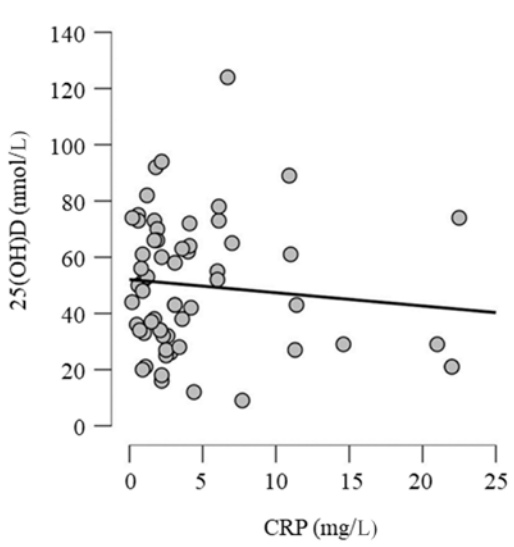

Fig. 2 – C-reactive protein (CRP) and 25-hydroxyvitamin D [25(OH)D].

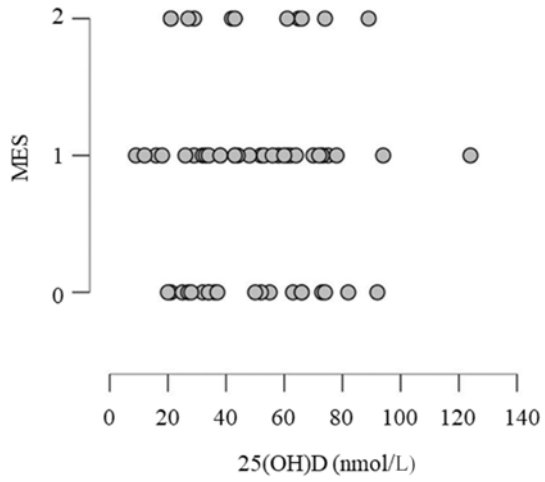

Fig. 3 – Mayo Endoscopic Score (MES) and 25-hydroxyvitamin D [25(OH)D].

Discussion

It is well established that the incidence of IBD is higher in regions with inadequate/reduced exposure to sunlight, which might be associated with vitamin D deficiency [12]. The prevalence of vitamin D deficiency in patients with IBD is higher compared to the general population, ranging around 45–50% in UC. However, routine 25(OH)D monitoring is not conducted in most cases [13]. Most commonly, the deficiency is associated with inadequate dietary intake and reduced exposure to sunlight due to immunosuppressive therapy. It has a seasonal character and is most pronounced in the winter/spring season [14].

In Serbia, a region with a moderate continental climate, our study was conducted in the winter/spring period. The results revealed inadequate serum levels of vitamin D in 91.3% of UC patients in clinical remission, with 51.6% categorized as deficiency (50–75 nmol/L) and 38.7% as insufficiency (< 50 nmol/L).

The prevalence of 25(OH)D deficiency (< 50 nmol/L) among the general population ranges from 24% in the USA to 37% in Canada and 40% in Europe and varies by age, region, and ethnicity [15]. The levels of 25(OH)D, according to vitamin D level classification, in our patients with UC, correspond with the data from the literature. The research conducted by Law et al. [16] on a population of 80 participants with UC revealed similar results: sufficient levels were determined in 10% of patients, while deficiency and insufficiency were detected in 56% and 34% of patients, respectively. According to the Del Pinto et al. [17] meta-analysis, which encompassed 14 studies and 1,891 patients (938 IBD and 953 controls), patients with IBD had 64% higher odds of vitamin D deficiency than the control group. The same group of authors conducted the meta-analysis according to the type of IBD. The analysis of seven studies on the prevalence of vitamin D deficiency in patients with UC revealed more than double the odds for vitamin D

deficiency compared to the healthy controls [odds ratio (OR) = 2.28; 95% confidence interval (CI): 1.18, 4.41; $p = 0.01$]. Taking these data into account, monitoring vitamin D levels in patients with UC would be significant for detecting insufficiency and timely supplementation, given that a favorable effect on the course of the disease has been demonstrated. It has been established that serum vitamin D level negatively correlates with endoscopic and histological inflammation and increases the risk of clinical relapse. As reported by Gubatan et al. [7], 25(OH)D level lower than 35 ng/mL increases the risk of clinical relapse in patients who are in stable clinical remission. Patients with lower vitamin D levels manifest higher clinical disease activity and higher values of inflammatory markers (FC and CRP) [18, 19]. Moreover, vitamin D deficiency is associated with increased disease extent and poorer therapeutic response to anti-tumor necrosis factor therapy [9, 20].

As stated in other available literature data, which is consistent with the results of our research, the correlation between 25(OH)D level and CRP value has not been established [21, 22]. According to the study by Garg et al. [23], cholecalciferol supplementation in patients with IBD did not decrease the values of FC or CRP despite the reduced clinical activity of the disease.

A study by Thomas et al. [24], which included 82 patients with UC, revealed decreased vitamin D and increased CRP values in patients with high endoscopic activity compared to those in remission. A statistically significant negative correlation between vitamin D and the endoscopic score was confirmed ($p = 0.047$), whereas a negative correlation between vitamin D and CRP was below the level of statistical significance ($p = 0.079$). In a study with 90 UC patients in clinical remission, Emami et al. [25] measured the concentration of visfatin, which exerts proangiogenic effects and is considered an indirect inflammation indicator. The obtained results indicated that supplementation of a 300,000 IU mono-dose vitamin D resulted in a moderate increase of visfatin level in a group with vitamin D insufficiency, unlike the group with normal vitamin D levels. This result suggests the anti-inflammatory effects of vitamin D in clinical remission, though its effects on the inflammation biomarkers (FC and CRP) in remission are still unknown.

Most studies of vitamin D effects on UC activity included a sample population with different stages of disease activity and patients with IBD in general, and the investigations of UC patients in clinical remission are still scant.

The importance of our research is in the fact that only patients in clinical remission were selected for the study, which was not the case in most of the available previous research. We fulfilled the aim of the study to assess the effects of vitamin D on the activity parameters only in clinical remission and hence contribute to a better understanding of the vitamin D function and its dynamics in this patient population. Moreover, this could contribute to the fact that vitamin D might have an important role as an inflammatory biomarker for noninvasive patient monitoring.

A major limitation of our study is the small sample size. However, several studies on this topic were also performed on a smaller sample. Therefore, the power of the study was calculated to approximate 0.88.

Conclusion

Vitamin D deficiency is a frequent condition in patients with UC in clinical remission. It requires continuous monitoring of 25-hydroxyvitamin D serum levels and consequent vitamin D supplementation due to its effects on calcium metabolism and the activity and course of the disease. Our research did not reveal any relationship between 25-hydroxyvitamin D serum level and values of biomarkers of inflammation (fecal calprotectin and C-reactive protein) or endoscopic disease activity (Mayo Endoscopic Score) in patients with ulcerative colitis in clinical remission, which weighs in favor of controversy whether the decreased level of vitamin D is a cause or the consequence of bowel inflammation, which is a topic of further research in this field.

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Diagnostic and therapeutic efficacy of urinary bladder hydrodistension in patients with bladder pain syndrome

Dijagnostička i terapijska efikasnost hidrodistenzije mokraćne bešike kod bolesnika sa sindromom bolne bešike

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Abstract

Background/Aim. Interstitial cystitis (IC)/bladder pain syndrome (BPS) is a condition with recurring discomfort or pain in the urinary bladder and the surrounding pelvic region without an identifiable disease. The aim of this study was to assess hydrodistension as a diagnostic and treatment procedure in patients with BPS. **Methods.** This prospective study included 45 patients who underwent cystoscopy with hydrodistension. The mean values for 24-hr voiding frequency, maximal voided urine volume, average voided urine volume, and minimal voided urine volume originated from the frequency volume chart. The values were compared between the time before hydrodistension and one, three, and six months after that. **Results.** By comparing the initial data and data in all three follow-up periods (after one, three, and six months), the statistical significance ($p < 0.046$) was found, and that: for 24-hr voiding frequency, it was 19.64 ± 3.56, 9.42 ± 1.71, 9.58 ± 1.45, and 12.2 ± 2.79, respectively; then, for the minimal voided urine volume ($p < 0.03$), it was 59.11 ± 23.72 mL, 114.89 ± 4.09 mL, 112.44 ± 100.86 mL, and 89.00 ± 29.45 mL, respectively; for an average voided volume ($p < 0.04$), it was 105.33 ± 18.29 mL, 186.89 ± 23.14 mL, 186.44 ± 21.44 mL, and 155.78 ± 30.78 mL, respectively. There was no significant statistical difference ($p < 0.1$) regarding the maximal voided urine volume between initial and follow-up interval data: 196.89 ± 43.68 mL, 312.89 ± 54.59 mL, 316.00 ± 49.47 mL, 266.67 ± 53.17 mL, respectively. **Conclusion.** Our results demonstrate that hydrodistension is a reliable diagnostic and therapeutic procedure.

Key words:
cystitis, interstitial; cystoscopy; diagnosis; treatment outcome; urinary bladder; urination disorders; urine.

Apstrakt

Uvod/Cilj. Intersticijski cistitis (IC)/sindrom bolne bešike (SBB) je stanje ponavljajuće nelagodnosti ili bola u mokraćnoj bešici i okolnom karličnom regionu bez prepoznatljive bolesti. Cilj rada bio je da se kod bolesnika sa SBB proceni značaj hidrodistenzije kao dijagnostičke i terapijske procedure. **Metode.** Prospektivnom studijom obuhvaćeno je ukupno 45 bolesnika podvrgnutih cistoskopiji sa hidrodistenzijom. Srednje vrednosti za parametre učestalost 24-časovnog mokrenja, zapremina maksimalno izmokrenog urina, zapremina prosečno izmokrenog urina i zapremina minimalno izmokrenog urina dobijene su iz „dnevnika mokrenja“. Ove vrednosti su upoređivane između vremena pre hidrodistenzije i jedan, tri i šest meseci posle toga. **Rezultati**. Poređenjem početnih podataka i podataka iz sva tri perioda praćenja (posle jedan, tri i šest meseci) utvrđena je statistički značajna razlika ($p < 0,046$), i to: za 24-časovnu učestalost mokrenja, bila je 19,64 ± 3,56, 9,42 ± 1,71, 9,58 ± 1,45 i 12,2 ± 2,79, redom; zatim, za minimalnu zapreminu urina ($p < 0,03$), bila je 59,11 ± 23,72 mL, 114,89 ± 4,09 mL, 112,44 ± 100,86 mL i 89,00 ± 29,45 mL, redom; za prosečnu zapreminu mokrenja ($p < 0,04$), bila je 105,33 ± 18,29 mL, 186,89 ± 23,14 mL, 186,44 ± 21,44 mL i 155,78 ± 30,78 mL, redom. Nije bilo statistički značajne razlike ($p < 0,1$) za vrednosti maksimalne zapremine izmokrenog urina između početnih i kontrolnih intervala: 196,89 ± 43,68 mL, 312,89 ± 54,59 mL, 316,00 ± 49,47 mL, 266,67 ± 53,17 mL, redom. **Zaključak.** Rezultati našeg istraživanja ukazuju na to da je hidrodistenzija pouzdana dijagnostička i terapijska procedura.

Ključne reči:
cistitis, intersticijalni; cistoskopija; dijagnoza; lečenje, ishod; mokraćna bešika; mokrenje, poremećaji; mokraća.

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Introduction

In the past, due to the absence of a commonly accepted definition for the condition affecting urinary bladder, confusion arose in defining and understanding patients' complaints. In 1808, Philip Syng Physick, a pioneer in medicine, was the first to mention bladder pain syndrome (BPS). This condition remained unnamed until 1987 when the National Institute for Diabetes and Digestive and Kidney Diseases introduced the term interstitial cystitis (IC) [1, 2].

In 2002, painful bladder syndrome (PBS)/BPS was defined by the International Continence Society (ICS) as "the complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased daytime and nighttime frequency, in the absence of proven urinary infection or other obvious pathology" [3]. Additionally, the European Society for the Study of IC/BPS (ESSIC) and its definition were incorporated in the nomenclature proposed by the International Consultation on Incontinence in 2010 [4, 5].

The estimated prevalence of BPS adds up to 0.06–20.0%, with a female predominance of 10 : 1 [6]. This discrepancy in available data can be explained by the fact that diagnosis of BPS is mainly determined by a reverse process of eliminating confusable diseases and excluding criteria and diagnostic procedures. Hydrodistension, initially reported by Bumpus [7] in 1930 and followed by Hand [8] in 1949, is widely accepted as a diagnostics tool included in guideline algorithms presented by relevant associations involved in treating BPS [4, 6, 9–11]. Besides that, hydrodistension is also performed as a treatment in managing BPS, which is of increased significance since other treatment options are often unavailable [12]. The aim of this study was to analyze the results of hydrodistension in 45 examined patients with BPS by determining the efficacy of hydrodistension as a diagnostic and therapeutic procedure.

Methods

Patients

This prospective study included 45 patients who underwent cystoscopy with hydrodistension as a diagnostic and treatment procedure for BPS at the Clinic for Urology, Military Medical Academy, Belgrade, Serbia, from April 2013 to January 2018. The study was conducted according to the Declaration of Helsinki and the protocol was approved by the local Ethics Committee. All patients signed a consent form.

Patients with a history of chronic pelvic pain (discomfort, pressure, burning sensation) in a period longer than six months, accompanied by bladder filling and an urge to void or increased frequency of urination were enrolled. The preoperative evaluation consisted of previous medical history, clinical examination, urine and urine culture analyses, abdomen and pelvis minor ultrasound with post-void residual urine measurement, as well as a 72-hr frequency volume chart (FVC).

Hydrodistension procedure

The cystoscopy with hydrodistension was carried out in an inpatient setting under general anesthesia. This low-pressure, short-duration hydrodistension is one of the most common treatments in BPS. Prior to the procedure, all patients were administered a single-dose antibiotic prophylaxis. They were placed in a dorsal lithotomy position. The bladder was filled through a 26 French (Fr) size electro resectoscope with 30° Hopkins® optic, using urologic irrigating solutions containing sorbitol (27 g/L) and mannitol (5.4 g/L) with an infusion height of 80 cm above the symphysis level (80 cm H_2O) without any additional pressure applied. The electroresectoscope was used for better visibility and less leakage during the procedure. The filling was terminated when the bladder capacity of 500 mL of the irrigating solution was achieved or when visible leakage occurred around the cystoscope. This was maintained for around 3 min, and the bladder was drained by assessing the bleeding through the color of the irrigation fluid. The volume of the drained fluid was measured, and the maximal bladder capacity was presented. Following the hydrodistension, the bladder was filled again to around two-thirds of its capacity for optimal vision and inspection of the bladder mucosae (anterior, posterior, lateral bladder walls and base of the bladder) to visualize the presence of glomerulations or even Hunner's ulcer. Glomerulations are commonly defined as small submucosal hemorrhages or petechial bladder bleedings. Hunner's lesion, previously known as Hunner's ulcer, represents a localized hyperemic part of the bladder mucosae and submucosae with central scaring surrounded by tiny blood vessels in a radiated fashion. It can be seen following cystoscopy with hydrodistension. Cystoscopic findings were classified as grade 0 – normal mucosa; grade I – petechiae in at least two quadrants; grade II – considerable submucosal bleeding (ecchymosis); grade III – diffuse global mucosal bleeding; grade IV – mucosal disruption, with or without bleeding/oedema. According to the ESSIC group classification, different types of BPS are defined. BPS types are defined using two symbols. The first one represents cystoscopic findings (1 – normal; 2 – glomerulations grade II or III; 3 – Hunner's lesion with or without glomerulations), while the second stands for the biopsy results (X – not performed; A – normal; B – inconclusive; C – inflammatory infiltrates). Our study defined BPS types as 1X, 2X, and 3X since no biopsy was performed during the cystoscopy and hydrodistension. An indwelling urinary catheter 16 Fr was left overnight, and all patients were discharged the following day.

The evaluation following cystoscopy with hydrodistension was conducted in an outpatient setting for one, three, and six months, respectively. This assessment included a urine culture and a 72-hr FVC, intended to record the volumes voided (VV), as well as the time of each visit to the toilet, both during the day and night. It aimed to determine the therapeutic effect of hydrodistension by analyzing FVC parameters. We examined the voiding frequency, as well as minimal, average, and maximal VV. These parameters were

then compared with the ones from FVC before hydrodistension.

Prior to the cystoscopy with hydrodistension, all patients were given detailed instructions about the procedure itself and what was planned to be performed.

Statistical analysis

Descriptive statistics, including mean, standard deviation, and median, were calculated for each variable using Statistical Package for the Social Sciences (SPSS) version 29.0 with significance set at $\alpha = 0.05$. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test implemented in SPSS. We applied the Friedman test to compare multiple related samples, and the Wilcoxon signed-rank test was performed for pairwise comparisons between associated samples. Differences between independent samples were assessed using the Mann-Whitney *U* test in SPSS. The Chi-square test (χ^2) was utilized to analyze categorical variables, with the likelihood ratio employed to assess goodness-of-fit. The Spearman correlation coefficient was computed to examine the relationship between non-normally distributed variables. Factor analysis with varimax rotation was performed to explore underlying patterns or dimensions within the dataset. The value of $p < 0.05$ was considered significant for all tests.

Results

The study included 45 patients. The average age was 57.78 (29–82) years. The baseline patient characteristics are presented in Table 1. Out of the 45 monitored patients, 40 (88.8%) were female and 5 (11.2%) were male. According to the ESSIC group classification of BPS, 2 (4.4%) patients were marked as type 1X, 41 (91.2%) as 2X, and 2 (4.4%) patients as 3X (Table 1, Figure 1).

Table 1

Demographic characteristics of the patients with BPS and their grades of BPS including the ESSIC dysfunction without biopsy

Parameters	Values
Age, years	57.78 (29–82)
Gender	
female	40 (88.8)
male	5 (11.2)
ESSIC classification	
1X	2 (4.4)
2X	41 (91.2)
3X	2 (4.4)

BPS – bladder pain syndrome; ESSIC – European Society Study of Interstitial Cystitis.
1X – normal without glomerulations; 2X – 2- and 3-grade glomerulations; 3X – Hunner's ulcers.
All values are given as numbers (percentages) except age which is given as median (range).

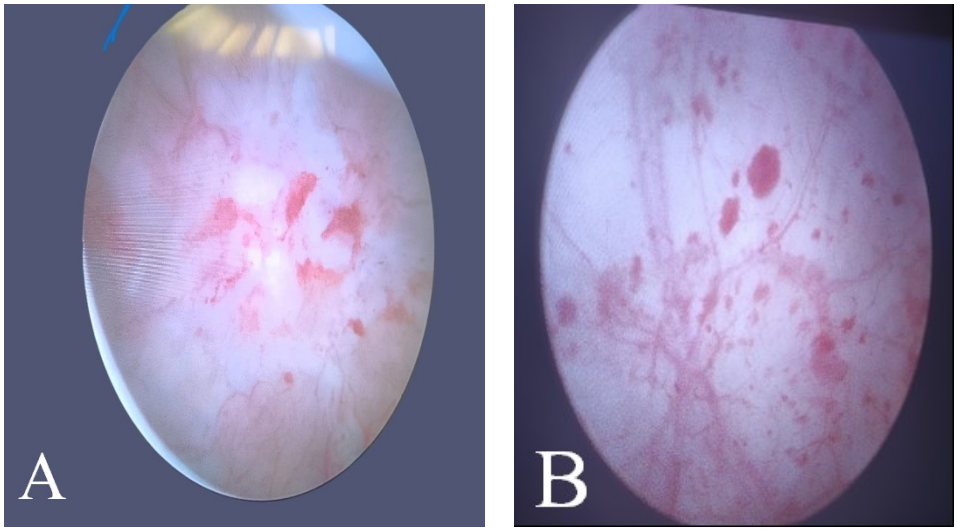

Fig. 1 – Cystoscopic findings of two patients that were marked as 3X according to the criteria of the European Society for Study of Interstitial Cystitis group classification of bladder pain syndrome (without biopsy performed show); A) Hunner's ulcer; B) grade 2 glomerulations.

FVC parameters, including the preoperative assessment and follow-up intervals, are presented in Table 2. The statistical analysis has reiterated a statistical significance ($p < 0.05$) by comparing the initial frequency and the frequency in all three follow-up periods (Table 2, Figure 2). The same has been confirmed when contrasted with minimal VV and the same parameter in all three follow-up periods (Figure 3). Comparison of the initial average VV with the same FVC parameters at one, three, and six months confirmed the statistical significance (Figure 4). However, no statistical difference was found when comparing the initial maximal FVC VV with the same parameter in the follow-up intervals (Figure 5).

Table 2

Frequency volume chart (FVC) parameters of examined patients with bladder pain syndrome

Parameters	Initial	Follow-up (months)			
		1	3	6	*p*-values
Frequency (24 hrs)	19.64 ± 3.56	9.42 ± 1.71	9.58 ± 1.45	12.2 ± 2.79	< 0.046
Maximal VV (mL)	196.89 ± 43.68	312.89 ± 54.59	316.00 ± 49.47	266.67 ± 53.17	< 0.100
Average VV (mL)	105.33 ± 18.29	186.89 ± 23.14	186.44 ± 21.44	155.78 ± 30.78	< 0.040
Minimal VV (mL)	59.11 ± 23.72	114.89 ± 4.09	112.44 ± 100.86	89.00 ± 29.45	< 0.030

VV – voided volume. All values are given as mean ± standard deviation.
Note: initial means before hydrodistension.

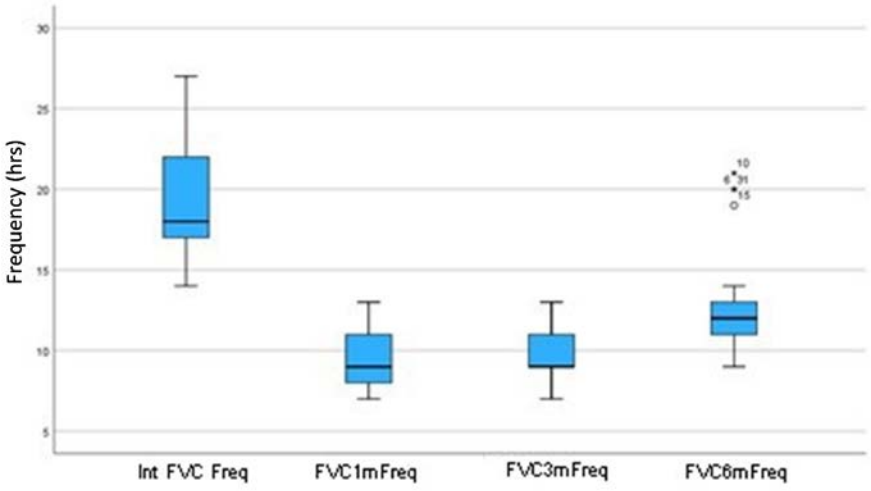

Fig. 2 – Frequency volume chart – 24-hr frequency.
Int – initial; Freq – frequency; m – months.
Note: The numbers above the 6-month value indicate the number of patients whose monitored values individually deviated from the range.

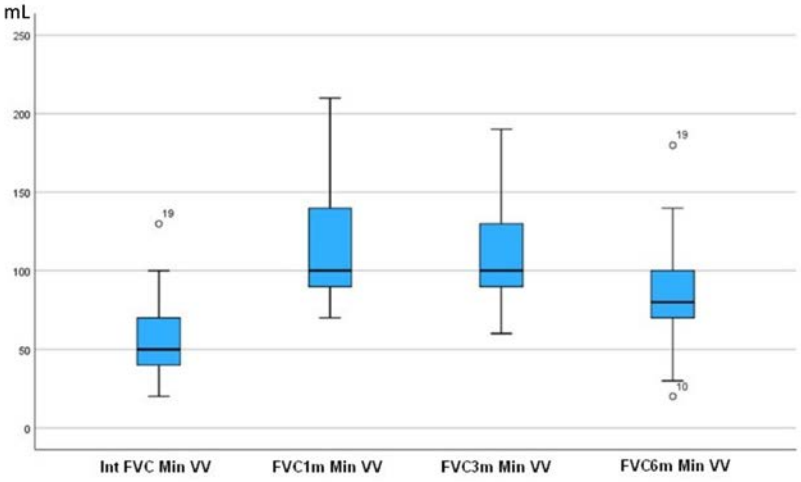

Fig. 3 – Frequency volume chart (FVC) – minimal voided volume (Min VV).
Int – initial; Freq – frequency; m – months.
Note: The numbers above the initial value and above and below 6-month value indicate the number of patients whose monitored values individually deviated from the range.

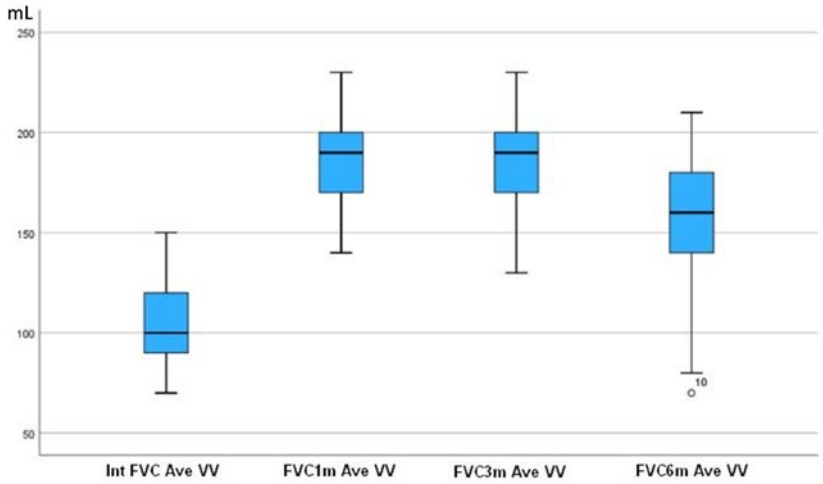

Fig. 4 – Frequency volume chart (FVC) – average voided volume (Ave VV).
Int – initial; Freq – frequency; m – months.
Note: The number below 6-month value indicates the number of patients whose monitored values individually deviated from the range.

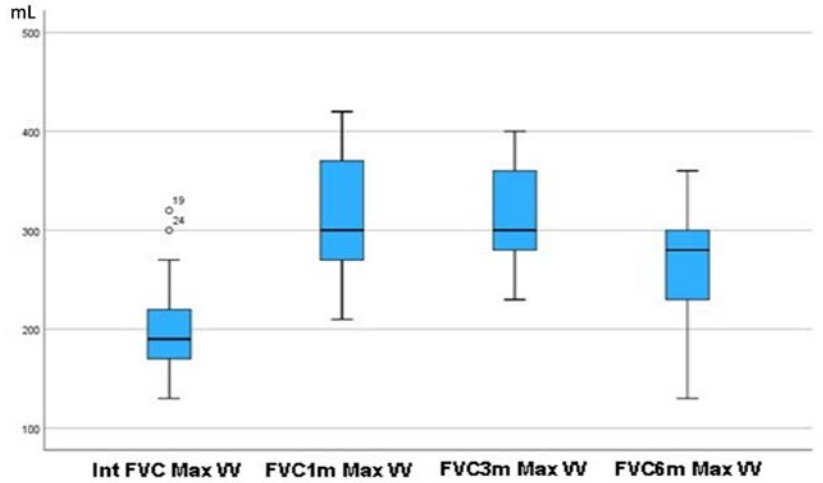

Fig. 5 – Frequency volume chart (FVC) – maximal voided volume (Max VV).
Int – initial; Freq – frequency; m – months.
Note: The numbers above the initial value indicate the number of patients whose monitored values individually deviated from the range.

In the monitored group of patients, symptoms failed to improve in all monitored follow-up intervals, in two patients with 1X and six patients with 2X following hydrodistension. Therefore, the treatment outcome by hydrodistension was favorable in 37 (82.22 %) patients in the first two periods. Eight more patients suffered from an unfavorable increase in symptoms after six months. The overall positive treatment outcome was favorable in 29 (64.44 %) patients at the end of the monitored intervals.

Discussion

BPS/PBS/IC is a chronic condition that may significantly impact patients' quality of life. BPS is a clinical diagnosis pertaining to pain symptoms in the bladder and pelvis, including other predominant urinary symptoms such as urgency and frequency. As previously mentioned, hydrodistension is broadly accepted as a diagnostics tool incorporated in guideline algorithms presented by relevant associations. According to the ESSIC criteria, evidence of positive signs of hydrodistension at cystoscopy is a prerequisite for diagnosing BPS [4].

Hydrodistension itself can be performed with or without concomitant bladder biopsies. The guidelines view this issue differently, including the necessity of performing them. It should be noted that bladder biopsies have not been routinely performed in the United States of America. Consequently, we have aligned with that view [9]. Therefore, we tend to perform hydrodistension as a single diagnostic procedure without concurrent biopsies. There is no difference in the baseline demographics in our study compared to the published data. This applies to the female/male ratio. According to the ESSIC criteria in our research, 41 (91.2%) diagnosed patients had 2X (2- and 3-grade glomerulations), 2 (4.4%) had

1X (normal without glomerulations), and 2 (4.4%) had visible Hunner's ulcers during hydrodistension. Besides visible glomerulations or Hunner's ulcers, mucosal bleeding after distension could be seen [13]. By definition, Hunner's ulcers were first reported by Guy L. Hunner [14] in 1915 as single or multiple erythematous mucosal patches, often accompanied by small vessels in a radiating fashion surrounding a central scar. According to current guidelines, fulguration is one of the treatment options for resolving Hunner's ulcers that are refractory to treatment by intravesical instillation solutions. These guidelines propose level of evidence C recommendation ("well-conducted case-control cohort studies with a low risk of bias and a moderate probability that the relationship is causal") or fulguration in instances with visible Hunner's ulcers at hydrodistension [15]. We have, therefore, conducted fulguration in all our patients with registered Hunner's ulcer lesions during cystoscopy with hydrodistensions.

The underlying principles for patients with BPS imply a decrease in their debilitating symptoms, improving their quality of life, and encouraging realistic patient expectations. Optimal management should involve multimodal behavioral, physical, and psychological techniques and proceed stepwise, starting with the most conservative one. Different grades of recommendation for all treatments of bladder hydrodistension and transurethral fulguration of Hunner's lesions have been discussed as the third-line option for treating BPS following the failure of the second-line therapies [10].

Having said this, we ought to emphasize that most patients were referred to our hospital without a defined condition. However, they were treated using the first- and second-line options without significant improvements. Different means can be used to assess symptoms before and following hydrodistension, such as validated symptom scores, O'Leary-Sant IC Symptom Index and the IC Problem Index and the Pelvic Pain and Urgency/Frequency Scale [16].

One of the available assessment tools is FVC, which evaluates the overall frequency, minimal, average, and maximal VV in 24 hrs. BPS patients typically have lower VV and higher voiding frequency than asymptomatic patients. Therefore, using an FVC is recommended for the initial evaluation. The recommended FVC is a three-day (72 hrs) FVC and we decided to apply it in our study. ICS defines median functional bladder capacity as the median maximum VV in everyday activities (as *per* FVC) [12, 17]. The initial frequency in our study was consistent with published data. Following hydrodistension, there is a significant decrease in frequency in all three monitored intervals, especially after one and three months. Regarding average FVC VV, there was a substantial increase in volume in all post-hydrodistension analyzed intervals ($p < 0.04$). The same was confirmed by comparing the initial and post-hydrodistension minimal FVC VV in all follow-up intervals ($p < 0.03$). No statistical significance was determined when comparing the initial maximal VV with values registered in the follow-up periods.

Since the symptoms failed to improve in all monitored follow-up intervals, the treatment outcome by hydrodistension was favorable in 37 (82.22%) patients in the first two periods. After six months, even eight more patients had additional symptoms. The overall positive treatment outcome was favorable in 29 (64.44%) patients at the end of the monitored intervals. This is higher than in available published data where a decrease in symptoms at three months following hydrodistension is registered in 53.8% of patients and in 25% of them at six months [18, 19].

Conclusion

Our results conclude that hydrodistension is a straightforward, broadly accepted, safe diagnostics procedure with low complication rates. Additionally, it is a therapeutic procedure providing significant symptomatic relief, with the possibility of repetition, should it be needed. Together, these results help reiterate/confirm that hydrodistension is a valuable diagnostic and therapeutic procedure with significant benefits for patients with severe BPS/PBS/IC. Unfortunately, the predictors of symptom response to hydrodistension have not been clearly identified. Our study considered some aspects of hydrodistension as a diagnostic and therapeutic procedure for the management of BPS/PBS/IC. Our ongoing research will enable us as providers to identify patients who are most likely to benefit from the procedure and potentially improve the treatment outcomes in those patients who suffer from this debilitating condition.

Conflict of interest

The authors declare no conflict of interest that could influence the work reported in this paper.

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Strengthening pharmacy practice: development and validation of the Resilience Scale

Jačanje apotekarske prakse: razvoj i validacija Skale otpornosti

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Abstract

Background/Aim. Despite the demanding nature of their roles in community pharmacies and their critical importance to patient safety, healthcare professionals, including pharmacists, are often not covered by the Resilience Scale. Therefore, the aim of the study was to develop and validate a resilience scale specifically tailored for pharmacists working in community pharmacies. **Methods.** The study involved the development and validation of a scale aimed at assessing psychological resilience among community pharmacists. The domains and items of the scale were considered from the aspect of reviewing the available literature. Content validation by subject matter experts and subsequent computation of the content validity index ensured the scale's content validity. Face validity assessment ensured alignment with the intended construct. The final scale was distributed to a sample of 504 community pharmacists, after which the scale was analyzed using statistical methods such as factor analysis, multiple regression, and reliability analysis. Additionally, test-retest reliability analysis was performed on 80 community pharmacists. **Results.** During the brainstorming sessions and focus groups, 95 items were generated within five domains – Confidence, Agility, Coping with stress, Interpersonal relations, and Developmental thinking. Following expert review and rigorous content and face validity analyses, 30 items with Content Validity Index and Face Validity Index values surpassing 0.80 were retained. The scale exhibited strong internal consistency, with Cronbach's alpha exceeding 0.9. Factor analysis confirmed the five-factor structure, with each component displaying high factor loadings and significant variable loadings on only one component. **Conclusion.** The Resilience Scale emerged as a promising tool for assessing community pharmacists' resilience, demonstrating robust psychometric properties. The study contributed validity evidence concerning content and internal structure, thereby enhancing the scale's credibility in evaluating resilience domains within the pharmacy profession.

Key words:
data interpretation, statistical; pharmacists; resilience, psychological; serbia; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Uprkos zahtevnoj prirodi njihovih uloga u javnim apotekama i kritičnom značaju za bezbednost pacijenata, zdravstveni radnici, uključujući farmaceute, često nisu obuhvaćeni Skalom otpornosti. Zbog toga je cilj rada bio da se razvije i validira skala otpornosti posebno prilagođena farmaceutima zaposlenim u javnim apotekama. **Metode.** Istraživanje je uključilo razvoj i validaciju skale usmerene na procenu psihološke otpornosti među farmaceutima zaposlenim u javnim apotekama. Domeni i stavke skale razmatrani su sa aspekta pregleda dostupne literature. Validacija sadržaja od strane stručnjaka za predmetnu oblast i naknadno izračunavanje indeksa validnosti sadržaja osigurali su validnost sadržaja skale. Procena pojavne validnosti (*face* validnosti) osigurala je usklađenost sa pretpostavljenim konstruktom. Konačna skala distribuirana je na uzorku od 504 farmaceuta zaposlenih u javnim apotekama, nakon čega je skala analizirana primenom statističkih metoda kao što su faktorska analiza, multipla regresija i analiza pouzdanosti. Dodatno, analiza pouzdanosti test-retest metodom izvršena je na uzorku od 80 farmaceuta zaposlenih u javnim apotekama. **Rezultati.** Tokom sesija grupnog promišljanja (*brainstorming* sesija) i fokus grupa generisano je 95 stavki u okviru pet domena – Samopouzdanje, Agilnost, Savladavanje stresa, Međuljudski odnosi i Razvojno mišljenje. Nakon stručnog pregleda i rigorozne analize validnosti sadržaja i pojavne validnosti zadržano je 30 stavki sa vrednostima indeksa validnosti sadržaja i indeksa pojavne validnosti iznad 0,80. Skala je pokazala snažnu unutrašnju konzistentnost, sa Cronbach-ovim alfa koeficijentom preko 0,9. Faktorska analiza potvrdila je petofaktorsku

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strukturu, pri čemu je svaka komponenta pokazala visoka faktorska opterećenja i značajno opterećenje stavki samo na jednoj komponenti. **Zaključak.** Skala otpornosti pokazala se kao odgovarajući alat za procenu otpornosti farmaceuta zaposlenih u javnim apotekama, demonstrirajući zadovoljavajuće psihometrijske karakteristike. Ova studija doprinela je dokazima o validnosti u vezi sa sadržajem i unutrašnjom strukturom, čime je povećan kredibilitet skale u proceni domena otpornosti u okviru farmaceutske profesije.

Ključne reči:
statistička analiza podataka; farmaceuti; rezilijentnost; srbija; ankete i upitnici.

Introduction

Resilience within psychology refers to an individual's capability to handle and bounce back from various life challenges, stressors, or unfavorable circumstances while maintaining mental well-being. In essence, it involves the capacity to adapt, recover, or sustain a positive outlook despite facing difficulties. Central to resilience are active coping strategies, adaptation skills, and cultivating internal resources to navigate life's adversities with resilience and emotional fortitude [1, 2]. Resilience, defined as the ability to bounce back from adversity and maintain psychological well-being, is an essential trait for individuals facing the challenges inherent in the pharmacy profession, particularly those working in community pharmacies. Pharmacists in community pharmacies encounter various stressors, including high workload, time pressure, dealing with patient health issues, and ensuring medication safety. Given the critical role of pharmacists in healthcare delivery, their resilience directly impacts service quality, patient outcomes, and pharmacist safety [3–5].

Examining resilience among pharmacists, especially post-pandemic, is of paramount importance. The prevalence of low resilience among the general population is twice as high compared to healthcare professionals [6]. Earlier research has traditionally tracked resilience through the phenomena of stress coping and burnout without specifically focusing on the distinct aspects of resilience dimensions viewed as a specific construct. Findings indicate that more than half of the participants reported high levels of anxiety, stress, and burnout, suggesting low levels of resilience [4, 5]. This underscores the need for researching and supporting resilience within the pharmaceutical profession. The experiences of healthcare professionals during the coronavirus disease 2019 (COVID-19) pandemic offer invaluable insights into understanding the rapid responsiveness of healthcare systems to changes and the potential for fostering resilient healthcare services on a global scale. It was evident that healthcare professionals exhibited remarkable adaptive abilities amidst the challenges posed by the COVID-19 pandemic. While certain adaptations were deemed advantageous for future organizational healthcare service modifications, others revealed deficiencies in healthcare system structures and capabilities, resulting in maladaptive adjustments [7]. Despite the recognized importance of resilience, there is a lack of standardized resilience assessment tools tailored specifically for pharmacists working in community pharmacies. Existing resilience scales (RSs) may not fully capture the unique stressors and coping mechanisms relevant to this population. Consequently, there is a pressing need to develop and validate an RS tailored to the context of public pharmacy practice. The development of a standardized RS for pharmacists in community pharmacies is crucial for several reasons. Firstly, it allows for a comprehensive assessment of pharmacists' resilience levels, enabling targeted interventions to enhance coping strategies and psychological well-being. Secondly, a validated RS can serve as a valuable tool for evaluating the effectiveness of resilience-building interventions and training programs tailored to pharmacists' needs. Thirdly, by understanding pharmacists' resilience levels, healthcare organizations can support their workforce better, leading to improved service quality, patient satisfaction, and medication safety outcomes. Additionally, the development of multidimensional instruments for assessing resilience is crucial, especially given the presence of similar phenomena such as stress and burnout. These instruments offer a more selective insight into specific components of resilience, allowing for a better understanding of how individuals cope with challenges and adversities. Their multidimensional nature contributes to a deeper understanding of resilience and the identification of key areas for interventions and support. Therefore, their existence enriches research and practice in the field of mental health and well-being, providing tools that target one of the most critical aspects of human resilience [8].

The aim of the research was to develop and validate a Pharmacist RS (PRS) tailored for pharmacists in community pharmacies at the primary level of healthcare. The study detailed the item generation, validation procedures, and psychometric evaluation of the scale using a sample of community pharmacists. Additionally, the significance of implementing a standardized RS for pharmacists was discussed, highlighting its potential to enhance service quality, improve patient outcomes, and safeguard pharmacist well-being and safety.

Methods

The study unfolded in two phases. The first phase identified resilience domains for community pharmacists and formulated corresponding items. The second phase validated these domains and items through content and face validation, followed by evaluating their factorial structure and internal consistency. Figure 1 shows the stages of scale development and validation.

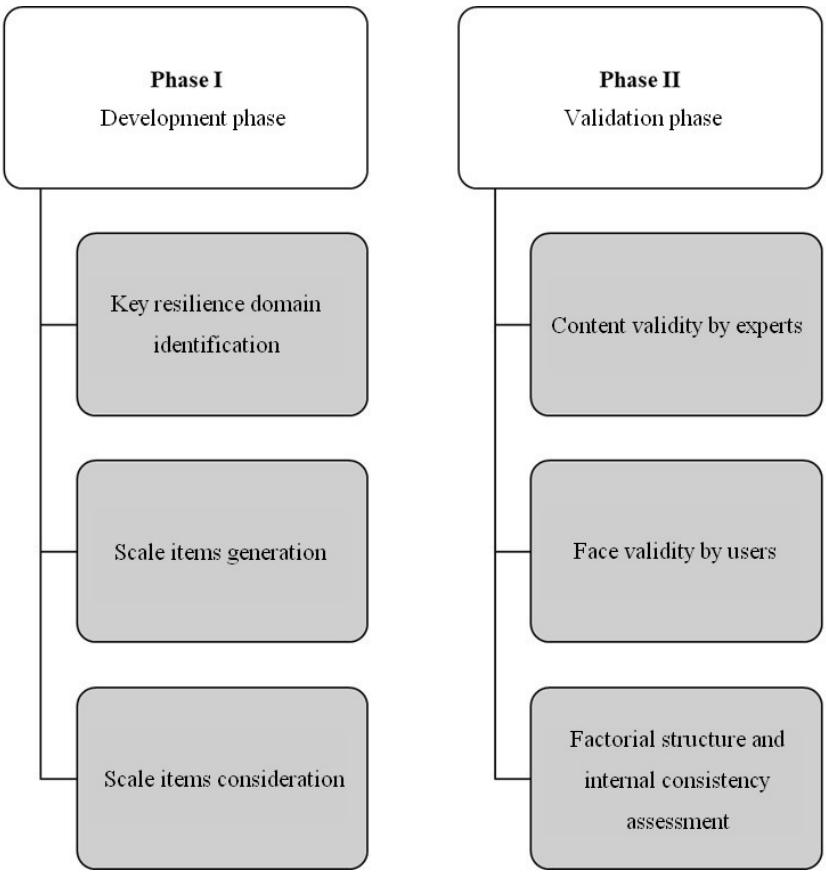

Fig. 1 – Stages of development and validation of the Pharmacy Resilience Scale.

Construction of the Pharmacist Resilience Scale

In the endeavor to evaluate psychological resilience for the study, a specialized scale was developed. The instrument was created following the methodology of psycho-social research concerning the development of measurement scales [9]. The study's initial phase was dedicated to pinpointing resilience domains among pharmacists in community pharmacies. These domains, derived from the integrated resilience model, encompass Control (maintaining composure under stress), Resourcefulness (utilizing available resources for solutions), Involvement (commitment to overcoming adversity), and Growth (continuous personal development amid challenges) [10]. Subsequently, items were crafted through a literature review and two collaborative brainstorming sessions with experts, including two psychologists, three pharmacy experts, and a scale development specialist. The process advanced through various stages, incorporating insights from two focus groups consisting of licensed pharmacists, a psychologist, and the study's primary investigator. Pilot testing highlighted areas for improvement, prompting iterative cycles of refinement that included formulation, testing, and revision.

The generation of items for the RS was based on a comprehensive literature review [1, 2], that involved an initial search in each database using selected keywords. Titles and abstracts were screened to identify potentially relevant studies, and full-text articles of the shortlisted studies were retrieved and reviewed. The methodology of the literature search, including details on the databases used, keywords, as well as inclusion and exclusion criteria, can be found in Appendix 1. Data extraction focused on resilience domains, item development processes, and validation techniques. The extracted data were synthesized to identify common themes and domains of resilience applicable to community pharmacists, such as Control, Resourcefulness, Involvement, and Growth.

The initial version of the scale was evaluated by a panel of content experts selected for their qualifications and extensive research background in resilience. A minimum of six experts participated in the content validation process, using a 4-point rating scale to assess each item's relevance to the resilience domains: 1 for irrelevant items, 2 for somewhat relevant, 3 for quite relevant, and 4 for highly relevant items. Experts also provided written feedback on items requiring modification or removal. The Content Validity Index (CVI) was computed based on two parameters. The first parameter was an Item-Level CVI (I-CVI). This index measured the proportion of experts who rated each item as 3 or 4, indicating its relevance out of the total number of experts. The second one was a Scale-Level CVI (S-CVI). The S-CVI was calculated as the average of the I-CVI scores across all items within the scale, reflecting the overall content validity of the instrument.

The minimum acceptable I-CVI value was set at 0.78, and the minimum acceptable S-CVI value was set at 0.80. Items with an I-CVI below 0.80 were rejected, while those with an I-CVI of 0.80 or higher were accepted [11].

Following the content validation process, face validation was conducted primarily to assess the clarity of instructions and language in the instrument, aiming to identify any ambiguities or multiple interpretations of the items. A minimum of ten test respondents were targeted for the face validation phase. The determination of the Face Validity Index (FVI) involved evaluating specific criteria. The first one was Item-Level FVI (I-FVI). This metric gauges the percentage of test respondents who assigned a clarity rating of 3 or 4 to each item. The second criterion was Scale-Level FVI (S-FVI). This index represents the mean of all I-FVI scores across items within a scale, such as a resilience domain.
The I-FVI was required to meet a minimum threshold of 0.80, while the S-FVI needed to reach at least 0.83. Items with an I-FVI below 0.80 were disregarded, and those with an I-FVI of 0.80 or higher were considered acceptable [12].

Scale results were categorized into Low, Moderate, and High resilience based on score ranges: 0–33% for Low resilience (insufficient resources and coping strategies), 34–66% for Moderate resilience (moderate resources and coping strategies), and 67–100% for High resilience (abundant resources and effective coping strategies). These criteria stem from resilience literature analysis and proposed classification standards [1, 2, 9].

The scale utilizes a 5-point Likert scale, spanning from "Never" to "Always", where respondents indicate their level of agreement or frequency. Scoring is determined by summing up the responses across all items. According to predefined criteria, individuals are then categorized into specific resilience levels: Low resilience (scores from 30–90), Moderate resilience (scores from 91–120), and High resilience (scores from 121–150).

The research instrument underwent official approval from the Pharmaceutical Chamber of Serbia, enabling its development and testing. All participating pharmacists were thoroughly briefed on the study's details, assured of their anonymity, and given a full explanation once again. Notably, no financial compensation was provided to any participant. The approval for the study was granted by the Ethics Committee of the Pharmaceutical Chamber of Serbia, No. 316/2-6, from August 3, 2022.

Procedure and statistical analyses

Licensed pharmacists working in community pharmacies were invited *via* email by the Serbian Chamber of Pharmacy to complete questionnaires assessing socio-demographic data and psychological resilience. Initially, 504 pharmacists completed the resilience questionnaire. During this phase, participants were given the option to revisit the questionnaire after three months. Informed consent was obtained from 120 individuals who agreed to participate in the follow-up evaluation. After three months, 80 respondents completed the questionnaire, forming the basis for further statistical analysis.

The scale's reliability was assessed to gauge its resistance to random errors, focusing on temporal stability (test-retest reliability) and internal consistency. Temporal stability was evaluated by administering the scale twice to the same subjects: initially and after three months. With a sample size of 80 in the second phase, additional parameters related to the distribution's normality were also examined for the test-retest analysis. Since the sample size in the second phase was 80, and given that this was the basis for the test-retest analysis, additional parameters of normality of the distribution were examined [9].

Subsequently, the correlation of the obtained results was calculated. Wilcoxon rank test was utilized to compare the mean values of the attributes of the same group of participants, i.e., resilience scores at two time points (measured twice). Another analysis confirming the temporal stability of the results is demonstrated by Spearman's rank correlation coefficient. Spearman's coefficient confirms the temporal stability of the results. High correlations indicate consistent measurement results over time, validating the scale's reliability and stability across different time points [9].

Internal consistency of the scale was assessed to measure item similarity and interconnectedness. Cronbach's alpha coefficient, commonly used for this purpose, indicates the average correlation among all scale items. Ideally, Cronbach's alpha should exceed 0.70 [9]. Skewness and kurtosis calculations are recommended for samples with fewer than 200 cases analyzed [13].

Multiple regression analysis was used to examine the scale model, assessing the predictive power of each subscale and their contribution to the overall model [9]. This analysis determined how much variance in the RS score is explained by each subscale individually, evaluating both the overall model and each subscale's statistical significance.

Factor analysis, specifically principal component analysis, was used to determine the underlying factors of the RS's 30 items. Criteria included a Kaiser-Meyer-Olkin value exceeding 0.6 and statistical significance in Bartlett's Test of Sphericity [14, 15], confirming the factorability of the correlation matrix. Subscale correlations were also calculated to affirm internal consistency. Sample size determination used GPower [9, 14, 15] with $\alpha = 0.05$ and effect size 0.5. Power analysis ensured a minimum sample size of 80% power to detect significant effects. The effect size of 0.80 indicated a substantial impact, boosting result confidence. *Post hoc* analyses validated findings across subgroups, reinforcing conclusions. Statistical calculations were conducted using the SPSS software package, version 29.0.1.

Results

Based on literature data, 95 items were generated through brainstorming sessions and focus groups, including Confidence, Agility, Coping with stress, Interpersonal relationships, and Developmental thinking domains. During this process, 28 items were discarded. Based on qualitative feedback from expert panels, 16 items were reviewed, and 13 items were discarded. Subsequently, 54 items underwent content validity analysis. Among these, ten items had an I-CVI of less than 0.8 and were consequently discarded, leaving 44 items for FVA. Based on the FVA, 14 items were dis-

carded (I-FVI less than 0.8), resulting in the creation of a final scale comprising 30 items. The S-CVI score was 0.81. The S-FVI yielded a satisfactory score of 0.71, slightly below that of the basic set. Despite this, the scale featuring this item structure was retained, as items with both I-CVI and I-FVI scores below 0.80 were discarded.

A questionnaire was administered to a sample of 504 pharmacists to assess the factorial structure of the scale. Among these participants, 92.5% were female, with an age range from 24 to 79 years [mean age = 41.43 years, standard deviation (SD) = 10.55]. Additionally, 58.3% of participants held managerial positions, and 67.5% expressed job satisfaction.

To evaluate the temporal stability of the scale, the questionnaire was administered to a sample of 80 pharmacists at the beginning and after three months. While the questionnaire was initially distributed to all 504 pharmacists who completed it, only 80 pharmacists responded to the follow-up assessment, resulting in a response rate of 15.87%. Among these respondents, 87.5% were female, with an age range from 27 to 52 years (mean age = 37.56 years, SD = 7.41). Furthermore, 63.7% of respondents held managerial positions, and 62.5% reported job satisfaction.

In the first dataset, the majority (50.6%) of respondents exhibited a high level of resilience, while smaller proportions showed moderate (41.5%) and low (7.9%) resilience levels, based on a sample size of 504 individuals. In the second dataset, the majority (53.8%) of respondents also demonstrated a high level of resilience, with moderate (36.3%) and low (10.0%) resilience levels being less prevalent, based on a sample size of 80 individuals.

The scale showed high reliability (Cronbach's $\alpha = 0.945$, n = 504; Cronbach's $\alpha = 0.934$, n = 80) and strong correlations with subscales, highest with Coping with Stress ($r = 0.919$, $p < 0.01$) and lowest with Interpersonal Relationship ($r = 0.564$, $p < 0.01$). Given that the level of significance was significantly greater than 0.05 (sigma = 0.637), based on the Wilcoxon rank test, it can be concluded that there was no difference in resilience test results after three months (z = -4.71). Additionally, the median (Md) score had not changed after three months (Md = 121) (Table 1).

A priori power analysis for the Wilcoxon signed-rank test was performed based on predefined parameters, including an effect size of 0.80, a power of 0.80, and an alpha error probability of 0.05. The analysis indicated that a minimum of 28 participants *per* group was needed to power the study adequately. With 80 participants included, the sample size exceeded this minimum requirement. Furthermore, a *post hoc* analysis revealed an effective test power of 0.99, surpassing the initially targeted 0.80. This heightened power level enhances the reliability of the study's results, ensuring its capability to detect true effect sizes with high confidence. These findings confirm that the sample size not only met but also exceeded the requirements for achieving statistically significant and reliable results, thereby strengthening the robustness of the study's conclusions.

Spearman's rho correlation demonstrated strong positive associations between the Resilience Score test and Resilience Score retest (r = 0.998, $p < 0.001$), as well as between the Resilience Level test and Resilience Level retest (r = 0.981, $p < 0.001$). Additionally, significant correlations were found between the Resilience Score test and Resilience Level test (r = 0.893, $p < 0.001$), and between the Resilience Score retest and Resilience Level retest (r = 0.896, $p < 0.001$).

The tolerance values presented in Table 2 indicate the proportion of variance in RS that remained unexplained by the variances of the included subscales. With values exceed-

Table 1

Analysis of temporal stability of Pharmacist Resilience Scale

Parameter	25th percentile	50th percentile (median)	75th percentile		Z	Asymp. Sig. (2-tailed)
Resilience (n = 80) Score test	109.25	121.00	128.75	resilience score retest – resilience score test	-0.471	0.637
Resilience (n = 80) Score retest	109.00	121.00	128.00			

n – number; Asymp. Sig. – asymptotic significance.
Note: Z is the result at baseline and after three months based on Wilcoxon signed ranks test and negative ranks.

Table 2

Multicollinearity assessment

Model		Correlations			Collinearity Statistics	
		zero-order	partial	part	tolerance	VIF
1	(Constant)					
	Confidence subscale	0.894	1.000	0.122	0.289	3.462
	Agility subscale	0.893	1.000	0.119	0.287	3.490
	Coping with stress subscale	0.919	1.000	0.167	0.274	3.644
	Interpersonal relationships subscale	0.723	1.000	0.086	0.553	1.807
	Developmental thinking subscale	0.881	1.000	0.144	0.330	3.028

VIF – variance inflation factor.
Note: Constant refers to the intercept of the model, representing the predicted value of the dependent variable when all predictors are zero. Part indicates the unique contribution of each predictor to the dependent variable after accounting for other predictors.

ing 0.10, indicating a low likelihood of multicollinearity among the subscales, it suggests that each subscale contributed individually to elucidating the overall model variance. Additionally, the Variance Inflation Factor value, below 10, reinforces these findings further, assuring that no subscale needs to be excluded from the model. GPower indicated a minimum requirement of 100 participants at a power of 0.80, while *post hoc* analysis demonstrated that the sample of 504 participants was sufficient and relevant with a power of 0.96.

Reviewing the scree plot, a clear break point after the second component was identified. Based on the Kaiser criterion, it was decided to retain five components for further investigation, as the five-component solution explained a total of 58.0% of the variance (compared to 61.5% for the six-component solution), with the contributions of the first component at 40.2% and the fifth component at 3.5%. To facilitate the interpretation of these five components, an oblimin rotation was conducted. Table 3 shows the correlation coefficients between different components extracted from principal component analysis with an oblimin rotation.

Table 3

Component correlation matrix

Component	1	2	3	4	5
1	1.000	0.183	0.422	-0.397	0.540
2	0.183	1.000	0.198	-0.207	0.197
3	0.422	0.198	1.000	-0.359	0.331
4	-0.397	-0.207	-0.359	1.000	-0.362
5	0.540	0.197	0.331	-0.362	1.000

Extraction method: principal component analysis.
Rotation method: oblimin with Kaiser normalization.

The rotated solution revealed a simple structure, with each of the five components having high factor loadings and each variable loading significantly on only one component. These findings support the use of five separate subscales as proposed in the previous analysis. Each factor exhibited a few variables with high loadings (correlations), while the remaining variables tended to have loadings near zero. This pattern is characterized by a small number of substantial loadings and a larger number of negligible or small loadings across each factor. While the scale items were initially designed to cover four domains, the results of the factor analysis revealed the presence of five factors. Factor analysis groups similar items based on their correlations. Thus, despite the initial anticipation of four domains, the analysis identified five factors as the optimal representation of the data's variability. These factors genuinely reflect the patterns within the data, with redistribution among the four domains that the scale measures.

Discussion

Literature focusing on scale validation emphasizes the necessity of high correlations between subscales and the total scale, as well as temporal stability of scale results [9], both of which this research has confirmed. This study suggests that RS maintained temporal stability, affirming its consistent measurement of intended constructs without significant changes in results over time.

Given the inevitability of the need for resilience measurement scales that are both selective and reliable while also containing subscales that cover comprehensive domains, this study has confirmed the validity of one such scale with five dimensions.

Many studies highlight the importance of validated RSs in various cultural contexts, providing essential tools for assessing and understanding resilience across different populations and settings [8]. The studies discussed provide valuable insights into the development, validation, and application of RS across different populations and contexts. Each study contributes to the growing body of knowledge on resilience and underscores the importance of understanding and assessing resilience in diverse professional settings. The Rushton Moral RS (RMRS) was refined to create a more concise scale, improve reliability, especially of the personal integrity subscale, and provide further evidence of validity [16]. The results of the study provide valuable insights into the development and validation of RS tailored for pharmacists working in community pharmacies. Drawing on the framework outlined in the study, it is evident that the scale underwent rigorous validation processes to ensure its reliability and validity.

Healthcare professionals often confront moral dilemmas, leading to moral distress when their integrity is tested. Therefore, a reliable tool to measure moral resilience is essential. RS developed for pharmacists in this study meets this need. Similar scales, like the RMRS and RS by Wagnild and Young [17], have been widely validated in diverse populations, emphasizing the importance of robust measurement tools in resilience assessment. Focusing on responses to moral adversity, personal and relational integrity, and moral efficacy has shown high reliability and validity, similar to RS by Wagnild and Young [17], which has been validated across various populations [17, 18]. Both scales address domains that are also present in the PRS. The Dispositional RS (DRS-15) in the Korean context and the Portuguese version of RS have also demonstrated satisfactory validity and reliability with similar domains [19, 20]. These findings, alongside the development and validation of the PRS, underscore the growing recognition of resilience measurement's significance across different contexts and populations. Utilizing validated scales enables effective assessment and intervention to support healthcare professionals, including pharmacists, promoting their well-being and adaptability in demanding work environments.

Other studies offer valuable insights into RS development, validation, and application in diverse health contexts. Liang et al. [21] explored the RS's measurement invariance in cancer care between the Americans and the Chinese, emphasizing the necessity of considering cultural differences in resilience assessment. In a separate study, Mueller [22] developed and tested a 10-item RS tailored for university students, identifying key factors such as social support and positive attitude. Wongpakaran et al. [23] introduced a resilience inventory based on inner strength, incorporating Buddhist principles, demonstrating good validity and reliability for nonclinical

populations. These studies highlight the importance of context-specific resilience assessments, emphasizing their vital role in identifying protective factors and fostering positive outcomes across diverse settings. They demonstrate that a general RS cannot be effectively applied in specific contexts [22, 23], thus justifying the need for a specialized scale in the community pharmacy setting.

Several studies offer insights into resilience in extraordinary circumstances. A rapid review highlighted limited research on resilience and self-efficacy among healthcare professionals during the COVID-19 pandemic, stressing the need for further investigation [24]. The PRS includes questions in the domains of agility and stress coping, making it suitable for use in emergencies.

A study by Chinese university students found good criterion validity for the Brief RS (BRS) and Brief Resilient Coping Scale (BRCS), with the BRS showing superior internal consistency and construct validity [25]. Another study validated the Chinese version of RS among disaster-exposed adolescents, demonstrating a 3-factor structure with good reliability and validity [26]. Researchers developed and validated the Indonesian Academic RS, showing high validity and reliability among junior high school students [27]. Confirmation of the suitability of RS for Spanish nursing students was emphasized [28]. These studies affirm the importance of comprehensive, context-specific scales that encompass domains suitable for emergencies while also monitoring resilience in everyday circumstances. The PRS embodies these qualities. High levels of burnout and secondary traumatic stress among the United Kingdom doctors were found, underscoring the importance of understanding resilience in healthcare settings [29]. These studies contribute to our understanding of resilience's role in mental health and professional functioning, highlighting the need for further research in challenging circumstances.

Several studies have developed and validated RSs specifically tailored for health professionals. Rahman et al. [30] created the Medical Professionals RS (MeRS) for medical officers, showing good psychometric properties and addressing the need for customized resilience measures in healthcare. McCoy et al. [31] found comparable reliability and validity between RS and its shortened version (RS-13) in nurses, emphasizing the importance of adapting RS for professional groups like nurses. Galanis et al. [32] validated the brief CD-RISC-10 in Greek nurses, providing a reliable instrument for assessing resilience in Greek-speaking populations.

Another study developed and validated the Work RS – Chinese version (WRS-C), demonstrating its reliability, validity, and measurement invariance across demographic groups, contributing to understanding resilience in occupational settings [33]. Wollny and Jacobs [34] validated the German versions of the CD-RISC-10 and CD-RISC-2, supporting their use as measures of trait resilience in German-speaking populations. Cajada et al. [35] critically examined RS, suggesting the need for further examination of its theoretical framework. Nguyet Trang and Thang [36] developed and validated the Vietnam Teachers' RS (VITRS) for Vietnamese teachers, offering a culturally sensitive tool for assessing teacher resilience. These studies enhance resilience research by offering validated scales for specific professional groups and cultural contexts, highlighting the need for instruments that are context-specific and demonstrate high factor saturation and internal consistency. They confirm the necessity of stable factor structures that cover relevant domains, allowing for resilience measurement in specific settings while accounting for environmental changes. The PRS meets these criteria.

Limitations of the study

One limitation of the study was the reliance on self-report measures, which may introduce response bias or social desirability bias. Participants may provide answers they believe are socially acceptable rather than reflecting their true experiences or feelings, potentially impacting the accuracy of the results. Additionally, the study's sample may not fully represent the diversity of pharmacists, potentially limiting the generalizability of the findings to other pharmacist populations. Moreover, the study's focus on pharmacists may restrict the applicability of the RS to other healthcare professionals or broader populations, warranting caution in extrapolating the results beyond the specific target group. Furthermore, while efforts were made to ensure the scale's temporal stability, the three-month follow-up period may not capture longer-term resilience level fluctuations. Finally, as with any validation study, there may be inherent limitations in the chosen statistical methods or in the interpretation of the results, necessitating further research to corroborate the findings and address any potential methodological shortcomings.

Directions for future research

Future research directions should prioritize further validation of resilience assessment instruments specifically tailored for pharmacists, going beyond merely examining resilience levels. This entails conducting additional validation studies to assess the psychometric properties of RSs, such as reliability, validity, and factorial structure, within the pharmacist population. Moreover, future studies could explore the applicability of RSs across different practice settings within pharmacies, such as community pharmacies, hospital pharmacies, and specialty pharmacies. This would involve examining whether RSs demonstrate consistent psychometric properties and factor structures across diverse pharmacy practice environments. Additionally, research efforts could focus on investigating the criterion validity of RSs for pharmacists by examining their associations with relevant outcomes, such as job satisfaction, burnout, turnover intention, and quality of patient care. Establishing these associations would provide further evidence of the utility and relevance of RSs in predicting important outcomes in pharmacy practice. Furthermore, future research could explore the responsiveness of RSs to interventions aimed at enhancing pharmacist resilience. Intervention studies could assess changes in resilience levels following participation in resilience-building programs or interventions, providing insights into

the effectiveness of such interventions in improving pharmacist well-being and performance. Lastly, given the importance of cultural context in shaping resilience perceptions and practices, future research could investigate the cultural validity of RSs for pharmacists across different cultural contexts. This would involve conducting cross-cultural validation studies to ensure that RSs are valid and reliable measures of resilience in diverse cultural settings.

By focusing on the validation of resilience assessment instruments specifically tailored for pharmacists and considering the unique challenges and contexts of pharmacy practice, future research can provide valuable insights into pharmacist resilience and inform evidence-based interventions to support pharmacist well-being and professional practice.

Conclusion

This study confirmed the scale's robust psychometric properties through rigorous statistical analyses, including measures of reliability, internal consistency, and factorial structure. High Cronbach's α coefficients and significant correlations between subscales and the total scale underscored strong internal consistency. Despite deviations from normality, the RS demonstrated temporal stability over three months, further reinforcing its reliability. Multiple regression analysis and principal component analysis revealed the predictive power of each subscale and a clear factorial structure, respectively, supporting the use of five separate subscales. These findings highlight the scale's utility as a valuable tool for assessing resilience among pharmacists. Moreover, the findings of this study align with the broader literature emphasizing the importance of validated RSs in diverse cultural contexts. The studies discussed in this context offer valuable insights into the development, validation, and application of RSs across different populations and settings. Each study contributes to expanding our understanding of resilience and emphasizes the significance of assessing resilience in various professional contexts. Collectively, these endeavors provide essential tools for evaluating and comprehending resilience across different populations and settings, ultimately contributing to the promotion of well-being and adaptive functioning in diverse professional settings.

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Appendix 1

Details of literature review

Element	Description
Databases Used	PubMed®
	PsycINFO®
	Scopus
	Web of Science
Keywords	“resilience”
	“psychological resilience”
	“community pharmacists”
	“stress management”
	“coping strategies”
	“occupational stress”
	“workplace resilience”
Inclusion Criteria	Articles published in peer-reviewed journals
	Studies conducted within the last 10 years
	Research focused on psychological resilience in healthcare professionals, specifically pharmacists
	Studies available in English
	Full-text articles accessible through the databases
Exclusion Criteria	Articles not peer-reviewed
	Studies focused on resilience in non-healthcare professions or unrelated fields
	Publications older than 10 years, unless they were seminal works in the field
	Abstracts or summaries without access to the full-text

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The influence of autoclave sterilization on the cyclic fatigue of M-wire rotary endodontic instruments

Uticaj sterilizacije u autoklavu na pojavu cikličnog zamora rotirajućih M-*wire* endodontskih instrumenata

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Abstract

Background/Aim. The most important moment in modern endodontics is the inclusion of rotating instruments made of nickel-titanium alloy in daily clinical work, which have supplanted stainless steel instruments due to their superior properties. The aim of this study was to investigate the influence of autoclave sterilization on cyclic fatigue (CF) in two types of rotating instruments produced by M-wire technology with different types of rotation. **Methods.** This research included two types of M-wire rotary instruments – 48 ProTaper Next® instruments with full rotation and 48 WaveOne® Gold instruments with reciprocating rotation. Each of the two groups of instruments was divided into four additional groups of 12 instruments depending on the number of cycles of sterilization in the autoclave. The instruments were tested in an artificial canal with a 2 and 5 mm radius of curvature. **Results.** Statistically significantly higher resistance to CF was observed with WaveOne® Gold compared to ProTaper Next® ($p < 0.001$) instruments, both in the non-sterilized group and after their exposure to the first, third, and fifth cycle of sterilization. The third and fifth cycle of sterilization significantly reduced resistance to CF in the WaveOne® Gold ($p < 0.001$) group. There was no statistically significant difference in CF resistance between sterilized and non-sterilized instruments of the ProTaper Next® group. **Conclusion.** Sterilization in an autoclave for instruments based on M-wire technology did not increase resistance to CF.

Key words:
alloys; dental instruments; disinfection; endodontics; equipment failure.

Apstrakt

Uvod/Cilj. Najvažniji momenat u savremenoj endodonciji je uključivanje rotirajućih instrumenata izrađenih od legure nikl-titanijum u svakodnevni klinički rad, koji su zbog svojih superiornijih osobina potisnuli instrumente od nerđajućeg čelika. Cilj ovog rada bio je da se ispita uticaj procesa sterilizacije u autoklavu na pojavu cikličnog zamora (CZ) kod dve vrste rotirajućih instrumenata, izrađenih M-*wire* tehnologijom, sa različitim tipovima rotacije. **Metode.** U istraživanje su bila uključena dva tipa rotirajućih M-*wire* instrumenata – 48 ProTaper Next® instrumenata sa punom rotacijom i 48 WaveOne® Gold instrumenata sa recipročnom rotacijom. Svaka od dve grupe instrumenata podeljena je u još četiri dodatne grupe od po 12 instrumenata u zavisnosti od broja ciklusa sterilizacije u autoklavu. Instrumenti su testirani u arteficijelnom kanalu sa radijusom krivine od 2 i 5 mm. **Rezultati.** Statistički značajno veća otpornost na CZ uočena je kod WaveOne® Gold instrumenata u odnosu na ProTaper Next® ($p < 0,001$) instrumente, kako u grupi nesterilisanih tako i nakon njihovog izlaganja prvom, trećem i petom ciklusu sterilizacije. Treći i peti ciklus sterilizacija su značajno smanjili otpornost na pojavu CZ u WaveOne® Gold ($p < 0,001$) grupi. Nije postojala statistički značajna razlika u otpornosti na CZ kod sterilisanih i nesterilisanih instrumenata u grupi ProTaper Next®. **Zaključak.** Sterilizacija u autoklavu kod instrumenata baziranih na M-*wire* tehnologiji nije povećala otpornost na CZ.

Ključne reči:
legure; stomatološki instrumenti; dezinfekcija; endodoncija; oprema, malfunkcija.

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Introduction

The highest quality endodontic therapy, in addition to the experience and knowledge of the practitioner, requires the use of instruments that effectively remove the paracanal layer of dentin and work in highly curved canals. A pivotal moment in modern endodontics was the incorporation of rotating instruments made of nickel-titanium (Ni-Ti) alloy into everyday clinical practice, as they have displaced stainless steel instruments due to their superior properties. Different forms of Ni-Ti alloy possess diverse characteristics, leading to variations in the behavior of instruments during use. With the aim of improving the characteristics of Ni-Ti instruments, various technologies of their production have been developed. Innovative M-wire technology is used to create instruments with remarkably elastic qualities. This process entails applying many heat treatments to the Ni-Ti wire during manufacturing [1, 2].

Subjecting the alloy to thermal treatments and the resulting changes in the alloy structure, manifested in an increased martensitic content, could explain the enhanced resistance to cyclic fatigue (CF) in rotary instruments. This resilience is observed when comparing instruments with full rotation the ProTaper® group, with the highest resistance demonstrated by the ProTaper Next® instrument obtained through M-wire technology [3]. Additionally, the effective performance of reciprocating instruments produced with M-wire technology, including WaveOne® Gold, in removing the paracanal layer of dentin systems has been noted in numerous studies [4].

Regardless of the numerous sets of rotating Ni-Ti instruments produced through various technologies, complications arising from instrument fractures continue to pose a significant challenge during endodontic treatment. These complications impact the procedure's efficiency, prolong treatment times, and require additional efforts from the practitioner to overcome them. While working with instruments, the varied anatomical and morphological characteristics of root canals could lead to the appearance of CF, resulting in instrument fractures [5]. Although instruments are exposed to forces during operation that may cause breakage, processes such as instrument cleaning and sterilization should not be overlooked, as they can indirectly influence the appearance of CF [6].

Single use of Ni-Ti rotary instruments, especially single file systems, ensures better cutting efficiency and reduces the risk of sudden fracture, as instruments almost always have some defects after use, in the form of loss of sharpness of the cutting edges and initial pits and cracks. For economic reasons, which cannot justify discarding the instruments after a single use, many therapists resort to sterilizing these instruments in order to reuse them [7].

Sterilization is a common procedure in medical practice, considering that instruments undergo this process almost daily to prevent the spread of infections and ensure patient safety. Repeated sterilization processes involving extreme temperature variations can contribute to various changes in Ni-Ti alloy. For some types of instruments made from this alloy, heat-induced changes can increase resistance to CF, while for others, sterilization affects a decrease in resistance to CF [8]. By studying the role sterilization plays in the occurrence of CF in specific types of instruments, it is possible to find solutions that effectively overcome this challenge and enhance the overall standard of endodontic treatment.

The aim of this study was to investigate the influence of autoclave sterilization on the CF in two types of instruments produced by M-wire technology with different types of rotation.

Methods

The study included two types of instruments manufactured with M-wire technology: 48 M-wire instruments with full rotation ProTaper Next® (Dentsply Sirona, Ballaigues, Switzerland) (#25, 0.06 taper) and 48 M-wire instruments with reciprocating movements WaveOne® Gold (Dentsply Sirona, Ballaigues, Switzerland) (#25, 0.07 taper). Each type was divided into four groups as follows: group I – 12 instruments that were not sterilized; group II – 12 instruments that have been sterilized once; group III – 12 instruments that have been sterilized three times; group IV – 12 instruments that have been sterilized five times.

For research purposes, sterilization was done in an autoclave (Cliniclave 45M, MELAG, Berlin, Germany) at a temperature of 134 °C for 35 minutes. Immediately after that, the instruments were tested for CF. To remove impurities, the fragments were ultrasonically cleaned and then analyzed using a scanning electron microscope.

The CF test was performed in an artificial canal with a curvature angle of 45° and a radius of curvature of 2 and 5 mm. Instruments were placed on the endomotor (X-smart plus, Dentsply Sirona, Ballaigues, Switzerland) and then put into the artificial canal, where one type (ProTaper Next® – 300 rpm, 2.0 torque Ncm) continuously rotated to the right and the other type (WaveOne® Gold 500 rpm) rotated reciprocally with a certain program.

The number of cycles to failure (NCF) was calculated by multiplying the number of rotations (r) until failure by the time required for the appearance of a crack, expressed in seconds (s), (r×s), and the result obtained was divided by 60. The size of the fractured fragment (FF) of the instrument was measured using a double-legged caliper.

The statistical analysis was conducted using the Student's *t*-test for independent samples and One-Way ANOVA in IBM SPSS version 26.0, with a statistical significance level set at $p < 0.001$.

Results

After testing the NCF value of the first instrument group, which was not exposed to the sterilization process, in an artificial canal with a radius of 2 mm, ProTaper Next® (1,249.50 ± 75.36 r×s) showed a statistically significant ($p < 0.001$) lower resistance to CF compared to WaveOne® Gold (2,223.33 ± 84.36 r×s). After the first and third cycle of sterilization and testing of instruments in the canal under the same conditions, a statistically significantly higher ($p < 0.001$) value of NCF was observed with WaveOne®

Gold (2,192.50 ± 129.67 r×s, 1,498.50 ± 100.01 r×s, respectively) instruments compared to ProTaper Next® (1,242.83 ± 65.55 r×s, 1,223.50 ± 48.44 r×s, respectively) instruments. Testing of the instruments after the fifth cycle of sterilization showed no statistically significant difference between these two types of instruments, given that the NCF values for WaveOne® Gold were 1,342.67 ± 91.71 r×s and for ProTaper Next® were 1,214.50 ± 67.00 r×s ($p = 0.02$) (Table 1).

Testing of instruments in the artificial canal with a 5 mm radius of curvature showed a statistically significant difference in the NCF value between WaveOne® Gold (2,434.00 ± 77.08 r×s, 2,362.00 ± 81.32 r×s, 1,647.83 ± 124.44 r×s, 1,454.83 ± 35.13 r×s, respectively) and ProTaper Next® (1,278.50 ± 52.16 r×s, 1,265.17 ± 33.71 r×s, 1,260.83 ± 66.77 r×s, 1,201.00 ± 94.21 r×s, respectively) instruments in group which was not exposed to the sterilization process, as well as after the first, third, and fifth cycle of sterilization ($p < 0.001$) (Table 2).

The use of the ANOVA test revealed that the sterilization procedure did not significantly affect the reduction in resistance to CF in the ProTaper Next® group. However, it was observed that for WaveOne® Gold instruments, the third and fifth cycles of sterilization significantly reduced resistance on CF compared to the control group and instruments subjected to only one cycle of sterilization (Tables 1 and 2).

The mean value of fragment length after the CF test in a canal with a radius of curvature of 2 mm was statistically significantly higher with WaveOne® Gold compared to ProTaper Next® in all tested groups ($p < 0.001$). Likewise, the same significance was observed for the canal with a radius of 5 mm ($p < 0.001$) (Figure 1).

Table 1

Values of the number of cycles to failure (NCF) of the instruments in the canal with a 2 mm radius of curvature

Number of sterilizations	NCF (r×s)		Independent *t*-test
	ProTaper Next®	WaveOne® Gold	
0	1,249.50 ± 75.36	2,223.33 ± 84.36	$t = 21.09$, df = 10, $p < 0.001$
1	1,242.83 ± 65.55	2,192.50 ± 129.67	$t = 16.01$, df = 10, $p < 0.001$
3	1,223.50 ± 48.44	1,498.50 ± 100.01	$t = 6.06$, df = 10, $p < 0.00$
5	1,214.50 ± 67.00	1,342.67 ± 91.71	$t = 2.76$, df = 10, $p = 0.02$
ANOVA	F = 0.38, df = 3, $p = 0.77$	F = 119.49, df = 3, $p < 0.01$	/
Post hoc Tuckey test $p < 0.001$	/	0 vs.3, 0 vs. 5, 1 vs. 3, 1 vs. 5	/

r – number of rotations; s – seconds; ANOVA – analysis of variance; F – test statistic of ANOVA; df – degree of freedom. All values are shown as mean ± standard deviation.

Table 2

Values of the number of cycles to failure (NCF) of instruments in the canal with a 5 mm radius of curvature

Number of sterilizations	NCF (r×s)		Independent *t*-test
	ProTaper Next®	WaveOne® Gold	
0	1,278.50 ± 52.16	2,434.00 ± 77.08	$t = 30.41$, df = 10, $p < 0.001$
1	1,265.17 ± 33.71	2,362.00 ± 81.32	$t = 30.52$, df = 10, $p < 0.001$
3	1,260.83 ± 66.77	1,647.83 ± 124.44	$t = 6.71$, df = 10, $p < 0.001$
5	1,201.00 ± 94.21	1,454.83 ± 35.13	$t = 6.18$, df = 10, $p < 0.001$
ANOVA	F = 1.65, df = 3, $p = 0.21$	F = 201.71, df = 3, $p < 0.01$	/
Post hoc Tuckey test $p < 0.001$	/	0 vs. 3, 0 vs. 5, 1 vs. 3, 1 vs. 5	/

All values are shown as mean ± standard deviation. For abbreviations, see Table 1.

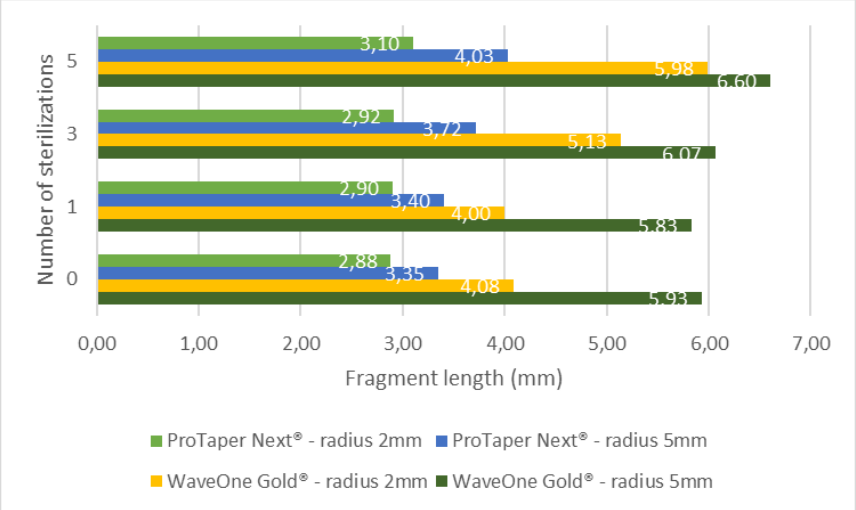

Fig. 1 – Mean values of fragment length after different sterilization cycles in canals with a 2 mm and 5 mm radius of curvature.

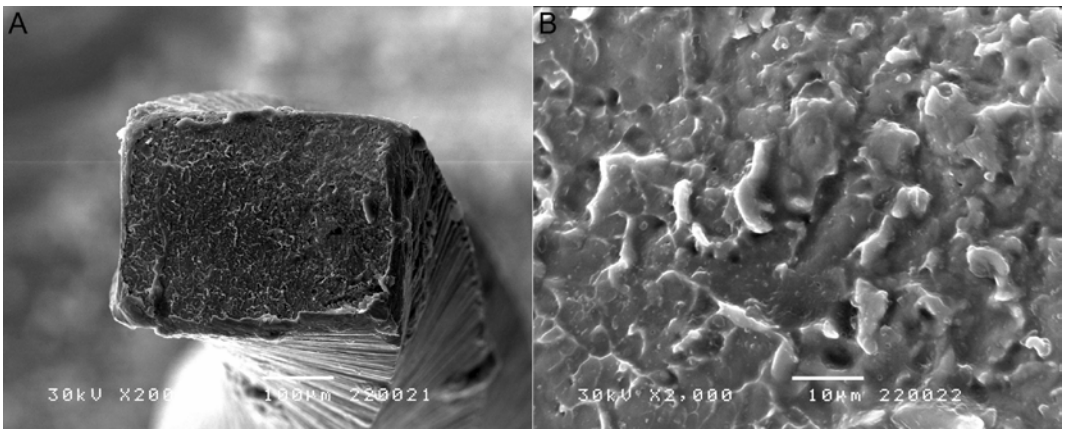

Fig. 2 – Micrograph of the cross-section of the ProTaper Next® fractured fragment after sterilization at A) ×200 and B) ×2,000 magnifications.

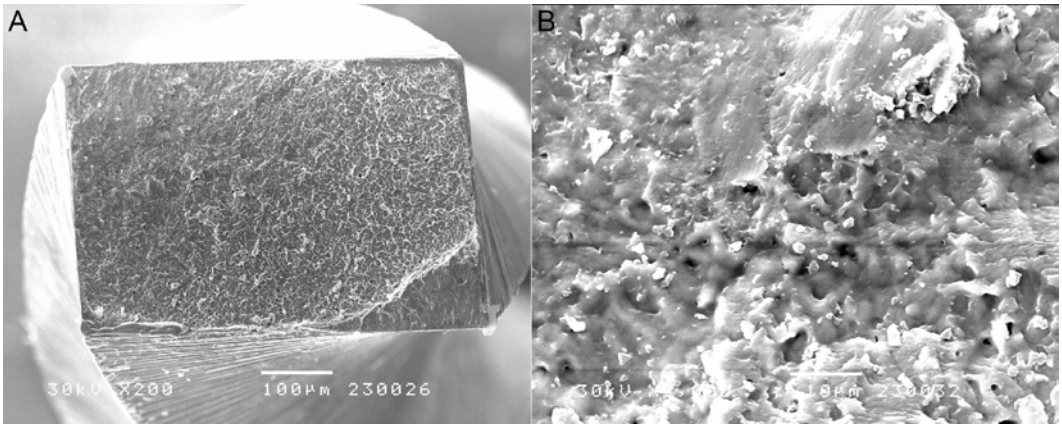

Fig. 3 – Micrograph of the cross-section of the WaveOne® Gold fractured fragment after sterilization at A) ×200 and B) ×2,000 magnifications.

Figure 2 represents the cross-section of the ProTaper Next® FF after autoclave sterilization, while the cross-section of the WaveOne® Gold FF is presented in Figure 3.

Discussion

The high operational costs associated with the use of not-so-affordable rotary endodontic instruments have influenced endodontic clinicians to subject these instruments to sterilization processes in order to prolong their functionality in clinical practice.

Numerous conducted studies have examined the impact of sterilization processes and their repetition on the appearance of microstructural defects on the working surface of instruments. These studies have determined that exposure to multiple sterilization cycles leads to an increased number of defects, consequently raising the risk of instrument fracture during clinical practice [9, 10]. However, there are also quite controversial findings that vary among different types of rotary instruments subjected to specific thermal processes during production [11]. Instruments made using M-wire technology are significantly more elastic and resistant to CF than conventional instruments due to Ni-Ti wire exposion to multiple heat treatments throughout the production process [3].

Nowadays, various tests are employed to assess the resistance of rotary instruments to CF to achieve safe clinical practices in endodontics. In this study, we utilized a static test known for its reliability as it provides valuable insights into the impact of design, taper, and manufacturing processes on CF [12]. Such instrument testing involves placing it in an artificial canal with a specific curvature angle, radius, and length and rotating it through either full rotations or reciprocal movements until the point of fracture.

The analysis of the results revealed a statistically significant difference in the resistance of WaveOne® Gold instruments before and after the third and fifth exposure to the sterilization cycle. With an increase in the number of sterilization cycles, Wave One® Gold instruments exhibited a consistent decline in NCF values. Contrary to these findings, the results of another study by De Ornelas Peraça et al. [13], who investigated the impact of sterilization on Reciproc Blue and WaveOne® Gold instruments, showed that the sterilization cycle did not affect the usage of WaveOne® Gold. Research conducted by Duque et al. [14] in 2020 investigated new and used instruments, including WaveOne® Gold, subjected to sterilization processes, followed by testing for CF. WaveOne® Gold exhibited a significant reduction in CF resistance after simulated clinical use and exposure to sterilization cycles. These findings align with the results of our study.

The obtained results have indicated that autoclave sterilization did not positively impact the CF resistance of the ProTaper Next® rotary instrument, and there was no statistically significant difference in CF resistance observed. The NCF values of ProTaper Next® consistently decreased both in the non-sterilized group and after the first, third, and fifth autoclave sterilization cycles. In contrast to our findings, the results of one study demonstrated an increase in the CF resistance of this instrument after the sterilization process [15]. In a recent study, ProTaper Next® exhibited variable NCF values with a slight increase after exposure to the first sterilization cycle, followed by a decline after exposure to the third and fifth sterilization cycles [16]. Additionally, according to findings from studies where instruments based on M-wire structure were examined, sterilization cycles did not affect fatigue resistance [17, 18].

Results from our study demonstrated a significant CF resistance of WaveOne® Gold compared to the ProTaper Next® instruments in all tested groups. The results of this study correlate with previously conducted studies [19–21]. WaveOne® Gold is a rotating system with reciprocal movements, and its unique parallelogram-shaped working part cross-section may help to explain these results [22]. Consequently, this type of rotational system exhibits different stress distribution behavior and, therefore, fracture patterns. The parallelogram-shaped cross-section of the WaveOne® Gold instrument establishes contact at a single point in the root canal, contributing to its superior resistance to CF compared to ProTaper® [19, 21]. In contrast to WaveOne® Gold, ProTaper Next® is characterized by full rotation movements and a rectangular cross-section that achieves contact at two points [20]. It is also worth noting that both WaveOne® Gold and ProTaper Next® have M-wire as their main structure. Still, WaveOne® Gold is made from a gold alloy as a result of consistent high-temperature heating and gradual cooling after the production process [23]. Therefore, it can be stated that this is one of the main reasons contributing to the higher resistance to CF in WaveOne® Gold compared to ProTaper Next®.

Hanbazaza and Abuhaimed [24], in their assessment of CF resistance for WaveOne® Gold and ProTaper® Gold instruments, employed various rotational directions and concluded that reciprocal movements enhance fatigue resistance for both instrument types. The study noted that WaveOne® Gold exhibited the highest resistance to CF when using movements of 150° counterclockwise and 30° clockwise. Furthermore, it was observed in the mentioned research that different directions of movement did not influence the length of FF. Testing instruments for CF in artificial canals at temperatures of 20° C and 37° C revealed that temperature does not play a role in increasing CF for instruments manufactured using M-wire technology. This is because the temperature used during the production of these instruments is much higher than body temperature [25].

The limitation of this study is reflected in the fact that it was conducted on a static model, which does not replicate a clinical setting. During work in root canals, the instruments are exposed to CF and also to torsional stress, which are the main causes of the file breakage [26]. However, torsional stress was not the subject of this study.

Conclusion

We may conclude from the acquired data that the resistance to cyclic fatigue cannot be increased by autoclave sterilizing rotary endodontic instruments produced by M-wire technology. While autoclave sterilization had a minor impact on ProTaper Next® decline in NCF values, it greatly decreased WaveOne® Gold resilience to cyclic fatigue. Furthermore, the instrument resistance to cyclic fatigue was higher in instruments tested in a larger radius of curvature.

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CASE REPORT

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Acrometastasis as a first sign of lung cancer

Akrometastaza kao prvi znak karcinoma pluća

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Abstract

Introduction. Bone metastases occurring distally to the elbow or knee joint are called acrometastases. Acrometastases make up only 0.1% of all bone metastases, but only 0.007% to 0.3% occur in the bones of the foot or hand. In 10% of patients, bone metastases occur as the first sign of a previously undiagnosed primary tumor. **Case report.** A 64-year-old male reported to the hospital due to painful swelling and redness of the fifth finger of the dominant hand. Osteolysis of the proximal phalanx of the fifth finger was diagnosed radiographically. A working diagnosis of osteomyelitis and phlegmon of the proximal phalanx was made. After incisional drainage, a tumor mass was observed intraoperatively. Amputation of the finger was performed at the level of the metacarpophalangeal joint. Pathohistological analysis revealed squamous cell carcinoma metastasis. After a computed tomography scan and bronchoscopy with biopsy, a diagnosis of squamous cell carcinoma of the bronchus was made. The patient was given chemotherapy. During the application of the second cycle, there was a fatal outcome. **Conclusion.** Pathohistological verification and determination of the location of the primary tumor are important when acrometastasis is found because starting novel immunotherapy and targeted therapy in a timely manner could change the median survival of these patients.

Key words:
amputation, surgical; carcinoma, squamous cell; diagnosis; drug therapy; finger phalanges; lung neoplasms; neoplasm metastasis; treatment outcome.

Apstrakt

Uvod. Metastaze u kostima koje se javljaju distalno od zgloba lakta ili kolena nazivaju se akrometastaze. Akrometastaze čine samo 0,1% svih metastaza kostiju, ali samo 0,007% do 0,3% njih javlja se u kostima stopala ili šake. Kod 10% bolesnika, metastaze kostiju javljaju se kao prvi znak prethodno nedijagnostikovanog primarnog tumora. **Prikaz bolesnika.** Muškarac star 64 godina javio se u bolnicu zbog bolnog oticanja i crvenila petog prsta dominantne šake. Radiografski je dijagnostikovana osteoliza proksimalne falange petog prsta šake. Postavljena je radna dijagnoza osteomijelitisa i flegmone proksimalne falange. Nakon izvršene incizione drenaže, intraoperativno je primećena tumorska masa. Izvršena je amputacija prsta na nivou metakarpofalangealnog zgloba. Patohistološkom analizom utvrđena je metastaza skvamocelularnog karcinoma. Posle kompjuterizovane tomografije pluća i bronhoskopije sa biopsijom, postavljena je dijagnoza skvamocelularnog karcinoma bronha. Bolesnik je započeo primanje hemioterapije. Tokom primene drugog ciklusa, došlo je do fatalnog ishoda. **Zaključak.** Kada se akrometastaza dijagnostikuje, potrebno je izvršiti patohistološku potvrdu i utvrditi lokalizaciju primarnog tumora, jer pravovremeno započinjanje inovativne imunoterapije i ciljane terapije može promeniti medijanu preživaljvanja tih bolesnika.

Ključne reči:
amputacija; karcinom, planocelularni; dijagnoza; lečenje lekovima; prsti, falange; pluća, neoplazme; neoplazme, metastaze; lečenje, ishod.

Introduction

Metastases in bones are common in oncological patients [1]. If they occur distally to the elbow or knee joint, they are called acrometastases [2]. Acrometastases account for only 0.1% of all bone metastases, but only 0.007 to 0.3% occur in the bones of the feet or hands [3, 4]. Bone metastases are most often detected during the treatment of the primary tumor, in certain cases synchronously with the detection of the primary tumor. In 10% of patients, they appear as the first sign of a previously undiagnosed primary tumor [5]. When the primary tumor is detected by the appearance of

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acrometastasis as the first sign, the prognosis is poor, and the median survival of these patients is about six months after diagnosis. The most common primary tumor locations that develop acrometastases are lung cancer, the gastrointestinal tract, and the genitourinary system [6].

Case report

A 64-year-old male presented to the emergency department with complaints of pain, swelling, and redness in the area of the fifth finger of the dominant hand, which started seven days earlier (Figure 1). He was previously examined by a vascular surgeon who, through ultrasonographic examination, diagnosed a hypoechoic homogeneous formation in the area of the proximal interphalangeal joint of the fifth finger. The circulatory status of the hand was normal. Upon admission to the emergency department, a blood laboratory analysis was performed, showing a slight increase in acute phase reactants and moderate anemia. The relevant laboratory results obtained at the time of admission are shown in Table 1. An X-ray of the hand was taken, which showed osteolysis of the proximal phalanx, proximal interphalangeal joint, and part of the proximal edge of the middle phalanx of the fifth finger (Figure 2). A working diagnosis of osteomyelitis of the proximal phalanx of the fifth finger with consecutive finger phlegmon was made. Incisional drainage was indicated, which was performed under total intravenous anesthesia. Intraoperatively, a tumor mass of hyalinized appearance, irregular shape, and soft consistency was observed, filling the subcutaneous tissue at the site of the proximal phalanx (Figure 3). Given the complete destruction of the proximal phalanx, proximal interphalangeal joint, and part of the middle phalanx, amputation of the finger at the level of the metacarpophalangeal joint was performed (Figure 4).

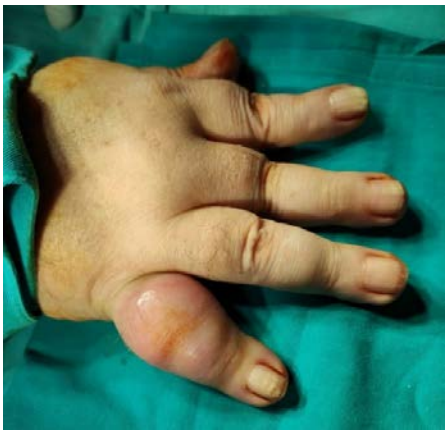

Fig. 1 – Preoperative appearance of the hand.

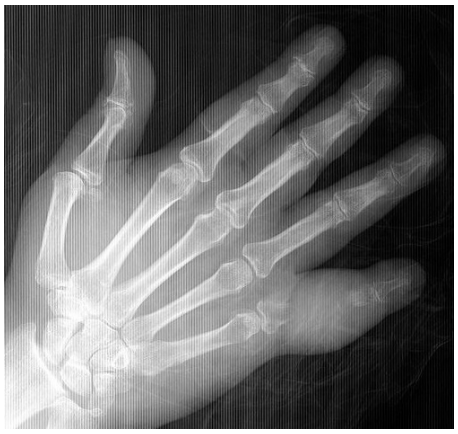

Fig. 2 – Preoperative X-ray of the hand.

Table 1

Laboratory results obtained at the time of admission

Parameter	Value	Reference range
WBC, $\times 10^9$/L	13.5	4.00–10.00
CRP, mg/L	60.2	< 5.0
[1]Fibrinogen, g/L	4.10	1.86–4.86
RBC, $\times 10^{12}$/L	3.2	4.20–6.00
Hb, g/L	90	130–170
HCT, L/L	0.351	0.400–0.540

WBC – white blood cells; CRP – C-reactive protein; RBC – red blood cells; Hb – hemoglobin; HCT – hematocrit.

[1] Using Clauss fibrinogen assay as a measure of function.

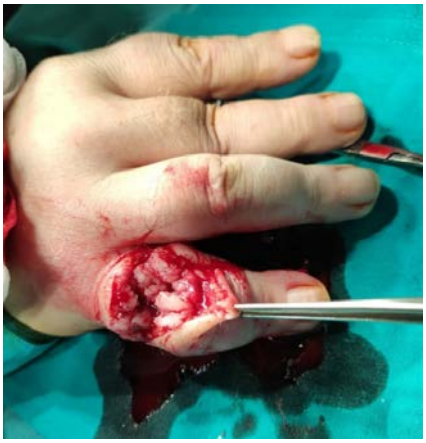

Fig. 3 – Intraoperative finding of the tumor mass.

Fig. 4 – Postoperative appearance of the hand.

The postoperative course was uneventful with primary wound healing. Pathohistological verification diagnosed *carcinoma planocellulare keratodes infiltrativum cutis et texti adiposi et ossis* (HG1) (Figures 5 and 6). Immunohistochemical testing registered 5% of PD-L1 positive tumor cells. After a computed tomography scan of the chest, a tumor change in the upper lobe of the right lung infiltrating the principal bronchus was diagnosed (Figures 7–9). Bronchoscopy was performed to biopsy the tumor change, and after pathohistological analysis of the biopsy, a diagnosis of squamous cell carcinoma of the bronchus was made. Skeletal scintigraphy did not detect other secondary bone deposits. The patient was started on chemotherapy with gemcitabine and cisplatin 20 days after the primary intervention on the hand. During the second cycle of treatment, two months after the diagnosis, the patient died due to massive hemoptysis.

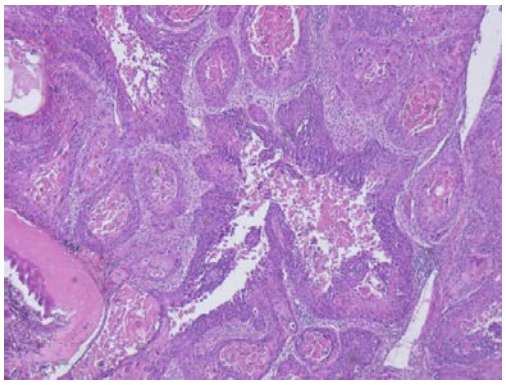

Fig. 5 – Metastasis in the soft tissue of the finger, hematoxylin and eosin staining (×5).

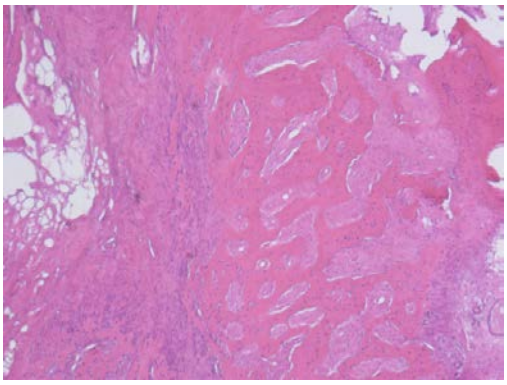

Fig. 6 – Bone metastasis, hematoxylin and eosin staining (×5).

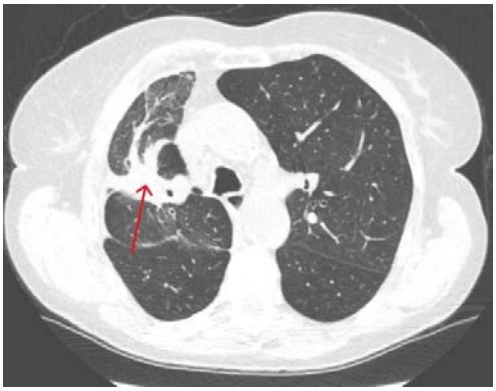

Fig. 7 – Transversal plane of the chest, computed tomography scan: the arrow indicates a tumor lesion in the upper right lobe.

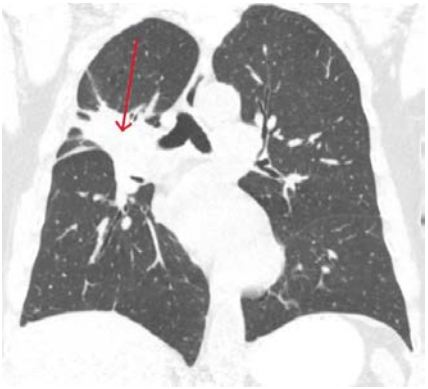

Fig. 8 – Coronal plane of the chest, computed tomography scan: the arrow indicates a tumor lesion in the upper right lobe with infiltration and compression of the main bronchus.

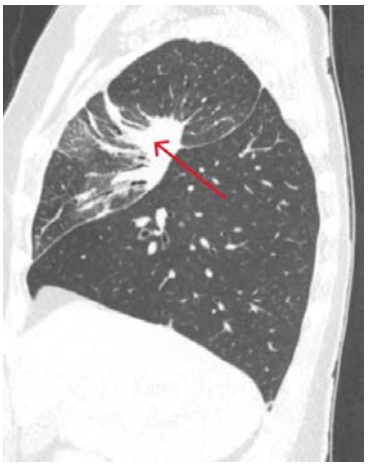

Fig. 9 – Sagittal plane of the chest, computed tomography scan: the arrow indicates a tumor lesion in the upper right lobe with infiltration and compression of the main bronchus.

Discussion

Bone metastases are characterized by tropism for bone marrow, which is primarily found in the vertebral bodies. The bones of the hand have a small content of bone marrow, making them a rare site for tumor metastasis. There are several theories about the pathways of metastasis, but the most accepted theory for the development of acrometastases is hematogenous dissemination. Among the mentioned primary tumor localizations that can develop acrometastases, lung tumors are the most common source. This is explained by the facilitated transport of metastatic tumor cells bypassing hepatic and pulmonary circulation [7]. Several authors have proposed the theory that acrometastases more frequently occur in the dominant hand, which was also the case in the presented patient. These theories have been explained by the increased blood flow to the dominant hand due to more intensive use compared to the non-dominant hand. More frequent use of the dominant hand causes repetitive microtrauma of soft tissue, which becomes less resistant to tumor emboli that are easily retained in the skeletal musculature and continue their growth. At the same time, such microtraumas induce the production of prostaglandins, which, as chemotactic factors, promote the migration and adherence of tumor cells [8, 9]. The clinical presentation of acrometastases most often occurs in the form of a painful, erythematous swelling that reduces the range of motion of adjacent joints. Differential diagnosis must consider the appearance of primary skin and soft tissue tumors, soft tissue infections, tenosynovitis, osteomyelitis, pyogenic granuloma, gout, inclusion cyst, and ganglion cyst [10]. Despite the lack of a treatment protocol for acrometastases, most authors agree that amputating the affected finger is the method of choice [7]. In the presented patient, ablative surgery resulted in the eradication of the developed severe phlegmon of the finger without affecting the already impaired function of the hand as a consequence of osteolytic destruction of the proximal phalanx and proximal interphalangeal joint.

Conclusion

Even though acrometastases are traditionally considered a rare entity, we believe they will be diagnosed more frequently in the future due to improved diagnostic and treatment protocols for oncology patients. In cases of suspected acrometastases, it is necessary to conduct adequate pathohistological and immunohistochemical detection analyses after surgical treatment. Additional radiological methods can determine the site of the primary tumor. The development of innovative immunotherapy and targeted therapy may enable longer survival and better disease control.

Conflict of interest

The authors declare no conflict of interest.

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The evolution of periodontology in the 19th and 20th century

Razvoj parodontologije u 19. i 20. veku

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Key words:
history of dentistry; history, 19th century; history, 20th century; periodontal diseases.

Ključne reči:
istorija stomatologije; istorija, 19. vek; istorija, 20. vek; periodontalne bolesti.

Introduction

Periodontology in the 19th century

The 19th century was the time when possible etiology and, therefore, prevention and treatment of periodontal disease have been proposed. Furthermore, the gained knowledge was transferred to clinical practice through the development of techniques and instruments for curettage, debridement, and scaling. This was the time when periodontal surgery started.

Leonard Koecker (1785–1850) was a dentist originally from Germany who later practiced in Baltimore (Figure 1) and emphasized the importance of dental health in his work [1]. In a paper published in the "Philadelphia Journal of Medicine and Physical Sciences" in 1821, he highlighted the inflammatory changes in the gums and the presence of tartar on teeth, which could lead to tooth loss. Koecker believed that poor dental health was linked to various disorders. His influential book *Principles of Dental Surgery*, released in 1826, advocated for the use of astringent powder and a horsehair toothbrush and a toothbrush placing bristles at the spaces of the teeth after each meal to maintain oral hygiene. He also supported the odontogenic focal infection theory early on, recommending the extraction of severely affected teeth and roots to prevent systemic infections [2]. Even though Koecker advocated for adequate oral hygiene practice, Levi Spear Parmly (1790–1859) was considered the father of oral hygiene and the inventor of the oral floss [3].

Fig. 1 – Leonard Koecker [1].

John Mankey Riggs (1811–1885) was the leading figure in the study of periodontal disease, and at that time, periodontal disease was commonly known as "Riggs' disease". Born in Seymour, Connecticut, Riggs was often credited as the first to practice exclusively in periodontics,

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thus marking him as the pioneer and the first specialist in this field (Figure 2) [4]. Riggs believed that periodontitis was an inflammatory response of gingiva to present subgingival calculus that caused necrosis and resorption of alveolar bone, eventually leading to pocket formation, increased tooth mobility, and loss. Riggs' publications strongly advocate for what is known as the conservative approach to periodontal therapy, which prioritizes oral prophylaxis and prevention [5]. Riggs emphasized the importance of maintaining oral cleanliness and opposed surgical interventions such as gingival resections, which were prevalent during his time [5, 6]. Riggs designed a series of six hand instruments that were not sophisticated and suitable for fine scaling. At a meeting of the Connecticut Valley Dental Association in 1867, Riggs delivered a presentation deemed fundamental in educating participants about his understanding of periodontal disease and the treatment methods he advocated for. His work was followed by L. Taylor, D. D. Smith, R. B. Adair, and W. J. Younger [2]. Riggs passed away in 1885 after a short respiratory illness [6].

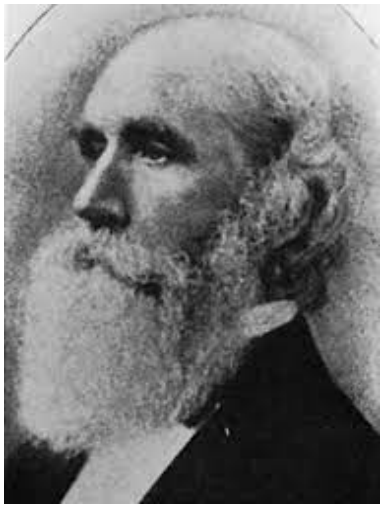

Fig. 2 – John M. Riggs [4].

William John Younger (1838–1920) proposed the concept of "dento-gingival reattachment", the development of granulation tissue following surgery. He also designed the scaling instruments that have served as a basis for modern instruments still in use today [2].

Greene Vardiman Black (1863–1915) was born in Winchester, Illinois (Figure 3) [7]. Even though he was an American pathologist, he made significant contributions to restorative dentistry. These contributions included the concept of polymicrobial nature and pathogenesis of dental caries, the design of cavity preparations, the development of alloys, and the invention of a foot-powered dental handpiece. However, his work had significant importance in the area of periodontology, too. His book *Special Dental Pathology* outlined a practical approach to diagnosing and treating periodontal disease with a particular focus on the detection of the early signs of periodontal disease. Black is often called the father of modern dentistry, sharing this title with Fauchard [2].

During the 19th century, many dental practitioners had limited formal training and lacked a comprehensive understanding of physiology. Dental clinicians, like Koecker [1], Riggs [4], and Black [7], believed that dental calculus played an important role in the development of inflammation and, consequently, periodontal disease. However, at that time, there was no universal consensus regarding this matter. Due to the lack of a precise understanding of the etiology of periodontal disease, therapy approaches ranged from teeth deposit removal, gingival massage, and dietary changes to teeth extraction.

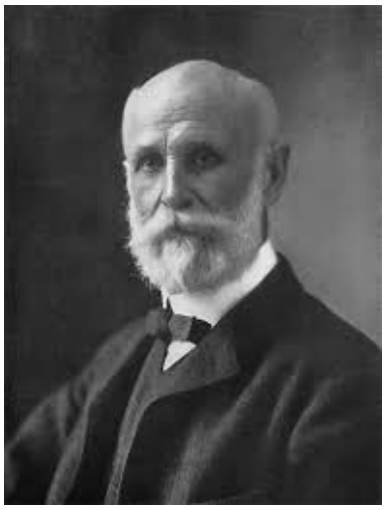

Fig. 3 – Greene V. Black [7].

Adolf Witzel (1847–1906) was the first to identify periodontal bacteria, but the pioneer of oral microbiology was Willoughby Dayton Miller (1853–1907) from Alexandria, Ohio. He practiced dentistry in Berlin, Germany, and collaborated in the laboratory of Robert Koch. Robert Koch was a follower of the theory of bacterial etiology of many diseases, such as pneumonia, cholera, puerperal fever, diphtheria, meningitis, plague, dysentery, and syphilis [2]. Miller transferred the knowledge he gained from Koch and his collaborators in the area of dentistry, and he was soon named the "father of dental prevention". He described the features of periodontal disease and their contribution to the disease development in his work titled *The Microorganisms of the Human Mouth*, in 1890, Miller outlined the features of periodontal disease and proposed the "non-specific plaque hypothesis". He believed that periodontal disease was not caused by one, but many bacterial species present normally in the oral cavity. Miller was among the first to suggest that antiseptics applied topically or *via* mouth rinsing might be useful in the treatment of periodontal diseases [5]. Although Miller did not identify and differentiate oral plaque [2], James Leon Williams (1852–1932) later did. Williams was the founder of the plaque hypothesis and described dental plaque as a "gelatinous accumulation of bacteria adhering to the enamel surface" [2]. However, he did not give evidence linking bacterial plaque to periodontal disease, but he laid the groundwork for this theory, which became mainstream 75 years later [5].

Joseph Lister (1827–1912) of England transferred the knowledge about microorganisms to the clinical and surgical practice and started the era of antiseptics (and later asepsis) in surgery [8].

French physician Jean Hyacinthe Vincent (1862–1950) identified the spirochete (*Borrelia vincentii*) and fusiform bacilli (*Bacillus fusiformis*) that are now associated with Vincent's angina [2, 5, 6]. In 1904, Vincent observed these microorganisms in cases of acute infection of the oral soft tissues, including the tonsils and pharynx, which he termed ulceronecrotic gingivitis [6].

Moritz Károlyi (1865–1945) published an original idea attributing a possible role of dental occlusion and trauma from occlusion in the etiopathogenesis of periodontal diseases. In the second half of the 19th century, there were several very important findings, which are described in the text that follows [5].

Invention of anesthesia

Horace Wells (1815–1848) was the first to use nitrous oxide anesthesia in 1844, and that was the first application of anesthesia to surgery. William Green Morton (1819–1868) used a combination of nitrous oxide and ether in 1846 in Boston. In 1905, Munich chemists Alfred Einhorn and Richard Willstätter introduced procaine [2, 5]. The combination of already-known local anesthesia with epinephrine (adrenaline), what quickly became a golden standard in local anesthesia, was introduced by Thomas Bell Aldrich (1861–1938) and Jōkichi Takamine (1854–1922) [9].

Invention of X-ray method

The discovery of radiographs was credited to the German physicist Wilhelm Conrad Röntgen, who lived from 1845 to 1923 (Figure 4) [10]. Charles Edmund Kells Jr. (1856–1928) was the first to demonstrate the use of Röntgen X-rays in dentistry in 1896 and the first to use dental X-ray on patients. The use of dental X-rays eventually led to the amputation of Kells' left arm. He is known as a person who introduced an era of accurate diagnosis of dental pathology. This invention, along with the discovery of anesthetics, dramatically changed the history of dentistry [2].

Fig. 4 – Wilhelm Conrad Röentgen [10].

Establishment of the first dental school

In 1840, Horace H. Hayden and Chapin A. Harris founded the first dental school named Baltimore College of Dental Surgery opened in Baltimore, Maryland [11].

Periodontology in the 20th century

Etiology of periodontal disease

In the 20th century, periodontics thrived in Europe, particularly in two leading centers: Vienna and Berlin. In Vienna, Bernhard Gottlieb (1885–1950) conducted extensive microscopic studies on human periodontal disease. He emphasized that merely removing calculus and other deposits was not enough; it was also crucial to eliminate the periodontal pocket. Gottlieb's scientific work sought to explain the biology of cementum, the tooth-eruption process, gingival epithelium attachment to the tooth, and traumatic occlusion. Balint J. Orban (1899–1960), a younger colleague of Gottlieb, continued his work and expanded his knowledge through detailed histological studies. In Berlin, prominent figures in periodontology included Oskar Weski (1879–1952) and Robert Neumann (1882–1958). Weski introduced the concept of the periodontium and its components: cementum, gingiva, alveolar bone, and the periodontal ligament. He also conducted pioneering studies on the relationship between histopathological changes in periodontal disease and radiographic images [12].

From the 1950s onward, the United States and Scandinavia played a leading role in both basic and clinical periodontal research, achieving significant progress in experimental pathology, microbiology, and immunology. Irving Glickman (1914–1972) emerged as a leading researcher in the United States during this period, while Jens Waerhaug (1907–1980) of Oslo, Norway, was a key figure in Scandinavia. His dissertation, *The gingival pocket; anatomy, pathology, deepening and elimination*, published in 1952, marked the beginning of a new era in understanding the biology of the periodontium [13-15]. The researchers at that time knew that the cause of periodontal disease was chronic inflammation, probably on the clinical and histologic levels. However, there was insufficient data for understanding inflammation or inflammation-resolving mechanisms to explain the etiology of periodontal disease [15].

The first experimental gingivitis studies conducted by Harald Löe (1926–2008) and his Scandinavian colleagues in the mid-1960s brought about a significant paradigm shift in the understanding of periodontal disease among both scientists and clinicians. Their research uncovered a robust correlation between the accumulation of dental plaque and the onset of periodontal disease in dogs. Furthermore, their findings illustrated that maintaining a daily tooth-brushing regimen was closely linked with the presence of clinically healthy periodontal tissues. As a consequence, teeth that

were not cleaned regularly developed gingival inflammation and, ultimately, periodontal disease. Subsequent research by other investigators further validated these results, indicating that experimental gingivitis in the majority of dogs progressed to periodontitis within four years if dental plaque was not removed on a daily basis [13-15].

Inspired by these findings, many clinical studies regarding the etiology and microbiology of periodontal disease were conducted [13]. Among many findings, the most significant ones were related to the presence of aerobic and anaerobic bacteria as causative agents of periodontal infection. It became clear that bacteria such as *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*, *Campylobacter rectus*, *Streptococcus intermedius*, and *Prevotella intermedia* were key members of the consortium of microorganisms responsible for periodontal disease. By the late 1990s, emerging evidence indicated that the intensity of inflammation and susceptibility to periodontal damage resulting from microbial challenges were influenced by the host response, which encompassed genetic polymorphisms [12, 16].

Treatment of periodontal disease

At the beginning of the 20th century, two major approaches to treating periodontal disease emerged. One approach continued to follow the beliefs of Riggs and William J. Younger that periodontal disease is caused by local irritation from dental calculus [12, 16]. They advocated for rigorous oral hygiene measures and were against gingival resection as a surgical approach. They believed that surgical intervention should be the last choice used in advanced cases only. The second approach defined the use of surgical resection of periodontal pockets followed by curettage of the underlying bone. The main goal of mechanical periodontal therapy was to efficiently clean the root surface. That concept was generally accepted. Later, mechanical periodontal therapy was enhanced by the application of antiseptics and antimicrobials as adjuncts, as they were found to be insufficient in eliminating periopathogens when used alone. At the same time, many microbiologists raised concerns about the possibility of microbial resistance to antibiotics, allergic reactions, and gastrointestinal disturbances. Consequently, topically applied antiseptics, particularly chlorhexidine, deserved their position as highly effective in the treatment and prevention of gingivitis [16].

As the field of periodontology advanced, periodontists began to utilize periodontal flap procedures, either alone or in conjunction with the insertion of bone-replacement graft materials, to address periodontal issues [12, 16]. Robert Neumann from Berlin and Leonard Widman (1871–1956) from Sweden first described flap surgery in detail, including osseous recontouring. A significant development in periodontal regeneration occurred with the introduction of guided tissue regeneration (GTR) procedures introduced in 1982. Sture Nyman and his colleagues were pioneers in a technique that involved placing a barrier membrane between the periodontal flap and a tooth slated for extraction in a patient with severe periodontitis. This method effectively excluded the gingival epithelium and connective tissue from the osseous defect, enabling pluripotent cells from the periodontal ligament to populate the wound. Histological studies showed the formation of new cementum, bone, and a functional periodontal ligament as a result of this approach [16].

Over the past two decades, researchers have refined the basic GTR approach by incorporating growth and differentiation factors, demonstrating an enhanced regenerative potential for periodontal tissues [12, 16]. Moreover, during the past two decades, researchers refined the basic GTR approach using growth and differentiation factors demonstrating enhanced regenerative potential of the periodontal tissues. While GTR has shown promise in regenerating the periodontium, achieving a completely normal periodontal status has not yet been achieved [16, 17].

Establishment of periodontal organizations

In 1914, in Cleveland, America, doctors Gillette Hayden (1880–1929) and Grace Roger Spalding (1881–1953) spearheaded the establishment of the first national organization, the American Academy of Oral Prophylaxis and Periodontology, which was later, in 1919, renamed the American Academy of Periodontology (AAP). The AAP focused on patient care, particularly the diagnosis and treatment of periodontal disease, while also promoting research and scientific development [5]. In 1930, the AAP launched "The Journal of Periodontology", and subsequently, several other journals were introduced, including "The Journal of Periodontal Research" (1966), "The Journal of Clinical Periodontology" (1974), "International Journal of Periodontics and Restorative Dentistry" (1981), and "Periodontology 2000" (1993). In Europe, in 1991, the European Federation of Periodontology (EFP) was founded. These organizations serve as a vital link between clinical and research centers with dental schools and private practitioners [14].

During the 1920s, the first peer-reviewed scientific journal dedicated to periodontal disease was initiated. Shortly after its inception, the journal began publishing randomized clinical trials to promote an evidence-based approach to clinical practice, thanks to the efforts of Sigurd P. Ramfjord and his team. Inspired by their work, many other scientists throughout the world conducted their own clinical investigations in an attempt to replicate their results [14, 15].

In 1947, periodontics was recognized as a specialty of dentistry by the American Dental Association [11].

Effects of periodontal disease on general health

In 1828, Riggs recognized the role of oral sepsis in rheumatic and other diseases. Later on, researchers revisited the idea that untreated periodontal infections might negatively impact overall health, potentially leading to conditions such as diabetes mellitus, myocardial infarction, stroke, and adverse pregnancy outcomes. Additionally, there

is growing evidence suggesting that other diseases may also contribute to the development of periodontal disease (bidirectional relationship). However, as of now, the precise relationship between periodontal disease and general health, the underlying risk factors, and the mechanisms involved remain unclear [16].

Implantology

In 1913, Edwin J. Greenfield, an American dentist, demonstrated the inaugural modern and effective dental implant, which laid the foundation for the flourishing implant dentistry we have today. The introduction of the first commercially viable water-driven turbine drill in 1953, pioneered by American dentist Robert Nelson, marked the beginning of the high-speed dental drill era. The Borden air-turbine drill, introduced in 1957 and now universally utilized by dentists, has significantly enhanced patient comfort and control during dental procedures [8, 12]. Per-Ingvar Brånemark (1929–2014) was a Swedish physician and researcher known as the "father of modern dental implantology" [12,16].

Conclusion

Clearly, we have learned a lot about periodontal disease and therapy over the past years. The major breakthroughs were the understanding of periodontal inflammation, fundamental microbiology in identifying and altering biofilms, and regeneration of periodontal structures. Further, implantology will continue to develop. As a result, it will be possible to expect healthy and functional dentition for a lifetime. The collaboration between researchers, clinicians, and educators remains crucial in advancing the understanding and management of periodontal diseases.

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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