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CONTENTS / SADRŽAJ

CASE REPORTS / KAZUISTIKA

Maj 2023. godine građani Srbije će pamtiti po dva zločina pod čijim teretom je čitava nacija u stanju šoka i neverice. U danima opšteg očaja i duboke tuge, milion pitanja koja je svako od nas sebi postavio slilo se u zajednički krik – Zašto? Gde smo pogrešili? Da li smo se pod pritiskom zahteva vremena u kome živimo promenili toliko da smo razorili ono što je vekovima čuvalo identitet ljudskih bića? Da li smo zaboravili vrednosti koje se uče u porodici, jednoj od najvažnijih tvorevina civilizacije? Zbog toga verujemo da ovog meseca posebnu pažnju zaslužuje Međunarodni dan porodice. Od 1994. godine, Međunarodni dan porodice obeležava se svake godine 15. maja. Posle ovih tragičnih događaja, više nego ikada ranije, važno je da se zapitamo šta možemo da uradimo da porodica opet postane ono što je nekada bila – okrilje u kome rastu zdravi ljudi.

In May 2023, the citizens of Serbia will remember two crimes under whose burden the entire nation is in a state of shock and disbelief. In the days of general despair and deep sadness, a million questions that each of us asked ourselves merged into a common cry – Why? Where did we go wrong? Have we changed so much under the pressure of the demands of the times we live in that we have destroyed what has preserved the identity of human beings for centuries? Have we forgotten the values taught in the family, one of the most important creations of civilization? Therefore, we believe that International Day of Families deserves special mention this month. Since 1994, International Day of Families has been celebrated on May 15 every year. After these tragic events, more than ever before, it is important to ask ourselves what we can do to make the family what it once was – a shelter where healthy people grow.

GENERAL REVIEW

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Outcome assessment of spa rehabilitation in ankylosing spondylitis

Procena ishoda banjske rehabilitacije bolesnika sa ankilozirajućim spondilitisom

Dušan Mustur

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Key words:
health resorts; physical and rehabilitation medicine; spondylitis, ankylosing; treatment outcome.

Ključne reči:
banje; medicina, fizikalna i rehabilitacija; spondylitis, ankilozirajući; lečenje, ishod.

Introduction

Ankylosing spondylitis (AS) is a chronic progressive autoimmune inflammatory systemic disease that usually starts at the sacroiliac joints and affects the joints and periarticular structures of the spine and chest. The disease begins at a younger age, and with time it causes postural changes, reduced lung capacity, muscle shortness, joint stiffness, and pain, significantly reducing the quality of life (QoL) and ability to function [1]. This illness is more common in men, the exact cause is still unknown, but the genetic marker HLA-B27 is present in more than 95% of patients with AS [2].

The treatment of patients with AS is very complex and consists of pharmacological, spa physical, and surgical treatment, and an important part of the treatment is also the education of patients. A treatment that would completely stop the progression of the disease has yet to be found. The main goals of AS therapy are reducing disease activity (inflammation), back stiffness, and pain and preserving and improving functional ability, i.e., preventing or at least slowing the progression of irreversible structural changes in the spine and other joints. The treatment of AS should be individually tailored to each patient according to their functional status and comorbidities and should start right after the disease is diagnosed before irreversible functional changes occur. Most people with AS do not need surgery, but they need to have a healthy lifestyle with a Mediterranean diet and follow a regular exercise program. That can help reduce pain, maintain flexibility, and improve their posture [1, 3].

Rehabilitation in ankylosing spondylitis

Rehabilitation in AS is a lifelong process. According to the definition of the World Health Organization (WHO), rehabilitation is a complex procedure of (re)training a disabled person after illness and injury for the highest possible physical, mental, social, and professional benefit according to their capabilities. The term disability means that a person has various functional limitations caused by illness, and handicap means a limitation or even inability to participate equally in social life. The development of medical rehabilitation helps people with significant disabilities to live and participate equally in daily life activities. Early and proper medical rehabilitation procedures reduce the number of completely disabled patients with AS from 25% to only 1%. Rehabilitation reduces the incidence of complications and the need for medications and hospitalization and leads to improved QoL in patients with AS [4].

Multimodal spa treatment

Spa treatment is a very important part of the overall management of AS. Since AS is an autoimmune process with no cure in sight, the treatment is focused on managing functional status, disease activity, and pain. Spa treatment is complex and includes balneotherapy (BT), climatotherapy, thalassotherapy, kinesitherapy, hydrokinesitherapy, sonotherapy, electrotherapy, and other therapeutic modalities according to special needs. It even includes changes in environment and lifestyle which lead to the activation and improvement of body adaptation mechanisms and potentials [5]. Physiotherapy procedures are prescribed by the doctor individually depending on the patient's health condition and possible contraindications. Bearing that in mind, Institute "Dr. Simo MIlošević", Igalo, Montenegro has four important natural factors: climate, peloid, seawater, and mineral water, which has enabled it to develop into one of the most modern center for physical medicine and rehabilitation in the region.

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Balneotherapy

BT is a combination of natural elements such as mineral waters, peloids, and gases in different world destinations, mostly in health resorts (spas), together with conventional physical therapy (kinesitherapy, sonotherapy, electrotherapy, etc.). Since Roman times, spa therapy has been applied in the treatment of different musculoskeletal conditions [6]. In European countries, BT is provided in health resorts with natural thermal baths (Niška Banja, Vrnjačka Banja, and Banja Koviljača in Serbia, Abano Terme in Italy, Hot Springs of Tiberias in Israel, etc.) or seawater baths (Igalo Spa in Montenegro, Dead Sea in Israel) [7]. For many diseases and injuries, BT is a desirable treatment since there are no serious side effects. BT has direct and indirect effects. Direct action includes the physical effects of water and peloids on the body (hydrostatic pressure, thrust, viscosity, resistance, friction). Balneo treatment also has thermal and chemical effects of substances absorbed through the skin [8]. Indirect effects come as a result of environmental and climate change, exercises, and social and psychological effects [4].

Climatotherapy

The climate is an essential factor that affects human health; it includes temperature, humidity, rainfall, wind, clouds, sunshine, etc. Wet and cold weather can intensify stiffness and pain in people with various rheumatic conditions. Rheumatic patients feel more comfortable in a warm, dry climate. The climate of Igalo is mild, coastal Mediterranean with a large number of sunny days and the air full of scents of diverse subtropical vegetation [4, 8].

Thalassotherapy

Thalassotherapy factors are coastal climate, seawater, solar radiation, aerosol, sea peloid, sand, and algae. Thalassotherapy is carried out in the warm summer months when its complex effect is most pronounced. It includes sunbathing on the seashore, swimming in seawater, swimming or exercising in seawater pools, but also walking by the sea and inhaling aerosols, or applying sea mud packaging or sand wraps.

In the natural area of the Adriatic Sea, the Igalo peloid (medicinal mud) is formed by mixing and depositing mineral-organic deposits of the river Sutorina and seawater sediment with its mineral and organic planktonic content. Igalo medicinal mud is an inorganic-organic, mostly mineral peloid with good physical and chemical properties. Its basic therapeutic effect is the thermal effect, and mud also has mechanical, biological-chemical, anti-inflammatory, and psychological effects [4]. Peloids can be prescribed as compresses or baths. Contraindications for peloids are rare, but we must emphasize that acutely inflamed joints should not be treated with any form of mud.

Physiotherapy is very important in treating AS, even today when biological drugs are used [9, 10]. In a systematic review, 28 studies were investigated (with a total number of 1,926 patients with AS). It was concluded that the most important part of the program is exercise (kinesitherapy) [11]. The main goal of the individually planned exercise program is to reduce pain and morning stiffness, improve spinal mobility, maintain and improve respiratory function, improve the patient's posture, increase muscle strength and endurance, and improve functional status and overall QoL [12]. The kinesitherapy program includes strengthening exercises for back and abdominal muscles, hip and knee extensors, stretching exercises (primarily pectoralis muscle, whose fibers shortening occurs due to the characteristic position of the patient), mobilization exercises, or exercises to maintain mobility of the spine and root joints. Breathing exercises with the "chest type" of breathing (inhale through the nose, exhale through the mouth) should be done every morning [12].

The use of hydrokinesitherapy is very common because water contributes to relaxation, and the recommended activities are swimming, mobilization in warm water, and walking in water. Exercise program, both in the gym and a swimming pool, improves functional status in patients with AS [13, 14]. Individual and group exercises are performed in a pool with a seawater temperature of 33–34°C. During the exercises, the beneficial effects of the aquatic environment are as follows: water temperatures, thrust, hydrostatic pressure, and the speed of the body's movement through the water.

Other physical modalities (electrotherapy, sonotherapy, magnetotherapy) are prescribed primarily for the good analgesic effect, to reduce pain and muscle stiffness, and patients are often prescribed manual massages within the spa physiotherapy program. Mineral water "Igaljka", with a temperature of 36–38 °C, is used at the Igalo Institute for pearl baths, mineral baths, and underwater shower massages as part of BT physical treatment. This mineral water is muriatic (sodium chloride) water, where sodium and chloride ions make up 70–80% of the total content of all ions [4].

Patients in spas often have traditional medicine procedures such as acupuncture, shiatsu, and tai chi; however, pilates and McKenzie and Heckscher exercises should be included [15].

Assessment tools

Leading experts from the Outcomes Measures in Rheumatology Clinical Trials (OMERACT) and Assessment of Spondylo-Arthritis (ASAS) International Society have created a core set to assess the effects of physical therapy. The core set includes several domains: pain, spinal stiffness, axial mobility, physical function, fatigue, and patient's global assessment [14]. To get the complete picture of the outcome of spa physical therapy, many studies assess the QoL and ASAS 20 improvement.

Pain should be assessed on the visual analog scale (VAS) or the numerical rating scale (NRS). Pain is a very unpleasant symptom associated with potential tissue damage and the most common reason for visiting a doctor [4]. Even though VAS and NRS scales are psychometric response scales, the double application shall produce objective information about the change in the intensity of pain after spa physical treatment [14, 16].

Spinal stiffness – patients should answer questions about how long they have been feeling stiffness in their back (in min, or VAS, NRS). When it is done twice (on admission and discharge), objective information about the change in the intensity of spinal stiffness is obtained after spa treatment [4, 16].

Spinal mobility – the range of motion in the spine in segments is assessed (occiput-to-wall distance, Otto's test, modified Schober index) or overall axial mobility with the Bath Ankylosing Spondylitis Metrology Index (BASMI) index. Occiput-to-wall distance shows whether the mobility of the cervical spine is reduced: if the distance is more than 0 cm, neck movements are reduced. Otto's test shows the mobility of the thoracic spine – normal values are 8 cm or more, and in advanced AS, it is significantly reduced. The modified Schober index shows the mobility of the lumbar spine, which is reduced if the result is less than 5 cm. BASMI shows the overall axial status of the patient with AS. The total BASMI score is obtained by measuring cervical rotation (in degrees), intermalleolar distance (in cm), lumbar flexion (modified Schober test, in cm), lateral lumbar flexion (in cm), and tragus-wall distance (in cm) [17]. Measured values are translated, using an algorithm, into values between 0–10, where a higher score shows more reduction in mobility of the spine [4, 9]. Spinal mobility measures correlate well with physical function, emotional role, mental health, and general health domains in the Medical Outcomes Study 36-item Short Form Health Survey (SF-36) questionnaire [18]. Impaired spinal mobility is associated with restricted pulmonary function [19].

Physical function – ASAS group experts suggest that physical function can be monitored using different questionnaires: Bath Ankylosing Spondylitis Functional Index (BASFI), The Dougados Functional Index (DFI), or Health Assessment Questionnaire for Spondyloarthropathies (HAQ-S) [9]. BASFI shows functional disability, it has 10 questions, eight of which concern activities referring to the functional anatomy (bending, changing position, standing, reaching, turning, and climbing steps), and two questions assess the patient's ability to cope with everyday life. BASFI uses a VAS scale with descriptors "easy" and "not possible", and a higher score means more functional disability [4, 14, 16]. BASFI strongly correlates with fatigue and global patient assessment in AS [20]. DFI consists of 20 questions about functional issues. A more recent version of DFI uses a 5-point Likert scale. BASFI is more sensitive to changes than DFI regarding functional status in patients with milder disease [21]. HAQ-S consists of 25 items, where 20 questions are from the original Health Assessment Disability Index (HAQ-DI) for arthritis patients, and the additional 5 questions are more specific to the issues of physical functioning and impairment specific to AS. A higher score means that the patient has more functional problems [9].

Fatigue often occurs in inflammatory rheumatic diseases and is associated with increased disease activity. It is determined on the 10 cm long VAS scale (or NRS), marked with "no fatigue" on the left and "maximum fatigue" on the right [4, 16]. Fatigue is increased by sleep disorders and depression. Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-F) is a short questionnaire covering thirteen questions on the level of fatigue that occurs when performing various activities during the previous seven days. FACIT-F uses a 4-point Likert scale. It was originally developed for assessing fatigue in people with anemia, but it is now widely used for different conditions, including AS [22].

The patient's global assessment refers to the current health condition of the respondents and is registered on the VAS or NRS scale. The Bath Ankylosing Spondylitis Global Score (BAS-G) is also a commonly used index. It assesses the general health of AS patients over a given time period. BAS-G contains two items on the VAS scale; a higher score means worst general health condition [9, 23].

QoL assessment – Ankylosing Spondylitis Quality of Life (AS-QoL) is the most frequently used disease-specific questionnaire. It is developed to assess the QoL in AS. This questionnaire shows the impact of disease on sleep, mood, motivation, independence, coping, activities of daily living, relationships, and social life. No/Yes answers are offered as responses, scored as 0/1. The final score is 0–18, where the highest score means the worst QoL. Still, a "golden standard" in QoL assessment in different diseases is the SF-36 questionnaire. SF-36 and EuroQol (EQ-5D) are generic questionnaires. SF-36 includes eight domains of QoL: Physical Function, Physical Role, Bodily Pain, Vitality, General Health, Social Function, Emotional Role and Mental Health. All scores are coded and transformed into eight 0–100 scales, where a higher value means better QoL. The use of these generic questionnaires allows comparison of QoL of patients with different diseases [24].

ASAS improvement criteria are commonly used to monitor the outcome of drug treatment or physiotherapy. ASAS improvement is calculated only once when the program is finished, and the final score is calculated in percentage. Five areas relevant to disease outcome are covered: patient global, back pain, functional impairment, morning stiffness, and the fifth one examines whether there is further deterioration in any given area. ASAS 40, ASAS 50, or even ASAS 70 can be achieved often in therapy with biologics [4].

Assessment of multimodal spa treatment

Despite the differences in clinical manifestations, multimodal spa rehabilitation is applied in different chronic inflammatory and degenerative arthritis [6, 24–28]. BT, together with climatic factors and other physical modalities, significantly reduces disease activity and improves functional status and QoL in rheumatoid arthritis [25, 29]. Rehabilitation in AS patients is a lifelong process. It should solve their overall life situation, i.e., their somatic, psychological, family, professional, and social problems. It represents a continuous process by which the patient maximizes their functional abilities. A multimodal spa treatment is a very important part of overall treatment in AS patients [1, 4, 20, 24, 30]. The program should be individually tailored based on clinical signs and symptoms, disease activity, functional status, deformities, posture, general health status, comorbidities, and patient preferences.

Since we cannot completely stop disease progression, treatments often focus on the management of symptoms, such as stiffness, pain, and mobility. BT improves the clinical course and slows the progression of the disease in patients with predominant axial involvement [31].

The consensus of the experts of the international ASAS group is that the optimal treatment requires a combination of drugs and non-pharmacological treatment measures and that they are of equal importance in rehabilitation [32]. A meta-analysis showed that all interventions in patients with AS and axial spondyloarthritis, both nonpharmacological and pharmacological significantly reduce pain and fatigue and improve physical function, spinal mobility, and patient global status when compared to control group [16]. Biologics are effective in AS; those patients already receiving biologics may also benefit from targeted physical therapy (improvement regarding pain, BASFI, BASDAI, chest expansion, modified Schober index) [33]. Still, approximately 20–40% of those receiving tumor necrosis factor inhibitor do not respond well to therapy [34]. Spa physical therapy reduces pain and the need to take analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) [35]. Contrary to BT, there are still many open questions regarding optimized treatment strategies and individual drug selection [36]. A Dutch AS patient study showed 40 weeks of prolonged benefits (measured with BASFI and EQ-5D) after 3 weeks of spa physical therapy compared to those with just regular exercises [37].

Dagfrund et al. [12] summarized data about the effects of different types of physical therapy. The conclusion is that the best care is provided when AS patients follow individually prescribed complex spa physical treatment with specific exercises program in a group. Patient education, active involvement, and motivation are of importance in AS [11]. Natural factors of the Niška Banja Spa increase axial mobility and decrease disease activity in AS [38]. A complex spa rehabilitation program that includes BT leads to a significant reduction of disease activity (BASDAI, ASDAS-CRP) and functional status (BASFI); it significantly improves the general index ASAS 20 [4, 39, 40].

Yurtkuran et al. [41] divided AS patients into three groups, the first had BT, the second BT and NSAIDs, and the third one had only NSAIDs for three weeks. Patients also had kinesitherapy – postural exercises and breathing exercises. Results were significantly better in groups with BT – they had less pain, less morning stiffness, and a better BASFI score.

Altan et al. [42] showed that three weeks of BT procedures together with exercises leads to significantly better health status (physical pain, patient's assessment of health status, functional status, disease activity) when compared to a group that had only exercises.

Conclusion

The treatment of AS is very complex and includes early diagnosis and initiation of adequate pharmacological therapy and application of multimodal spa physical treatment. Three or four weeks of a complex spa rehabilitation program showed favorable cost-effectiveness and cost-utility ratios compared with standard exercise treatment alone.

The spa rehabilitation program should be strictly individualized, primarily according to the stage and disease activity, previous treatment, and the patient's general condition. The best results are achieved in those treated with optimal pharmacological therapy before a spa treatment. BT procedures should be applied patiently, with discipline, and accurately at the right time, respecting the principles of chronotropism. Patients should not take baths right after the application of peloids because they can lose the positive chemical effect of mud. Exercises in the gym and pool have a central place in the rehabilitation of AS patients. Prolonged rest leads to muscle weakness, increased morning stiffness, decreased range of motion, reduced respiratory index, accelerated ankyloses, or contractures. Different analgesic procedures (heat, ice, interfering currents, ultrasound, transcutaneous electrical nerve stimulation – TENS) are often prescribed. Massage relaxes muscles and has a whole-body relaxing effect. The goals of multimodal spa physical therapy are numerous – to reduce pain, inflammation, stiffness in joints, and muscle spasm, accelerate the resorption of edemas and exudates, prevent contractures and ankyloses, strengthen muscles, preserve posture, and improve functional ability, health, and mood.

A recommended core set to assess complex spa physical therapy includes several domains: pain, axial stiffness, spinal mobility, physical function, fatigue, and patient's global assessment. Many studies use QoL questionnaires and ASAS 20 improvement, which leads us to believe that the proposed core set can be extended with these instruments. All the instruments are standardized and easy to use, and their application has reduced the potential bias of the interviewers to the smallest possible extent since most of the questionnaires are filled out independently. The BASMI index contains objective parameters (occiput-wall distance, lateral flexion, modified Schober index, etc.), so the bias of the interviewers cannot be entirely eliminated. Survey for patients may show their health condition better than it really is, but this is practically eliminated in tests as they are done two or more times. In the future, large studies with a low risk of bias are needed.

Natural factors are complex; they act simultaneously and should not be separated. However, when applied together, they lead to positive effects on AS patients that persist for several months after the end of the spa program, as shown by "cost-benefit" studies.

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Short-stay thyroid surgery for older patients: is it safe?

Operacija tireoidne žlezde sa kratkotrajnim boravkom u bolnici kod starijih bolesnika: da li je bezbedna?

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Abstract

Background/Aim. The incidence of nodular thyroid disease increases significantly with age as well as the incidence and aggressiveness of thyroid cancers. The aim of the study was to determine whether thyroid surgery for geriatric patients is safe in a short hospital stay surgery setting. **Methods.** In this retrospective study, medical histories of all operated geriatric patients (65 years and older), in whom a total thyroidectomy (TT) or hemithyroidectomy (HT) was performed from January 2012 to December 2018, were analyzed. A total of 976 patients were operated on for thyroid diseases in the mentioned period, out of which 247 geriatric patients fulfilled the inclusion criteria. Patients with thyroid reoperations and simultaneous neck lymph node dissections were excluded from the study. The geriatric patients were divided into two groups: the HT group (33 patients) and the TT group (214 patients). Each of these two geriatric groups, HT and TT, had two additional paired control groups. Control group I consisted of younger subjects from 20–44 years, and control group II included middle-aged subjects from 45–64 years. **Results.** All three TT groups – geriatric, control I, and control II, had 214 patients each, and all three HT groups had 33 patients each. In all three HT groups, the average hospital stay was 24 hrs, while in the TT geriatric group, 150 (70.1%) of 214 patients spent 24 hrs at the hospital. In the geriatric population, the incidence of neck swelling and increased drainage output were higher compared to both control groups, and thus the need for longer hospitalizations. When the age was compared, it was shown that subjects with each subsequent year of intervention had a 22% lower chance of developing complications, and regarding the pathohistological finding, benign thyroid hyperplasia was less likely to develop complications compared to malignant hyperplasia. **Conclusion.** According to the study, TT can be safely performed within the concept of a short hospital stay in patients under 65 years, while in the elderly, hospitalization days may be extended due to more frequent surgical and nonsurgical complications. Speaking of HT, the short hospital stay is safe for all age groups.

Key words:
aged; length of stay; minor surgical procedures; postoperative complications; risk factors; thyroidectomy; thyroid gland.

Apstrakt

Uvod/Cilj. Incidenca nodularnih bolesti štitaste žlezde značajno raste sa starenjem, kao i incidenca i agresivnost karcinoma štitaste žlezde. Cilj rada bio je da se utvrdi da li je operacija štitaste žlezde bezbedna kod starijih bolesnika u okviru koncepta kratkotrajnog postoperativnog boravka u bolnici. **Metode.** U studiji retrospektivnog tipa, analizirane su istorije bolesti svih operisanih bolesnika starijih od 65 godina kojimah je urađena totalna tireoidektomija (TT) ili hemitireoidektomija (HT) u periodu od januara 2012. do decembra 2018. godine. U navedenom periodu ukupno je operisano 976 bolesnika zbog bolesti štitaste žlezde, od kojih je 247 gerijatrijskih bolesnika ispunilo kriterijume da budu uključeni u studiju. Bolesnici kod kojih je bila rađena reoperacija štitaste žlezde, kao i bolesnici kod kojih je istovremeno bila urađena tireoidektomija i disekcija limfnih čvorova vrata, bili su isključeni iz studije. Gerijatrijski bolesnici bili su podeljeni u dve grupe – u prvoj grupi bili su bolesnici kod kojih je izvršena HT (33 bolesnika) a u drugoj su bili bolesnici kod kojih je izvršena TT (214 bolesnika). Svaka od ove dve gerijatrijske grupe, HT i TT, imala je po dve uparene kontrolne grupe. Prvu (I) kontrolnu grupu činili su mlađi bolesnici, od 20–44 godina, a drugu (II) kontrolnu

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grupu činili su bolesnici srednjih godina, od 45–64 godina. **Rezultati.** Sve tri grupe kod kojih je bila izvršena TT – gerijatrijska grupa i I i II kontrolna grupa, imale su po 214 bolesnika i svaka od tri grupe kod kojih je bila urađena HT imala je po 33 bolesnika. Kod bolesnika iz sve tri grupe kod kojih je bila izvršena HT, prosečan boravak u bolnici bio je 24 časa, dok je u gerijatrijskoj grupi bolesnika kojima je bila urađena TT, 150 (70,1%) od 214 bolesnika provelo 24 sata u bolnici. U gerijatrijskoj populaciji dolazilo je češće do pojave otoka u predelu vrata i povećane drenaže u odnosu na obe kontrolne grupe, pa je samim tim i postojala potreba za dužom hospitalizacijom. Poređenjem godina starosti, pokazano je da ispitanici sa svakom kasnijom godinom intervencije imaju za 22% manje šanse za nastanak komplikacija, kao i da kod benignih bolesti štitaste žlezde postoje manje šanse za nastanak komplikacija u odnosu na maligne bolesti. **Zaključak.** Prema rezultatima dobijenim u ovoj studiji, TT se može bezbedno sprovesti u okviru koncepta kratkog boravka u bolnici kod bolesnika mlađih od 65 godina, dok se kod starijih bolesnika dani hospitalizacije mogu produžiti zbog češćih hirurških i nehirurških komplikacija. Kod HT, kratkotrajni postoperativni boravak u bolnici je bezbedan za sve starosne grupe.

Ključne reči:
stare osobe; hospitalizacija, dužina ; hirurgija, mala; postoperativne komplikacije; faktori rizika; tireoidektomija; tireoidna žlezda.

Introduction

The incidence of nodular thyroid disease increases significantly with age as well as the incidence and aggressiveness of thyroid cancers [1, 2]. Since global and regional population aging trends are on the rise, including thyroid diseases, it is essential to establish adequate guidelines and protocols to provide optimal care for these patients [3].

Short-stay surgery represents a modern, safe, and effective approach to surgical treatment with numerous benefits for patients, their relatives, hospitals, and the healthcare system in general. This concept is less stressful for patients, especially when it comes to the youngest and oldest age groups, which are heavily dependent on their loved ones in daily life [4, 5]. Despite numerous evaluations on this topic, there is still a concern that thyroid surgery in the elderly may lead to more frequent complications than in the younger population, which led some authors to favor multi-day thyroid surgery for adequate preoperative preparation and longer postoperative follow-up [6, 7]. The higher percentage of comorbidities in the geriatric population could be one of the factors affecting the incidence of intra- and postoperative complications and, therefore, the period of inpatient stay. Although most authors consider geriatric thyroidectomy a safe procedure that fits into the concept of short-stay surgery [8, 9], there is still insufficient literature data regarding the safety of this approach.

The aim of this study was to determine if thyroid surgery for geriatric patients is safe in a short hospital stay surgery setting.

Methods

In this retrospective study, medical histories of all operated geriatric patients (65 years and older), in whom a total thyroidectomy (TT) or hemithyroidectomy (HT) was performed from January 2012 to December 2018 in our hospital, were analyzed. In the aforementioned period, 976 patients were operated on for thyroid diseases within the short-stay surgery setting, out of which 247 geriatric patients fulfilled the inclusion criteria. In 214 geriatric patients, a TT was performed, while HT was conducted in 33 geriatric patients. The analyzed geriatric patients were divided into the HT and TT group; each of these groups was paired with two control age groups, control I (20–44 years) and control II (45–64 years), in a numerical ratio of 1: 1: 1. For TT, each group had 214 patients, and for HT, each group had 33 patients.

Patients with thyroid reoperation, as well as patients who underwent thyroidectomy and neck lymph node dissection at the same time, were excluded from the study. All patients were prepared for short-stay thyroid surgery as outpatients in a standard manner and were admitted to the surgery department on the day of surgery. Standard preoperative preparation has implied anamnesis, clinical examination, blood analyses, biochemical analysis, laboratory analysis for thyroid hormones, anti-thyroid autoantibodies (anti-thyroid peroxidase and anti-thyroglobulin antibodies), thyroglobulin, and calcitonin, ultrasound imaging of the neck, electrocardiogram, lung X-ray, cardiologic examination, and an ear, nose, and throat specialist examination. In certain cases, a part of the preoperative workup also included an X-ray of the neck for tracheal positioning, thyroid scintigraphy, percutaneous biopsy, and appropriate specialist findings for other conditions patients might have.

Discharge was planned 24 hrs after the operation, with an overnight stay, with the possibility of an extension of stay in case of complications. All operations were performed by a single surgeon through a neck-based incision using ultrasound scissors.

According to the short-stay protocol, patients were prepared completely before arrival and admission to the hospital [10, 11]. After that, patients were admitted on the day of planned surgical treatment and discharged the same day or 24 hrs after the surgery (short-stay concept).

Protocols within the modern one-day surgery provide a contemporary, safe, and effective approach to surgical treatment with numerous benefits for both the patients and their relatives, hospitals, and the health system in general. Treatment within one-day surgery gives greater benefits and comfort to the patient, to whom the entire treatment and care are subordinated, unlike standard hospital care, where there are much more serious patients for treatment. Patients who are operated on in well-organized day hospital wards receive treatment that is better adapted to their needs, which allows

them to be discharged on the same day and return to home surroundings for easier recovery.

Data were collected from medical histories, operating protocols, histopathological findings, laboratory findings, and outpatient examinations. The following data were processed: demographic (age and gender), hospital stay, postoperative laboratory findings [parathyroid hormone (PTH) – in the first hour postoperatively, serum calcium – 24 hrs postoperatively], clinical findings, pathological findings [American Society of Anaesthesiologists (ASA) score, pathohistological finding, postoperative indirect laryngoscopy], surgical complications (neck swelling, drainage output > 100 mL/24 hrs, bleeding of the operative wound requiring reoperation) and nonsurgical complications (hypertensive crisis, exacerbation of the chronic obstructive pulmonary disease, etc.). The serum calcium levels were measured 24 hrs after the operation, whereby hypocalcemia was defined as a value less than < 2.0 mmol/L.

Descriptive statistical methods, methods for testing statistical hypotheses, and methods for analyzing the relationship between outcomes and potential predictors were used to analyze the primary data. Descriptive statistical methods were measures of central tendency, measures of variability, and relative numbers. The methods used to test the statistical hypotheses were the χ^2 test, Fisher's exact probability test, analysis of variance, Kruskal-Wallis test, and Mann-Whitney *U* test. Logistic regression was used to analyze the relationship between binary outcomes and potential predictors. Predictors of hospitalization days were analyzed by univariate and multivariate ordinal logistic regression. Statistical hypotheses were tested at a level of statistical significance (alpha level) of 0.05. The model of multivariate logistic regression included those predictors of complications that were statistically significant in the models of univariate logistic regressions at the level of significance of 0.1 and those that, based on previous research, are considered significant for complications. All data were processed in IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL, USA) software package.

This retrospective observational cohort study was done in accordance with current Good Clinical Practice guidelines, the Declaration of Helsinki, and the Ethics Committee approval of the Clinical Center "Dr. Dragiša Mišović – Dedinje", Belgrade, Serbia (No. 01-2905/1 from 21 March, 2022).

Results

Out of the total 976 patients that were operated on for thyroid disease within the short-stay surgery setting, 247 geriatric patients fulfilled the inclusion criteria. In 214 geriatric patients, a TT was performed, while HT was conducted in 33 geriatric patients.

In all investigated groups of patients, women appeared more frequently than men, with no statistically significant difference between the groups ($p = 0.114$; $p = 0.109$, respectively). According to the ASA classification system, the eldest group with HT (Table 1) had a statistically significant higher percentage of patients with the ASA III category compared to the controls ($p < 0001$). The same appeared in the eldest TT group ($p < 0.001$).

As shown in Table 2, in the geriatric HT group, the most frequent histopathological diagnosis was benign goiter (including incidentally found differentiated microcarcinoma) with no statistically significant difference compared to the controls ($p = 0.222$). In the TT groups, benign goiter was also the most frequent pathohistological diagnosis in all three groups (78.5%, 67.3%, and 61.2%, respectively). Diffuse thyroid hyperplasia (Graves' disease) appeared most frequently (14%) in the younger control group, as expected (compared to the medium age control group, 4.2%, and the geriatric group, 0%). A statistically significant difference was found between the TT age groups with regard to the histopathological findings ($p < 0.001$).

In all three HT groups, the average hospital stay was one day ($p = 0.133$), without surgical complications needing

Table 1

American Society of Anaesthesiologists (ASA) classification system in relation to age and type of surgery

ASA score	Hemithyroidectomy			Total thyroidectomy		
	65+	45–64	65+	45–64	65+	45–64
I	0 (0.0)	4 (12.1)	10 (30.3)	3 (1.4)	5 (2.3)	37 (17.3)
II	20 (60.6)	26 (78.8)	23 (69.7)	154 (72.0)	188 (87.8)	173 (80.8)
III	13 (39.4)	3 (9.1)	0 (0.0)	57 (26.6)	21 (9.8)	4 (1.9)
Total	33 (100)	33 (100)	33 (100)	214 (100)	214 (100)	214 (100)

65+ – patients older than 65 years; 45–64 – middle-aged group of patients; 20–44 – younger group of patients. Results are presented as numbers (percentages) of patients.

Table 2

Pathohistological (PH) findings in relation to age and type of surgery

PH finding	Hemithyroidectomy			Total thyroidectomy		
	65+	45–64	65+	45–64	65+	45–64
Benign goiter	28 (84.8)	30 (90.9)	32 (96.9)	168 (78.5)	144 (67.3)	131 (61.2)
Chronic thyroiditis	2 (6.1)	3 (9.1)	1 (3.1)	17 (7.9)	29 (13.6)	24 (11.2)
Graves' disease	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (4.2)	30 (14.0)
Thyroid carcinoma	3 (9.1)	0 (0.0)	0 (0.0)	29 (13.6)	32 (14.9)	29 (13.6)
Total	33 (100)	33 (100)	33 (100)	214 (100)	214 (100)	214 (100)

65+ – patients older than 65 years; 45–64 – middle-aged group of patients; 20–44 – younger group of patients. Results are presented as numbers (percentages) of patients.

reoperation or nonsurgical complications and without mortality. Two patients out of the middle-aged control group (p = 0.327) had neck swelling caused by subcutaneous seromas, which were solved by needle puncture in an ambulatory setting.

In the TT geriatric group, 150 (70.1%) of 214 patients spent 24 hrs in the hospital, while 176 (82.2%) patients of the middle-aged control group and 178 (83.2%) of the younger control group spent a similar time in the surgical ward. The arithmetic mean of the hospital stay was 1.5 days (range 1 to 7) for the geriatric group, while it was 1.3 (range 1 to 7) and 1.4 days (range 1 to 6) for the control groups, respectively. A significant statistical difference was found in terms of hospital stay between the geriatric and the middle-aged group (p = 0.011) as well as between the geriatric and the younger control group (p = 0.010).

There was no statistical difference between the age groups in the case of postoperative serum calcium (p = 0.107) and PTH levels (p = 0.756).

In the TT groups, the number of postoperative complications showed a statistically significant difference between the age groups (Table 3) in terms of neck swelling (10.3%, 4.7%, 2.3%, respectively; p = 0.001), drainage output (6.1%, 2.3%, 0.5%, respectively; p = 0.002), while nonsurgical complications (1.9%, 0%, 0%, respectively; p = 0.036) occurred more frequently in the geriatric group. Four geriatric patients had nonsurgical complications which prolonged the hospital stay (hypertensive crisis in three patients and exacerbation of chronic obstructive pulmonary disease in one patient). Neck swelling implied subcutaneous seroma or hematoma, which did not require reoperation. In two patients within the middle-aged control group (45–64 years), postoperative bleeding required surgical revision of hemostasis.

For overall 642 patients combined, complications appeared more frequently in patients with thyroid carcinoma (Table 4) (p = 0.001). We also found that complications, in general, occurred less frequently as the team acquired more understanding of this surgical concept (Table 5) (p < 0.001).

The model contains five predictors for potential complications, listed in Table 6, which were compared for 642 respondents (of which 164 had an outcome of interest). The whole model (with all predictors) was statistically significant (p < 0.001). There is no significant multicollinearity between predictors.

Table 3

Type of complication in relation to age for total thyroidectomy groups

Type of complication	Age			*p*-value
	65+	45–64	20–44	
Neck swelling	22 (10.3)	10 (4.7)	5 (2.3)	0.001
Drainage output > 100 mL/24 hrs	13 (6.1)	5 (2.3)	1 (0.5)	0.002
Hypocalcaemia < 2.0 mmol/L	21 (9.8)	20 (9.3)	28 (13.1)	0.396
iPTH < 3 pg/mL	28 (13.1)	22 (10.3)	23 (10.7)	0.619
Unilateral vocal cord palsy	2 (0.9)	1 (0.5)	2 (0.9)	1.000
Bilateral vocal cord palsy	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Bleeding into the operative wound requiring reoperation	0 (0.0)	2 (0.9)	0 (0.0)	1.000
Nonsurgical complications	4 (1.9)	0 (0.0)	0 (0.0)	0.036

iPTH – intact parathyroid hormone; 65+ – patients older than 65 years; 45–64 – middle-aged group of patients; 20–44 – younger group of patients. Results are presented as numbers (percentages) of patients.

Table 4

Pathohistological (PH) findings in relation to complications

PH finding	With complications	Without complications	Total
Benign goiter	106 (23.9)	337 (76.1)	443 (100)
Chronic thyroiditis	15 (21.4)	55 (78.6)	70 (100)
Graves' disease	5 (12.8)	34 (87.2)	39 (100)
Thyroid carcinoma	38 (42.2)	52 (57.8)	90 (100)
Total	164 (25.5)	478 (74.5)	642 (100)

Results are presented as numbers (percentages) of patients.

Table 5

Year of operation in relation to complication rates

Year of operation	With complications	Without complications	Total
2012	48 (41.1)	69 (58.9)	117 (100)
2013	25 (39.7)	38 (60.3)	63 (100)
2014	19 (19.2)	80 (80.8)	99 (100)
2015	27 (34.6)	51 (65.4)	78 (100)
2016	12 (13.8)	75 (86.2)	87 (100)
2017	24 (20.5)	93 (79.5)	117 (100)
2018	9 (11.1)	72 (88.9)	81 (100)
Total	164 (25.5)	478 (74.5)	642 (100)

Results are presented as numbers (percentages) of patients.

Table 6

Multivariate logistic regression of complication occurrence as a dependent variable

Independent variable	RC	*p*-value	OR (95%CI)
Age categories	-0.325	**0.012**	0.72 (0.56–0.93)
Gender	0.646	0.054	1.91 (0.99–3.67)
Year of operation	-0.247	< **0.001**	0.78 (0.71–0.86)
Patohistological finding			
Benign goiter	-0.700	**0.005**	0.50 (0.31–0.82)
Chronic thyroiditis	-0.871	**0.020**	0.42 (0.20–0.87)
Graves' disease	-0.828	0.131	0.44 (0.15–1.28)
Thyroid carcinoma		reference category	
ASA	0.328	0.171	1.39 (0.87–2.22)

ASA – American Society of Anaesthesiologists; RC – regression coefficient; OR – odds ratio; CI – confidence interval.

Bolded values are statistically significant.

Table 7

Ordinal logistic regression of the number of hospitalization days as a dependent variable

Independent variable	Ordinal logistic regression			
	univariate		multivariate	
	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
Age categories	0.70 (0.56–0.89)	**0.003**	0.77 (0.59–1.01)	0.060
Gender	0.73 (0.39–1.37)	0.329		
Year of operation	0.77 (0.70–0.85)	< **0.001**	0.77 (0.70–0.85)	< **0.001**
Patohistological finding				
Benign goiter	0.48 (0.30–0.78)	**0.003**	0.58 (0.35–0.95)	**0.031**
Chronic thyroiditis	0.30 (0.14–0.67)	**0.003**	0.37 (0.16–0.83)	**0.016**
Graves' disease	0.22 (0.07–0.66)	**0.007**	0.50 (0.16–1.58)	0.239
Thyroid carcinoma	reference category		reference category	
ASA	1.48 (0.97–2.25)	0.068	1.54 (0.94–2.52)	0.087

ASA – American Society of Anaesthesiologists; OR – odds ratio; CI – confidence interval.

Bolded values are statistically significant.

In the multiple logistic regression model, the following are statistically significant predictors of complications: older patients (p=0.012) with the odds ratio (OR) of = 0.72, which shows that patients in the younger age category have a 28% lower chance for the occurrence of complications, with control of all other factors in the model; earlier year of operation ($p < 0.001$), with OR = 0.78, which shows that respondents with each subsequent year of operation have a 22% lower chance of complications, with control of all other factors in the model; histopathological finding of benign goiter (p = 0.003), with OR = 0.50, which shows that patients with benign goiter have a 50% lower chance of complications; chronic thyroiditis (p = 0.020), with OR = 0.42, which shows that subjects with chronic thyroiditis have a 58% lower chance of developing complications, both compared to thyroid carcinoma as a reference category.

The multivariate ordinal logistic regression model includes those predictors of hospital length that were statistically significant in the univariate logistic regression models at a significance level of 0.1 and which, based on previous research, are considered significant for hospitalization length (Table 7). Due to multicollinearity, the complication variable was not included in the models.

In the multivariate ordinal logistic regression model with the number of days of hospitalization as a dependent variable, statistically significant predictors of longer hospitalization are earlier years of operation ($p < 0.001$) and histopathological finding, where benign goiter (p = 0.031) and chronic thyroiditis (p = 0.016) have a significantly lower chance of longer hospitalization compared to thyroid carcinoma as a reference category.

Discussion

The main obstacle for short-stay thyroid surgery is the possibility of postoperative bleeding in the thyroid lodge and in the closed paratracheal space with consequent compression on the cervical trachea, which can lead to asphyxia. In about 50 to 75% of cases, this complication occurs in the first six to eight hours after surgery [12], even though there are cases described in which this complication occurred several days after thyroidectomy [12–15]. In our study, this complication occurred in two patients of the middle-aged group with TT, where one of these patients needed a reoperation two days after surgery (the bleeding site was the anterior jugular vein). In general, in about 80 to 97% of patients, postoperative bleeding occurs in the first 24 hrs after surgery [13, 15–19]. Godballe et al. [18] claimed that the relative risk of bleeding in patients aged 50 and over is 1.5 times higher than in those younger than 50. Bergenfelz et al. [20] showed in their work that the group of patients with postoperative bleeding had an average age of 60 years, which, compared to the average age

of 48 years of the group of patients without postoperative bleeding, proved to be statistically significant. Patients on anticoagulant or antiplatelet therapy (which appears more often in the elderly) tend to have late postoperative bleeding, according to a larger number of authors [21–23].

Another limiting factor for the implementation of a short-stay surgery approach in thyroid surgery is hypocalcemia, which occurs in 5% to 35% of cases and is usually clinically manifested 12 to 72 hrs after surgery [24–26]; fortunately, in the majority of cases, it is temporary. Hypocalcemia 24 hrs after TT operation occurred in all three age groups, with similar percentages and no statistically significant difference. Given this fact, hypocalcemia is the most common complication after TT, which was the case in our series as well (10.8%). Postoperative hypocalcemia, as a complication after HT, does not occur since there are always parathyroid glands on the contralateral side of the thyroid gland, whose function is not compromised by surgery.

In order to predict which patient will develop hypocalcemia after TT, for the timely initiation of replacement therapy with calcium and vitamin D preparations, as well as discharge from the ward, we decided to determine early postoperative PTH as a predictor of hypocalcemia. In our study, early postoperative PTH values lower than 3 pg/mL appeared in 11.4% of patients after TT, with no significant difference between the age groups. In a multicenter study, Noordzij et al. [27] concluded that postoperative PTH with values lower than 10 pg/mL predicted hypocalcemia. In the case of normal values of postoperative PTH, the probability of severe hypocalcemia is minimal, which can accelerate the discharge of these patients from the ward.

The rarest complication after thyroid surgery, which could be a limiting factor for a short stay, is bilateral paralysis of the vocal cords, with a frequency of about 0.2% to 0.6% [20, 28]. This complication is potentially life-threatening and is obvious immediately after the operation; it requires an adequate assessment of airway patency and airway sufficiency and, quite often, placement of a temporary tracheostomy. Fortunately, we had no case of bilateral paralysis of the vocal cords in our study. The most common cause of poor vocal cord mobility after thyroidectomy is recurrent nerve neurapraxia, which in most cases is a temporary complication with complete recovery expected after a few weeks. However, the frequency of permanent recurrent nerve injury should not exceed 1% [29]. We had no case of recurrent nerve injury in the HT groups, while in the TT groups, we had no significant difference between the age groups in unilateral paralysis of the vocal cords, with an overall rate of 0.78%. Unilateral injury to the recurrent nerve results in hoarseness of the voice, which does not require an extended hospital stay.

While the average hospital stays for all HT groups in our study was one day with no significant complications in all patients, the oldest TT age group showed a statistically significant longer hospital stay in comparison to the younger age groups as a result of a higher rate of surgical and nonsurgical complications. Schwartz et al. [30] and Tartaglia et al. [31] showed in their works that complications occur more often in the elderly, while Canonico et al. [6] and Seybt et al. [32] claim that there is no significant difference between the age groups. In the study of Gervasi et al. [2], age over 70 is an independent factor for complications after each surgical procedure under general anesthesia. Mekel et al. [33] claim that postoperative complications increase significantly with the patient's age, from 9% in the control group (mean age 50.1 years) to over 24% in octogenarians.

According to our first logistic regression model, statistically significant predictors of complications, except age, are pathohistological findings and the surgeon's experience. In numerous studies, it is stated that a higher percentage of complications is associated with a higher percentage of thyroid malignancy in the elderly [34–37], which corresponds to the results of our study. This fact we also confirmed in our second multivariate ordinal logistic regression model with the number of days of hospitalization as a dependent variable, where thyroid malignancy manifested as a significant predictor of longer hospital stay.

The admission of patients for thyroidectomy into the short-stay surgery protocol should be kept for experienced endocrine surgeons and teams, i.e., those who operate on more than 100 thyroidectomies per year [38, 39]. In our study, all operations were performed by one surgeon and their team, and the complication rate was lower as more experience was gained each year. This fact we also confirmed in our second multivariate ordinal logistic regression model with the number of days of hospitalization as a dependent variable, where an earlier year of operation showed as a significant predictor of longer hospital stay.

Conclusion

According to our study, TT can be safely performed within the concept of a short hospital stay in patients under 65, while in the elderly, hospitalization days may be extended due to more frequent surgical and nonsurgical complications. On the other hand, HT is safe for all age groups. Short-stay thyroid surgery, especially for geriatric patients, should be carried out by endocrine surgeons with experience with this kind of surgical setting in order to lower the length of hospitalization.

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Effect of neurofeedback training on auditory evoked potentials' late components reaction time: a placebo-control study

Efekat treninga nervnog sistema povratnim informacijama (*neurofeedback*) na reakciono vreme kasnih komponenti auditivnih evociranih potencijala: placebom kontrolisano istraživanje

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Abstract

Background/Aim. Neurofeedback (NFB) training of sensorimotor rhythm (SMR) contributes to improving cognitive performance and increasing attention. SMR power is increased when a person is focused and task-oriented. The shorter reaction time (RT) of the P300 auditory evoked potentials (AEPs) is associated with better attention. Hence, the increase in SMR power after NFB SMR training should decrease the RT in a cognitive task. The aim of the study was to examine the ability of healthy individuals to modulate the SMR of electroencephalographic (EEG) activity between 12 and 15 Hz during 20-day NFB training sessions. In addition, the effect of NFB SMR training on RT was investigated. **Methods.** Participants were divided into experimental and control groups, with 24 subjects (12 males and 12 females) in each group, aged between 25 and 40 years. Participants in the experimental group were trained with authentic NFB SMR training, while in the control group, false (placebo) training was applied. AEPs were registered on five occasions: before NFB training, after 5, 10, and 20 training sessions, and one month after the last training. **Results.** The results showed that a series of 20 NFB SMR training sessions increased the amplitudes of the SMR. RT in the experimental group was significantly shortened, while in the control group, it was not observed. Moreover, the increase in the power of the EEG signal of the SMR showed a negative correlation with RT, but only in a subgroup of male subjects. **Conclusion.** The obtained results indicate the effects of NFB training on the improvement of the attention process expressed by RT.

Key words:
attention; brain; cognition; electroencephalography; event-related potentials, p300; evoked potentials, auditory; feedback, sensory.

Apstrakt

Uvod/Cilj. Efekat treninga nervnog sistema povratnim informacijama – *neurofeedback* (NFB) trening senzomotornog ritma (SMR) doprinosi poboljšanju kognitivnih sposobnosti i povećanju pažnje. Snaga SMR se povećava kada je osoba usmerena na određeni kognitivni zadatak. Kraće vreme reakcije (VR) auditivnih evociranih potencijala (AEP) P300 povezano je sa boljom pažnjom. Stoga se očekuje da nakon NFB SMR treninga dođe do povećanja snage SMR i posledično do smanjenja VR u kognitivnom zadatku. Cilj rada bio je da se ispita mogućnost zdravih osoba da moduliraju SMR elektroencefalografske (EEG) aktivnosti između 12 i 15 Hz, tokom 20-dnevnih sesija NFB treninga. Pored toga, proučavan je i efekat NFB SMR treninga na VR. **Metode.** Ispitanici su podeljeni u eksperimentalnu i kontrolnu grupu, sa po 24 ispitanika (12 muškog i 12 ženskog pola) životnog doba između 25 i 40 godina. Ispitanici u eksperimentalnoj grupi trenirani su autentičnim NFB SMR treningom, dok je u kontrolnoj grupi primenjivan lažni (placebo) trening. AEP su registrovani u pet navrata: pre primene NFB treninga, posle 5, 10, i 20 sesija treninga, kao i jedan mesec nakon

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poslednjeg treninga. **Rezultati.** Rezultati su pokazali da serija od 20 NFB SMR treninga povećava amplitude SMR. U eksperimentalnoj grupi bilo je značajno skraćeno VR, dok u kontrolnoj grupi to nije zabeleženo. Takođe, povećanje snage EEG signala SMR bilo je u negativnoj korelaciji sa VR, ali samo u podgrupi ispitanika muškog pola. **Zaključak.** Dobijeni rezultati ukazuju na efekte NFB treninga na poboljšanje procesa pažnje, izraženo pomoću VR.

Ključne reči:
pažnja; mozak; saznanje; elektroencefalografija; potencijali povezani sa događajima, p300; evocirani potencijali, auditorni; povratna informacija, senzorna.

Introduction

For many years, cognitive training with neurofeedback (NFB) has proven to be a useful noninvasive and nonpharmacological method in improving numerous cognitive performances. NFB, a form of biofeedback, represents a form of neuromodulation in which individuals have information about the state of electroencephalographic (EEG) activity (brain waves) with the ability to control and self-regulate brain activity through the paradigm of operant conditioning. The modification of brain activity occurs not only through the feedback of operant conditioning but also through the modification of an individual's perception of their physiological state. Thus, two processes are involved in NFB – unconscious through operant conditioning and conscious cognitive self-perception [1].

NFB protocols are based on amplifying, inhibiting, or harmonizing certain EEG rhythms. Sensorimotor rhythm (SMR) NFB training is used as a therapeutic method in various types of disorders such as attention deficit hyperactivity disorder (ADHD) and epilepsy [2–8]. Research studies on healthy individuals, as well as on patients with brain damage, have also found positive effects of NFB SMR training protocols on cognitive functions [8–17]. NFB has also been employed in the treatment of anxiety and traumatic brain injury and in the recovery of patients with impaired motor performance [18]. Recently, the use and research in the field of EEG-NFB have expanded to a healthy population, as is the case in memory training, attention, and other cognitive abilities in young adults or the elderly population [19–22]. The method is used to improve athlete performance training, creativity, or even optimize microsurgical skills [23].

SMR or SMR waves training refers to cognitive function, better focus, and increased attention and concentration. SMR or SMR waves are beta waves in the frequency range between 12–15 Hz that occur in the sensorimotor region of the brain regulated by the thalamocortical loop [24]. SMR is observed when a person is immobile but mentally focused and attentive.

With the NFB SMR protocol, the subject trains to gain control in terms of increasing the amplitude of SMR waves, which subsequently results in improved cognitive performance in terms of increased attention and better focus. P300 cognitive evoked potential is frequently considered a neurophysiological marker of auditory attention [25]. P300 is an endogenous cognitive neuroelectric phenomenon that occurs under the influence of endogenous stimuli and depends on the state of alertness, concentration, and type of task that the subject is obliged to perform. Event-related potential (ERP) components are represented by a series of positive and negative waves (N100, P100, N200, P200, and P300) of different duration and amplitudes, of which the most significant is P300. Cognitive potentials with long latency are bioelectrical responses to thalamic and cortex activity [26]. The amplitude and latency of ERP components reflect the processes of perception, attention, cortical inhibition, memory updates, and other cognitive activities [27]. Latency [expressed in milliseconds (ms)] is defined as the time from the stimulus presentation to the point of maximum amplitude. The higher amplitudes and shorter latencies and reaction time (RT) of the P300 component are associated with better attention [28].

Studies of NFB training in a healthy young population have shown that the SMR protocol could be an effective method for improving attention and perceptual ability, reducing RT, and increasing semantic working memory [13].

Previous studies have found an association between increased SMR power and improvement of attention as well as increased SMR power and RTs in cognitive tasks, mostly in groups of participants with a variety of neurocognitive disorders. SMR power is increased when a person is focused and task-oriented. Hence, the increase in SMR power after NFB SMR training should decrease RT in a cognitive task.

So far, no studies have used a blind placebo-controlled study design in analyzing the effects of NFB SMR training on RT in auditory ERPs. Therefore, the aim of our study was to examine whether healthy subjects aged 25 to 40 years can modulate the lower-beta frequency band (12–15 Hz), called SMR, through 20 NFB SMR training sessions and influence RT compared to the placebo-control group of peers.

Methods

Participants

The study involved 48 healthy participants of both sexes (24 males and 24 females), 25 to 40 years old. The participants were recruited from the Institute for Experimental Phonetics and Speech Pathology and the Life Activities Advancement Center in Belgrade, Serbia, whose Laboratory for Cognitive Research conducted the experiments. Participants were without hearing or speech disorders, with no prior or current neurological or psychiatric illness (based on the participant's verbal report). All participants were right-handed, according to the Edinburgh Handedness Inventory. Each participant gave their written informed consent before the experimental procedure. This study was approved by the Ethics Committee of the Institute for Experimental Phonetics and Speech Pathology "Đorđe Kostić" in Belgrade, on February

12, 2019 under the number 22/19 according to the Declaration of Helsinki.

Participants were divided into the control (placebo) and experimental (treatment) groups. Each group consisted of 24 subjects (12 males and 12 females) aged 25 to 40 years. Each of the 24 participants of the experimental group had 20 NFB SMR training sessions, while the participants of the control group had a placebo NFB training.

Auditory event-related potentials recording

The auditory event-related potentials (aERP) were recorded using a standard oddball go/no-go paradigm. To obtain the P300, an auditory "oddball" paradigm with two tones was used, with 80% of non-target and 20% of target stimuli. Participants had a task to react by pressing a control button with the right hand's thumb each time they heard a tone that differed from other tones that were mostly presented. A total of 80% of each presented tone had a frequency of 1,000 Hz, and 20% of tones were oddballs with a frequency of 2000 Hz. The tones were randomly presented to the participants. The participants listened to the tones using earphones. Three Ag/Ag-Cl ring electrodes for aERP registration were positioned according to the International 10–20 System of Electrode Placement at the Fz (frontal midline), Cz (central midline), and Pz (parietal midline) regions. The reference electrode was set to the ear lobes, and the ground electrode was on the forehead. The impedance was kept below 5kΩ with no more than 1kΩ difference between electrodes. The software has its own implemented tool for artifact rejection. Each recording section that had more than 20% of rejected trials due to excessive artifacts was discarded and redone. Each participant underwent the experimental procedure in the morning hours (9–11 am). For each participant, averaged amplitude (μV) and latency (ms) of N100, N200, and P300 waves were obtained for each electrode (Fz, Cz, and Pz). The aERP were recorded at the beginning (t1), after 5 (t2), 10 (t3), and 20 (t4) NFB SMR treatments, as well as one month after the last NFB SMR treatment (t5). The aERP were recorded using a Nihon Kohden Electroencephalograph (model EEG-4314F) and Neuroscan Acquire 4.0 software.

Neurofeedback sensorimotor rhythm protocol training

The task for participants of the experimental group was to perform NFB SMR training, thus increasing the amplitude of SMR. Each participant took part in 20 sessions of NFB SMR protocol training three times a week for 33 min: 2 min of the resting-state period (watching a blank computer screen) at the beginning, four training trials, each lasting 6 min, and 2 min resting state at the end.

During the trials, the participants look at the physiological responses on the screen in the form of pictures and video games. The information that comes from this process is feedback, which is reflected in changes in the image or sound of the video game used for training. For the control group, the games are designed to let the participants advance in the game if they can bring the physiological function being rehearsed to the desired level. After each trial, participants had a one-minute break. While the experimental group had to improve the amplitude of the SMR during 20 instrumental conditioning sessions, the control group received false feedback. The control group had the same test protocol and amount of treatments. The training design of the control group was identical and differed only in the frequency setting where the respondents did not receive feedback related to their achievement. For a detailed description of the placebo control study design, see Lansbergen et al. [29].

The NFB SMR training was performed using BioTrace software for Nexus – 10B2015. The electrode was set to a Cz region (central midline-vertex region). After 5, 10, and 20 NFB SMR training sessions, as well as one month after the last session, participants were re-registered with aERP using the same procedure as at the beginning.

Statistical analysis

This study had a small sample with the obtained data that did not have a normal (Gaussian) distribution. Hence, the groups (experimental and placebo) were compared for NFB SMR power and RT using nonparametric statistics – Kruskal Wallis test for exploring the effect of time point (before NFB, after 5, 10, 20 sessions, and one month after the last training session) and Wilcoxon signed ranks test for *post hoc* multiple comparisons reporting Z score and *p*-value. The Wilcoxon signed ranks test was used to compare male and female participants. Finally, we have used the Pearson correlation coefficient to probe an association between RT and NFB SMR power in the Cz region in the experimental group. In each comparison, a 95% confidence interval was used.

Results

The first level of analysis was to explore the effect of group (experimental – treatment vs. control – placebo) on NFB SMR power. The Kruskal Wallis test found a significant effect of group on NFB SMR power in the Cz region after 10 sessions: H (47) = 3.244, $p < 0.01$, and 20 sessions: H (47) = 4.205, $p < 0.001$. No differences between the experimental and control group were found after 5 sessions. In addition, in the experimental group, the *post hoc* Mann Whitney *U* test found a statistically significant difference in NFB SMR power between the 5th and 10th session: Z = 3.776, $p < 0.01$; between 5th and 20th session: Z = 4.713, $p < 0.001$; as well as between 10th and 20th session: Z = 2.859, $p = 0.02$. The results show a statistically significant linear increase in NFB SMR power as a result of NFB SMR training sessions in the Cz region in the experimental (treatment) group. No such trend was found for the placebo control group (Figure 1).

The next level of analysis was to explore the effect of NFB SMR training on average RT in both groups (experimental – treatment vs. control – placebo). The Kruskal Wallis test found a significant effect of group on RT at the following time points: t1 – H (47) = 2.672, $p = 0.02$; t2 – H (47) = 3.165, $p < 0.01$; t3 – H (47) = 3.822, $p < 0.001$; t4 – H (47)

= 3.047, $p < 0.01$. The next level of analysis was to explore the effect of NFB SMR training sessions on RT in the experimental and placebo group separately. In the experimental group, *post hoc* Mann Whitney *U* test found a statistically significant difference in RT between t0 and t1: Z = 2.427, $p = 0.02$, between t1 and t2: Z = 2.344, $p = 0.03$, and between t2 and t3: Z = 2.859, $p < 0.01$.

The results show a statistically significant linear decrease in RT as a result of NFB SMR training sessions in the Cz region in the experimental (treatment) group. No such trend was found for the placebo control group (Figure 2).

In the experimental group, the *post hoc* Mann Whitney *U* test found a statistically significant difference between male and female participants in RT at each time point: t0 – Z = 2.105, $p = 0.02$; t1 – Z = 2.237, $p = 0.018$; t2 – Z = 2.336, $p = 0.018$; t3 – Z = 2.291, $p < 0.01$; t4 – Z = 2.341, $p < 0.01$.

Male participants had shorter RT compared to females (Figure 3).

In the experimental group, *post hoc* Mann Whitney *U* test found a statistically significant difference between male and female participants in NFB SMR power in each of three-time points – after 5 sessions: Z = 4.236, $p < 0.01$; after 10 sessions: Z = 2.382, $p = 0.018$; as well as after 20 sessions: Z = 2.116, $p = 0.018$. Male participants had higher NFB SMR power compared to females (Figure 4).

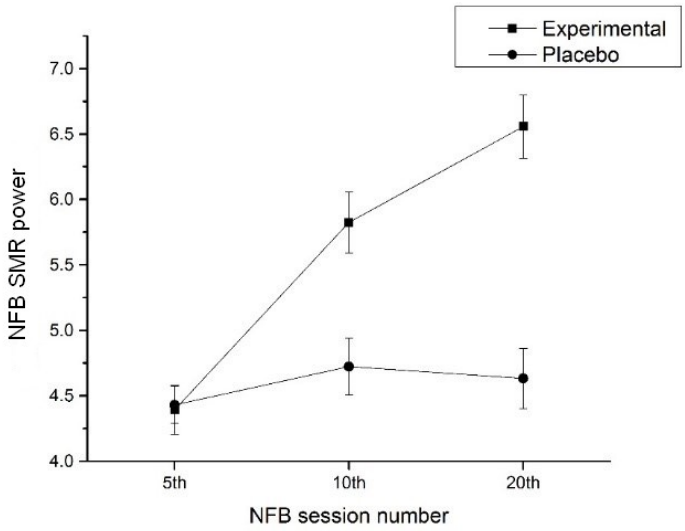

Fig. 1 – The effect of neurofeedback (NFB) sensorimotor rhythm (SMR) training on the NFB SMR power after 5, 10, and 20 NFB sessions in experimental and placebo groups.

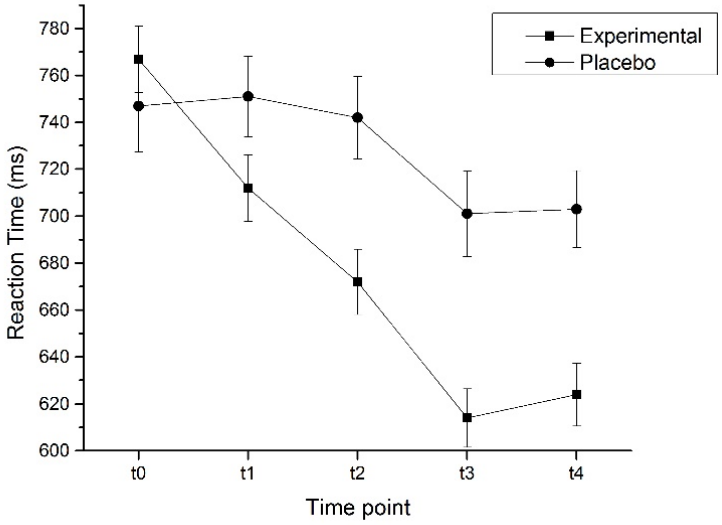

Fig. 2 – The effect of neurofeedback sensorimotor rhythm training on average auditory evoked potentials reaction time in the central midline region for each group (experimental and placebo) and at each time point.

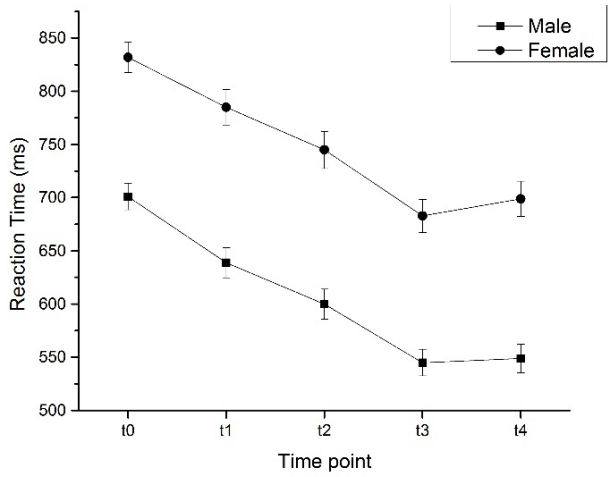

Fig. 3 – The effect of neurofeedback sensorimotor rhythm training on the average auditory evoked potentials reaction time in the central midline region in male and female participants from the experimental group.

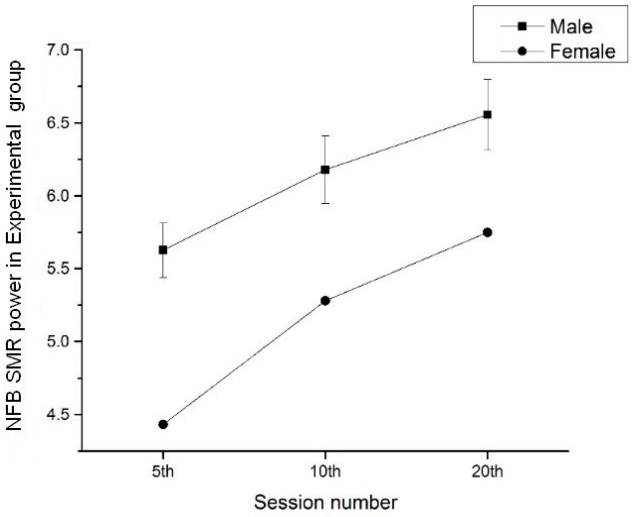

Fig. 4 – Comparison of neurofeedback (NFB) sensorimotor rhythm (SMR) power between male and female participants in the experimental group.

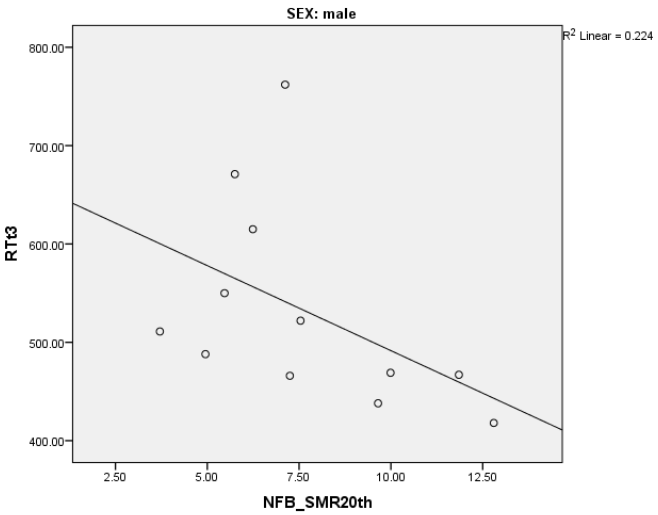

Fig. 5 – Correlation between neurofeedback (NFB) sensorimotor rhythm (SMR) power and reaction time (RT) after 20 NFB SMR sessions in male participants from the experimental group.

The final level of analysis was to probe a potential association between NFB SMR power and RT after 20 NFB sessions in male participants from the experimental group (Figure 5). A Pearson correlation coefficient (linear R^2) showed a significant negative correlation between NFB SMR power in the Cz region and RT after 20 NFB sessions: $R^2 = 0.024$, $p = 0.02$. Results showed a linear reduction of RT with the increase in NFB SMR power in male participants from the experimental group.

Discussion

The aim of this study was twofold. We first examined whether healthy subjects could modulate their EEG activity using NFB training. Second, we examined the effect of NFB SMR training on RT. Finally, we examined the correlation between NFB SMR power and RT.

The study showed that the subjects of the experimental group were able to increase their EEG activity within NFB training in the trained frequency range of 12–15 Hz.

Several studies have shown that subjects can learn to self-regulate different parameters of EEG activity (amplitude and coherence of EEG signals) through NFB training [30–32].

The study of Doppelmayr and Weber [33] showed that subjects who had SMR training were able to modulate the EEG in the trained frequency bands as opposed to the control and theta/beta ratio groups. In addition, only the SMR group was able to achieve better results in RT tasks. In a study by

Vernon et al. [13], healthy subjects were able to increase SMR activity after only eight NFB sessions, which was associated with an improvement in memory tasks. Gadea et al. [34] showed that healthy women were able to improve SMR waves, and this was positively associated with improved performance in a test that measures executive attention. In the study by Parsaei et al. [35], there was an increase in SMR waves and a significant improvement in RT in the experimental group of older men but not in the control group, which had a false NFB. In our study, the effect of NFB SMR training was examined in the experimental group on RT. NFB SMR training caused a reduction in RT in both male and female participants observed as a group. However, the increase in NFB SMR power had a statistically significant negative correlation with the RT only in male participants. That is probably the explanation for the shorter RT in male participants compared to female ones.

The oddball paradigm was used to generate P300 potential. It is the auditory discrimination test, which involves the use of two types of tones: high-frequency arrhythmic tone and low-frequency rhythmic tone. The difference between the two tones is in frequency and intensity [36]. The respondent is presented with two types of auditory stimuli: the "rare" or "unexpected" arrhythmic tone, which represents the target stimulus and differs in frequency from the "standard" or "expected" tone and occurs about it in random order. The participant is required to respond to an "unexpected" tone (pressing a key) and ignore the "standard" tone, i.e., to recognize target stimuli in a series of stimuli that differ in one feature (volume, duration) and are less probable than the standard ones. The oddball experimental paradigm requires the attention and concentration of respondents.

Components can be analyzed in terms of their latency and amplitude. Registration of these potentials shows a sequence of peaks with negative-positive-negative-positive polarity (N1-P2-N2-P3) at intervals of 80 and 350 ms after stimulation [25].

Latency represents the time interval, that is, the period from the moment of stimulation to the appearance of maximum amplitude, i.e., the peak of ERP. Latency reflects the speed of processing of sensory stimuli as a consequence of distinction from the other stimuli. Therefore, shorter latencies are considered to reflect more effective mental performance compared to longer latencies.

A large number of studies talk about the positive effects of NFB SMR on selective attention, auditory attention, phonological awareness [37, 38], RTs, and memory [39, 40]. Bielas and Michalczyk [41] demonstrated an improvement in attention capacity after the beta protocol of NFB training (12–22 Hz), with an active electrode set to Cz in the elderly population. Analysis of RT of the subjects after NFB training showed a significant improvement. In contrast, the difference in RT in the control group that did not have NFB training was not significant. In children and adolescents with focal epilepsy, SMR training significantly reduced RT [42]. In the Kober et al. [11] study of subjects after stroke, the experimental but not the control group showed a linear increase in SMR strength during training, which was associated with improvements in memory and attention. In addition, the increase in SMR led to a more pronounced stimulus processing, which is shown by the increased amplitude of N1 and P3 evoked potential. In an extensive Kaiser and Othmer [43] study, NFB training on a large number of subjects produced significant improvement in attention and impulse control in 85% of subjects. Egner and Gruzelier [44] investigated the different effects of SMR (12–15 Hz) and beta (15–18 Hz) NFB training on different performances. In their research, SMR training resulted in increased perceptual sensitivity and better attention, and low beta rhythm training gave faster RT. In this study, as in ours, the differentiation of cognitive performance in relation to gender shows that male subjects had a faster RT compared to female subjects, which is consistent with the results of Adam et al. [45] and Botwinick and Thompson [46].

Much research has been devoted to studying the effects of gender differences in RT, and it is often stated that men have faster and less variable RT than women. One possible explanation is that gender differences in RT variability may be due to the influence of sex hormones on the brain and, implicitly, can be expected in adults but not in children [47, 48]. Recently, interest in RT has been focused on medium RT and intraindividual RT variability, i.e., the consistency of an individual's response. Intraindividual variability, although highly correlated with mean RT, is a discrete measure of cognitive performance. A small number of studies have investigated gender differences in intraindividual variability in RT and show that women are less consistent than men [49]. Our study is in line with these findings. After NFB SMR training, healthy male subjects showed a significant association between NFB SMR power and cognitive evoked potential RT.

EEG-NFB training is a promising technique that helps an individual learn to modulate brain activity in order to achieve cognitive and behavioral enhancement.

Conclusion

This study showed the possibility of increasing the power of SMR by using the NFB training protocols. This result was confirmed using a placebo-control study group that showed no such effect. Further, the increase in the SMR power was followed by a decrease in RT in auditory evoked potentials. However, these results are limited to a positive effect of NFB SMR training on auditory attention only. Further studies should include different modalities of attention (visual, for instance) as well as different age groups (including children and adolescents).

Conflict of interest

The authors declare no conflict of interest.

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The levels of circulating long non-coding *RNA GAS5* in prostate carcinoma patients: a single-center study

Nivoi cirkulišuće *lncRNA GAS5* kod obolelih od karcinoma prostate: iskustvo jednog centra

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Abstract

Background/Aim. Prostate carcinoma (PCa) is second occurring carcinoma that affects the male population. Although PCa incidence rates are high, most cases have a favorable prognosis, with comfortable long-term life quality. The aim of the study was to compare long non-coding RNA (*lncRNA*) growth arrest-specific transcript 5 (*GAS5*) plasma levels between healthy individuals and patients with PCa, and also between PCa patients with different prognostic scores. **Methods.** The present study included a total of 40 patients with PCa and a control group of 20 healthy individuals. PCa patients were divided into two subgroups (20 patients each) based on the prognostic criteria of the American Joint Committee on Cancer. The patient data were collected and analyzed; *lncRNA GAS5* levels were quantified using the real-time polymerase chain reaction method. Statistical analysis was conducted using the IBM SPSS Statistics 26.0 computer program (IBM, USA, 2019). **Results.** The relative quantification of *lncRNA GAS5* expression levels showed down-regulation in PCa patients compared to healthy individuals; however, the difference was marginally statistically significant ($p = 0.056$). With further analysis of the given results, we concluded that the expression level of *lncRNA GAS5* was not significantly different in the first patient subgroup and the healthy individuals ($p = 0.268$). Patients from the second subgroup had significantly lower plasma levels of *lncRNA GAS5* than healthy individuals ($p = 0.033$). The difference in the level of *lncRNA GAS5* expression between patients with favorable prognoses (Group 1) and the ones with worse prognostic scores (Group 2) did not indicate statistical significance ($p = 0.275$). In both Group 1 ($p = 0.805$) and Group 2 ($p = 0.454$), the plasma levels of *lncRNA GAS5* were not significantly different in comparison to the age (≤ 65 vs. > 65 years). **Conclusion.** One of the main objectives of PCa research is identifying novel and more efficient biomarkers. Conducted research provides strong evidence about the significance of *lncRNAs GAS5* in PCa, as well as the correlation between decreased expression of *lncRNA GAS5* and poor prognosis in various tumors.

Key words:
biomarkers; prognosis; prostate neoplasms; rna, long noncoding.

Apstrakt

Uvod/Cilj. Karcinom prostate (KP) je po učestalosti drugi karcinom u muškoj populaciji. Premda je incidenca obolevanja od KP visoka, kod najvećeg broja bolesnika prognoza je povoljna, sa zadovoljavajućim kvalitetom života dugoročno. Cilj rada bio je da se utvrde razlike u nivoima *long non-coding RNA* (*lncRNA*) *growth arrest-specific transcript 5* (*GAS5*) u plazmi zdravih ispitanika i bolesnika sa KP, kao i između obolelih od KP u različitim prognostičkim stadijumima. **Metode.** U studiju je bilo uključeno 40 bolesnika sa KP i 20 zdravih osoba (kontrolna grupa). Bolesnici sa KP su, na osnovu prognostičkih kriterijuma *American Joint Committee on Cancer*, bili podeljeni u dve podgrupe (u svakoj po 20 bolesnika). Podaci o bolesnicima su prikupljeni i analizirani, a *lncRNA GAS5* je kvantifikovan korišćenjem metode lančane reakcije polimeraze u realnom vremenu. Statistička analiza podataka izvršena je pomoću programa IBM SPSS Statistics 26.0 (IBM, USA, 2019). **Rezultati.** Izmereni nivo ekspresije *lncRNA GAS5* bio je niži kod obolelih od KP u odnosu na zdrave osobe, mada granično statistički značajan ($p = 0,056$). Daljom analizom dobijenih podataka, utvrđeno je da razlika u nivou ekspresije *lncRNA GAS5* u prvoj podgrupi bolesnika i zdravih osoba nije bila

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statistički značajna (p = 0,268). Bolesnici iz druge podgrupe imali su značajno niže vrednosti *lncRNA GAS5* u odnosu na zdravu populaciju (p = 0,033). Razlika u nivou ekspresije *lncRNA GAS5* između bolesnika sa povoljnijom prognozom (Grupa 1) i bolesnika sa lošom prognozom (Grupa 2) nije bila statistički značajna (p = 0,275). U oba slučaja, u Grupi 1 (p = 0,805) i u Grupi 2 (p = 0,454), vrednosti *lncRNA GAS5* u plazmi nisu pokazale razliku u odnosu na starost bolesnika (≤ 65 vs. > 65 godina).

Zaključak. Jedan od glavnih ciljeva u istraživanju KP je pronalaženje novih i efikasnijih biomarkera. Sprovedeno istraživanje pruža jake dokaze o značaju *lncRNAs GAS5* u KP, kao i o povezanosti niskih vrednosti ekspresije *lncRNA GAS5* sa lošom prognozom kod različitih tumora.

Ključne reči:
biomarkeri; prognoza; prostata, neoplazme; rnk, duga nekodirajuća.

Introduction

Following lung cancer, prostate carcinoma (PCa) is the second leading carcinoma in males, accounting for 14.1% of all newly diagnosed cancers and 6.8% of all cancer-related deaths [1]. Its incidence in the countries with higher human development index (HDI) is 37.5 *per* 100,000 in comparison to 11.3 *per* 100,000 in countries with lower HDI [1]. The average age at the time of PCa diagnosis is 66, with the incidence and mortality increasing with age [2]. PCa is not an aggressive disease, but it usually metastasizes to bones and lymph nodes [3]. Despite high incidence, PCa has a favorable prognosis, with high quality of life [4]. The five-year survival rate for localized-stage cancer is 99.3%, with less than 6% progression to metastatic disease. The five-year survival rate for distant stage PCa has improved in the last decades and includes 32.3% of all patients [5].

There are more than 20 tumor markers currently used in tumor diagnostics, but only prostate-specific antigen (PSA) is used in prostate cancer [6]. In order to detect an early stage of asymptomatic PCa, PSA (normal serum level > 4.0 ng/mL) as a primary tumor marker is usually used in combination with digitorectal examination. However, studies have shown that levels of PSA in the serum are more specific for benign prostatic hyperplasia, so only 25% of people with increased levels of PSA will develop PCa [7]. There is a constant need for discovering novel biomarkers with higher specificity and sensitivity, which could be used in early diagnosis and follow-up of patients with PCa.

Nowadays, increased attention is directed toward examining the impact of long non-coding RNAs (*lncRNAs*) on cancer pathology [8–10]. Long non-coding RNAs, often called "genomic dark matter", are non-protein-coding transcripts with a length of more than 200 nucleotides. Their role in the human genome is mostly unknown, but novel studies have shown the involvement of *lncRNAs* in regulating cell proliferation and apoptosis. They can also interact with promoter or enhancer sequences to modulate gene expression and, consequently, act as tumor suppressors or oncogenes [10–13]. The finding, which indicates that *lncRNA* from tumor cells can be detected in the plasma, has prompted the idea of using *lncRNA* as a biomarker in cancer patients [9]. Indeed, accumulated knowledge on *lncRNA* has indicated their possible usage as diagnostic/prognostic markers and also as therapeutic targets in many tumors [14].

One of the well-known *lncRNAs* is growth arrest-specific transcript 5 (*GAS5*), a protein non-coding RNA of about 630 nucleotides, initially described as a tumor suppressor. This *lncRNA* is encoded by the *GAS5* gene (1q25), a member of the 5′-terminal oligopyrimidine (5′-TOP) gene family, comprised of 12 exons and 11 introns [15]. The *GAS5* introns are transcribed into 10 box C/D small nucleolar RNA (*snoRNA*) molecules involved in the epigenetic regulation of gene expression [9, 10]. Previous studies have not proven the connection between the expression levels of *lncRNA GAS5* and patients' age [16, 17]. Expression of *lncRNA GAS5* is decreased in growing tissue but increased in periods of dormancy, so its lower expression can predict a worse prognosis [18]. There are several tumors linked with lower expression of *lncRNA GAS5*, such as colorectal cancer, non-small-cell lung cancer (NSCLC), breast cancer, gliomas, and others [10]. Recent data have shown that the *lncRNA GAS5* levels were significantly downregulated in tissues of different tumors and patient plasma [19]. Furthermore, the *lncRNA GAS5* levels were reduced significantly in the PCa tissues and cell lines [20]. However, at this moment, published data on the expression of plasma *lncRNA GAS5* levels in PCa patients are deficient.

The aim of the study was to assess the variability of *lncRNA GAS5* plasma levels between healthy individuals and patients with PCa, between PCa patients with different prognostic scores, and the impact of patients' age on the *lncRNA GAS5* expression.

Methods

Type of study and patients

The present opservational, prospective, and case-control study included a total of 40 patients with PCa treated operatively or conservatively at the Clinic for Urology, Military Medical Academy (MMA), Belgrade, Serbia in 2021 and a control group of 20 healthy individuals. This research protocol was approved by the Ethics Committee of MMA (Approval No. from 26 April 2018), according to the principles of the Declaration of Helsinki. All of the participants signed an informed consent to participate in the study.

The PCa patients were divided into two subgroups (20 patients each) based on the prognostic criteria of the American Joint Committee on Cancer (AJCC) that includes Tumor, Nodes, Metastasis (TNM) classification, serum concentration of PSA, and tumor grade (Gleason score – GS) [21]. Group 1 included patients with favorable prognostic scores 1 and 2 according to AJCC criteria, while Group 2 included patients with AJCC unfavorable prognostic scores 3 and 4. Control

Table 1

Age, family history of prostate carcinoma, body mass index, and prostate-specific antigen level in the control group

Characteristic	Control group
Age (years)	37.2 ± 7.4
Family history of prostate carcinoma	
positive	1 (5)
negative	19 (95)
Body mass index	27.56 ± 3.67
Prostate-specific antigen (ng/mL)	0.90 ± 0.79

Results are shown as average ± standard deviation except family history, which is shown as numbers (percentages).

Table 2

Clinical characteristics of prostate carcinoma patients

Characteristic	Group 1	Group 2	p^1
Age (years)	64.5 ± 6.8	67 ± 5.9	0.189
Family history of prostate carcinoma			
positive	6 (30)	5 (25)	0.718
negative	14 (70)	15 (75)	
Body mass index	25.92 ± 3.49	27.14 ± 3.51	0.277
Prostate-specific antigen (ng/mL)			
≤10	13 (65)	7 (35)	0.058
>10	7 (35)	13 (65)	
Gleason grade			
1, 2, 3	20 (100)	17 (85)	0.231
4, 5	0 (0)	3 (15)	
Pathologic t stage			
2	20 (100)	3 (15)	**< 0.001**
3, 4	0 (0)	17 (85)	
Pathologic n stage			
N0	20 (100)	16 (80)	0.106
N1	0 (0)	4 (20)	
Involvement of bones, seminal vesicles, or locally advanced disease			
present	0 (0)	2 (10)	
absent	20 (100)	18 (90)	0.487

[1]*p*-values were calculated by chi-squared test or two-tailed Fisher exact test (when the characteristics were present in less than five patients); only values for body mass index and average age were compared using a two-tailed *t*-test.
Bolded value is statistically significant. Results are shown as numbers (percentages) except age and body mass index which are shown as average ± standard deviation.

group, Group 1, and Group 2 were matched with regard to PCa risk factors [family history, body mass index (BMI), smoking status, alcohol intake, and physical activity], excluding age. Individuals younger than 18 years of age and patients with the presence of other/secondary malignancies were not included in this study. Data about age, the presence of PCa in the family, BMI, and PSA level in the control group are presented in Table 1; clinical characteristics of PCa patients included in the present study are shown in Table 2. In parallel, the independent cohort for validation of *lncRNAs GAS5* quantification included 11 healthy subjects and 13 PCa patients (prognostic score 1–4). In the independent cohort, the average age of the healthy controls was 36.7 ± 8.6 years, while of the PCa patients, it was 66.2 ± 5.9 years.

Quantification of lncRNA GAS5

Peripheral blood was collected in tubes with ethylenediaminetetraacetic acid and delivered to the laboratory of the Center for Clinical Pharmacology, MMA. In order to separate plasma, blood was centrifuged at 1,200 g for 10 min at 4 ºC and subsequently at 12,000 g for 10 min at 4 °C. Plasma samples were stored at -40 ºC until RNA isolation (Isolate II RNA Mini Kit, Bioline, UK). Concentrations of total RNA were calculated after spectrophotometry at 260 nm (NanoPhotometer NP60, Implen, USA). In order to evaluate the integrity of isolated RNA, reverse transcription polymerase chain reaction (RT-PCR) and amplification of four different-in-length DNA sequences were employed for 30 randomly chosen samples (10 controls and 20 PCa).

The isolated total RNA (2 μg) was transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, UK) according to the manufacturer's instructions (25 ºC for 10 min; 37 °C for 120 min; 85 °C for 5 min; hold at 4 °C).

For further quantitative Real time-PCR (q-PCR) runs, 3 μL of cDNA was used. The qRT-PCR was performed using *GAS5* (sense: 5'-CTTGCCTGGACCAGCTTAAT-3', anti-

sense: 5'-AAGCCGACTCTCCATACCT-3') or housekeeping gene β-actin (sense: 5'-ACCCACACTGTGCCCATC TA-3', antisense: 5'-CGCAACCGCTCATTGCC-3') specific primers (Invitrogen, Thermo Fisher Scientific, UK) [22, 23], and Power SYBR® Green PCR Master Mix (Applied Biosystems, Thermo Fisher Scientific, UK), according to the manufacturer's instructions. The amplification was run through an initial denaturation (95 ºC for 5 min) followed by 50 cycles at 95 ºC for 15 sec and 60 ºC for 1 min on Step One Plus Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, USA). Relative quantification was performed by the comparative $2^{-\Delta\Delta}$Ct method, using healthy controls as calibrators.

Statistical analysis

The statistical analysis using the program IBM SPSS Statistics 26.0 (IBM, USA, 2019) was performed. All continuous variables were described in the form of mean ± standard deviation (SD). Comparisons of parametric variables between two groups were performed by independent samples *t*-test. Comparisons of parametric variables between the three groups were performed with the ANOVA test. The normality of data distribution was tested by the Kolmogorov-Smirnov test. Attributive variables were described as a proportion of the total number of patients. The Chi-squared (χ^2) test or Fischer's exact test was used for comparing categorical variables. All the analyses were evaluated at the level of statistical significance of $p < 0.05$.

Results

In order to validate the results of *lncRNA GAS5* expression in controls and PCa patients, we performed a validation study in an independent cohort (11 controls and 13 PCa patients with prognostic scores 1–4). As shown in Figure 1, levels of *lncRNA GAS5* were concordant between the validation cohort and the study population. In the validation cohort, levels of *lncRNA GAS5* were lower in PCa patients than in healthy subjects (0.78 ± 0.6 vs. 1.1 ± 0.41; $p = 0.055$).

The relative quantification of *lncRNA GAS5* levels in the plasma of PCa patients and healthy controls demonstrated a difference of marginal statistical significance ($p = 0.056$) between the groups, whereby *lncRNA GAS5* levels were lower in PCa patients (0.81 ± 0.52) than in controls (1.11 ± 0.59). Considering prognostic groups, levels of *lncRNA GAS5* were not significantly higher in the healthy controls compared with the patients from Group 1 (1.11 ± 0.59 vs. 0.91 ± 0.54; $p = 0.268$). However, patients from Group 2 had significantly lower plasma levels of *lncRNA GAS5* than the controls (0.72 ± 0.52 vs. 1.11 ± 0.59; $p = 0.033$). Group 1 had higher levels of *lncRNA GAS5* than Group 2, but the difference between the groups is not statistically significant (0.91 ± 0.54 vs. 0.72 ± 0.52; $p = 0.275$) (Figure 2).

The average age of the healthy controls was 37.2 ± 7.4 years, and of the PCa patients, it was 66.9 ± 6.5 years (64.5 ± 6.8 years for Group 1 and 67 ± 5.9 years for Group 2).

LncRNA GAS5 levels between the two prognostic groups of PCa patients (Group 1 vs. Group 2) were not significantly different ($p = 0.275$); *p*-values were obtained by independent samples *t*-test (Figure 3).

Plasma levels of *lncRNA GAS5* were not significantly different between the patients aged 65 or below and patients older than 65 years in both Group 1 (0.87 ± 0.94 vs. 0.94 ± 0.67; $p = 0.805$) and Group 2 (0.61 ± 0.5 vs. 0.79 ± 0.51; $p = 0.454$) (Figure 4). The *lncRNA GAS5* levels were not significantly different between Group 1 and Group 2 when analyses included only patients ≤ 65 years of age (0.82 ± 0.45 vs. 1.15 ± 0.75; $p = 0.247$) or patients older than 65 years (0.91 ± 0.54 vs. 0.91 ± 0.61; $p = 0.578$).

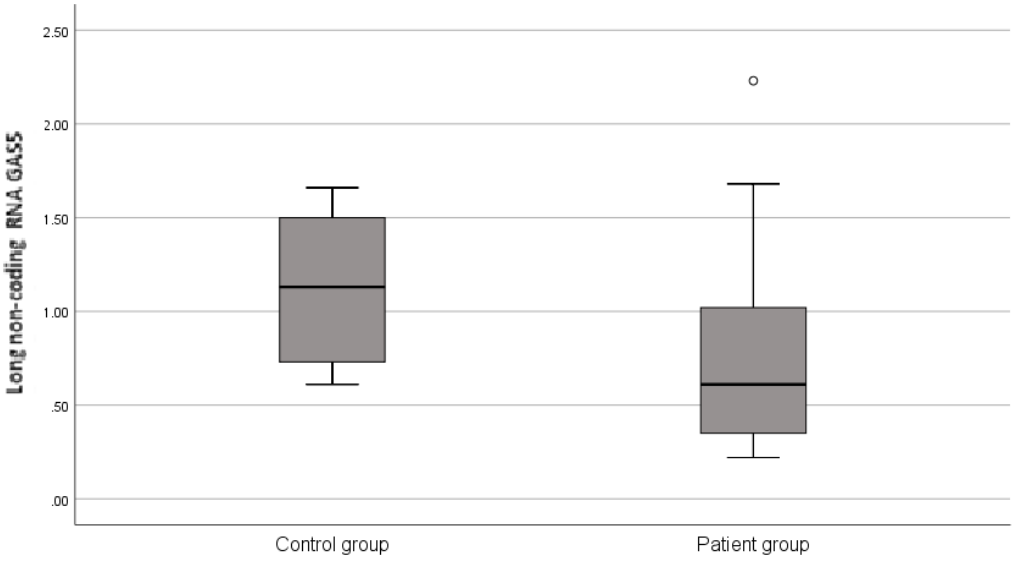
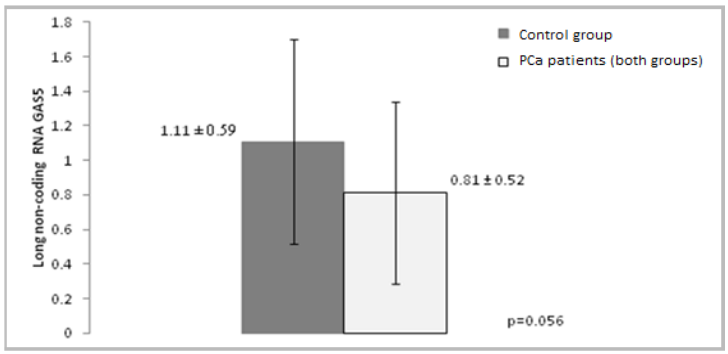
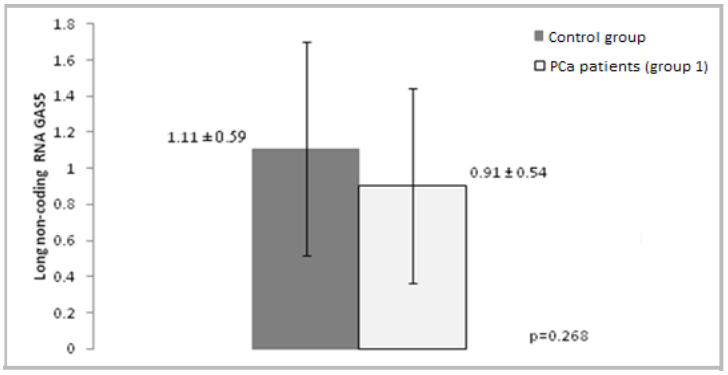


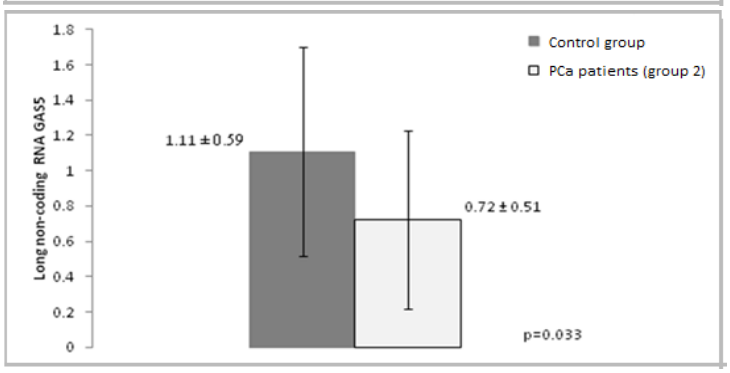
Fig. 1 – *LncRNA GAS5* levels in the independent cohort of healthy subjects and prostate carcinoma (PCa) patients. Levels of *lncRNA GAS5* were lower in PCa patients than in healthy subjects (0.78 ± 0.6 vs. 1.1 ± 0.41; $p = 0.055$). *p*-value was obtained by Mann-Whitney *U* test.



A)



B)



C)

Fig. 2 – *Lnc RNA GAS5* levels in prostate cancer (PCa) patients and controls: A) The difference in *lncRNA GAS5* levels between the controls and PCa patients was of marginal statistical significance ($p = 0.056$); B) The difference in *lncRNA GAS5* levels between controls and PCa patients from Group 1 (patients with favorable prognosis) was not statistically significant ($p = 0.268$); C) *LncRNA GAS5* levels between controls and PCa patients from Group 2 (patients with poor prognosis) were significantly different ($p = 0.033$).
***p*-values were obtained by independent samples *t*-test.**

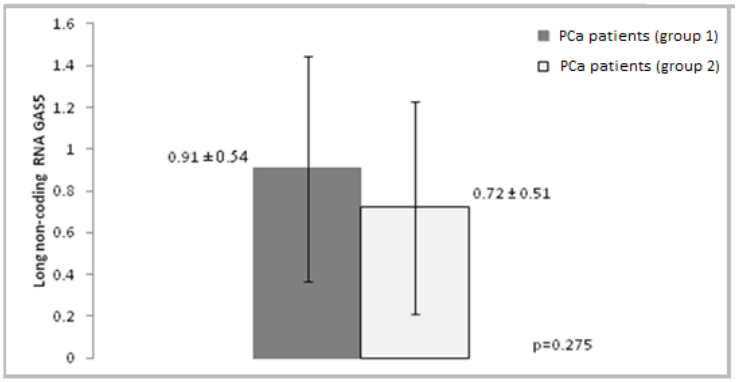


Fig. 3 – *LncRNA GAS5* levels in prostate carcinoma (PCa) patients with different prognostic scores (Group 1 vs. Group 2); $p = 0.275$.
***p*-value was obtained by independent samples *t*-test.**

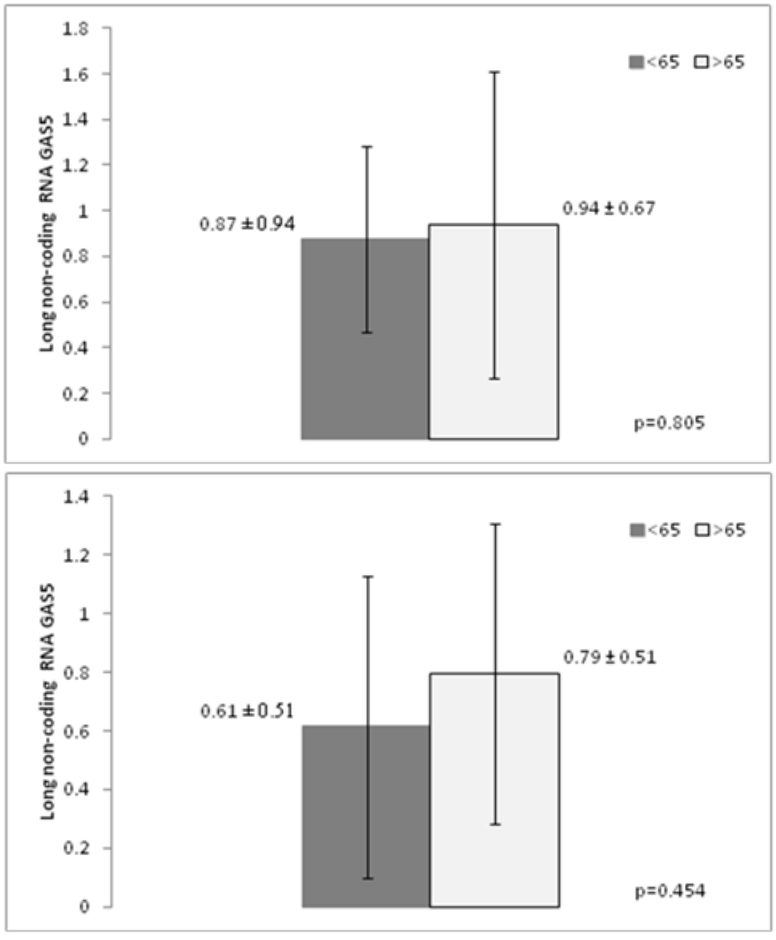


Fig. 4 – *LncRNA GAS5* levels in prostate carcinoma (PCa) patients of different age groups. Levels of *lncRNA GAS5* were not significantly different in patients ≤ 65 years compared to patients > 65 years of age nor in Group 1 (p = 0.805) (A), nor in Group 2 (p = 0.454) (B). p-values were obtained by independent samples t-test.

Discussion

As mentioned earlier, findings of relatively stable *lncRNAs* plasma concentration have implied the possible usage of circulating *lncRNAs* as a noninvasive diagnostic biomarker in different pathologic conditions. The research was conducted for various tumor types, including NSCLC [9], breast cancer [24], diffuse large B-cell lymphoma (DLBCL) [25], malignant mesothelioma [16], and many nonmalignant diseases, such as type 2 diabetes mellitus [26]. In this research, the expression of *lncRNA GAS5* was quantified in plasma samples of PCa patients.

We found that *lncRNA GAS5* expression levels were downregulated in the PCa patients compared to healthy individuals (p = 0.056); however, the difference was of marginal statistical significance. The possible reason for marginal statistical significance was the relatively small sample size. Similar results were obtained by Vesovic et al. [9] and Tan et al. [19] in NSCLC patients, as well as Han et al. [24] in breast cancer patients. However, Senousy et al. [25] reported that *lncRNA GAS5* expression was significantly downregulated ($p < 0.0001$) in the overall DLBCL patients compared to the control group. In patients with malignant mesothelioma, Weber et al. [16] reported a low sensitivity of 14% when *lncRNA GAS5* was used as a single marker, a combination of calretinin and mesothelin showed a sensitivity of 64%, but a panel composed of *lncRNA GAS5*, calretinin, and mesothelin reached a sensitivity of 73% (at a predefined specificity of 97%). Similarly, Tan et al. [19] found a strong association of *lncRNA GAS5* levels with carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) (p = 0.017 and p = 0.001, respectively) and suggested that a combination of circulating *lncRNA GAS5* levels with CEA and CA19-9 was more advantageous for the diagnosis of the early stage of NSCLC. On the other hand, in the research of Senousy et al. [25], a panel of HOX transcript antisense intergenic RNA (*HOTAIR*) and *lncRNA GAS5* demonstrated good results for response assessment in DLBCL patients treated with rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP).

It is evident that the level of *lncRNA GAS5* expression varies across tumor types; the results of the present study indicate that *lncRNA GAS5* as a single marker was not significantly downregulated in PCa patients with favorable prognosis. However, we can only assume that in combination with PSA and other potential biomarkers, *lncRNA GAS5* could be used in PCa diagnostics.

Furthermore, our results showed that the level of *lncRNA GAS5* expression was not significantly different be-

tween patients with favorable prognosis and the healthy individuals ($p = 0.268$) nor between patients with different prognostic scores ($p = 0.275$). However, levels of *lncRNA GAS5* were significantly different between patients with AJCC unfavorable prognostic scores 3 and 4 and healthy individuals ($p = 0.033$). Vesovic et al. [9] did not observe a difference in *lncRNA GAS5* expression between early-stage tumor patients (TNM stage I/II) and healthy controls; however, there was an apparent difference in the TNM stage III patients. Moreover, they found that circulating *lncRNA GAS5* was a potent predictor of tumor size since patients with tumors > 3 cm had a significantly diminished expression compared to patients with tumors ≤ 3 cm. These results were expected since *lncRNA GAS5* is a well-known tumor suppressor.

The origin of *lncRNAs* in the bloodstream remains unexplained. Xue et al. [20] showed that *lncRNA GAS5* levels were reduced significantly in the PCa tissues compared to healthy tissue ($p < 0.05$); overexpression of *lncRNA GAS5* significantly decelerated tumor growth through the inactivation of a serine/threonine-specific protein kinase/the mammalian target of rapamycin (AKT/mTOR) signaling pathway ($p < 0.05$). In NSCLC patients, Tan et al. [19] showed that *lncRNA GAS5* decreased in NSCLC tissues compared to noncancerous tissues ($p < 0.001$). Furthermore, there was a weak connection between 55 paired plasma samples and cancer tissues obtained from the same individuals with NSCLC ($p > 0.05$). Despite the connection being weak, it could be concluded that changes in the levels of circulating nucleic acids were associated with tumor burden and malignant progression and that circulating *lncRNA GAS5* originated from the self-secretion, apoptotic, or necrotic tumor cells [27].

In our study, we also analyzed the patient's age and determined a statistically significant difference between the PCa patients and the healthy individuals ($p < 0.001$), which is expected considering that PCa is a common disease in older males. Furthermore, our patient age correlates with literature data, with cited average age at the moment of diagnosis being almost 66 years [24]. We found that the level of *lncRNA GAS5* expression does not differ significantly between tested patients below and over 65 years of age and also between patients younger and older than 65 years of age in Groups 1 and 2 (independent samples test, $p = 0.805$, $p = 0.454$, $p = 0.247$, and $p = 0.578$, respectively). Differing *lncRNA GAS5* expression between age groups has not been determined in other research so far, which correlates with our results [8, 21].

Conclusion

One of the main priorities in PCa research is the identification of novel biomarkers. Conducted research provides strong evidence about the significance of *lncRNAs* in PCa, as well as the correlation between decreased expression of *lncRNA GAS5* and poor prognosis in various tumors. The results of this study are based on a relatively small patient size. Future prospective studies on a larger scale could show the true value of the plasma *lncRNA GAS5* expression level in PCa patients either as a single marker or in combination with other biomarkers.

Acknowledgment

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Conflict of interest

The authors declare no conflict of interest.

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Evaluation of the retinal morphological and functional findings in optic neuritis related to multiple sclerosis

Procena rezultata morfološkog i funkcionalnog ispitivanja mrežnjače kod bolesnika sa optičkim neuritisom u sklopu multiple skleroze

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Abstract

Background/Aim. Unilateral optic neuritis (ON), and its pathological substrate, retrobulbar neuritis (RBN), is a common presentation of multiple sclerosis (MS). The aim of the study was to determine the diagnostic and prognostic value of structural and functional examination using novel 'swept-source' optical coherence tomography (SS-OCT) and OCT angiography (OCTA) techniques in patients with MS who experienced RBN. **Methods.** For examining retinal structural and functional changes in both the affected and nonaffected eye of patients with MS, novel techniques, OCT and OCTA, were used. The obtained results were compared with the results of the same examination on the left and right eye of the healthy controls. **Results.** Using OCT, significant differences in the structural integrity and thickness of retinal layers between the eye in which RBN had been detected and the contralateral, nonaffected eye were found (83.73 ± 18.36 vs. 98.67 ± 11.84; $p = 0.013$). On the other hand, the functional examination of the macular vascular plexus did not show significant differences between the affected and the nonaffected eye in these patients (41.86 ± 1.52 vs. 42.52 ± 1.40; $p = 0.228$). Interestingly, comparing the nonaffected eye of patients with RBN and healthy controls, a significant difference in the thickness of the retinal layers between the contralateral eye of the patient and both healthy eyes of healthy subjects was found. OCT examination showed particularly significant thinning of the macular ganglion cell-inner plexiform layer (mGCIPL) (61.07 ± 5.04 vs. 67.53 ± 4.57; $p < 0.001$). **Conclusion.** Overall, our research showed that OCT and OCTA offer an unprecedented opportunity for a safe, reliable, and repetitive assessment of structural and functional retinal changes as invaluable diagnostic and prognostic tools, paving the way for a better understanding of pathogenic mechanisms underlying inflammatory demyelinating and neurodegenerative diseases. In addition, mGCIPL may be a particularly sensitive and reliable biomarker of pathological changes in MS and perhaps in other neurodegenerative diseases.

Key words:
angiography; diagnosis; multiple sclerosis; optic neuritis; tomography, optical coherence.

Apstrakt

Uvod/Cilj. Jednostrani optički neuritis (ON), kao i njegov patofiziološki supstrat, retrobulbarni neuritis (RBN), česta je manifestacija multiple skleroze (MS). Cilj rada bio je da se utvrdi dijagnostički i prognostički potencijal nove metode funkcijskog i strukturnog ispitivanja oka, tzv. „*swept-source*" optičke koherentne tomografije (SS-OKT) i OKT angiografije (OKTA), kod bolesnika sa prethodnom epizodom RBN u okviru MS. **Metode.** Za ispitivanje strukture i funkcije mrežnjače bolesnika sa MS, kako u oku na kome je dijagnostikovan ON, tako i na kontralateralnom oku, korišćene su nove metode, OKT i OKTA. Dobijeni rezultati upoređivani su sa rezultatima istovetnog ispitivanja na levom i desnom oku zdravih ispitanika. **Rezultati.** Korišćenjem OKT, pokazane su značajne razlike u strukturnom integritetu i debljini pojedinih slojeva mrežnjače u oku bolesnika sa RBN, u poređenju sa kontralateralnim okom (83,73 ± 18,36 vs. 98,67 ± 11,84; $p = 0,013$). Sa druge strane, nisu pokazane značajne razlike u perfuziji horoidnog pleksusa ni u jednom oku tih bolesnika (41,86 ± 1,52 vs. 42,52 ± 1,40; $p = 0,228$). Interesantno, poređenjem oka obolelih koje nije bilo zahvaćeno RBN sa zdravim kotrolama, pronađena je značajna razlika u debljini slojeva mrežnjače između kontralateralnog oka bolesnika i oba zdrava oka zdravih ispitanika. Pregled pomoću OKT pokazao je

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posebno značajno istanjenje unutrašnjeg pleksiformnog makularnog sloja mrežnjače (61,07 ± 5,04 vs. 67,53 ± 4,57; $p < 0,001$). **Zaključak.** Naše istraživanje pokazalo je da OKT i OKTA, kao dragocene metode u dijagnostici i prognozi, pružaju odlične mogućnosti za bezbednu, pouzdanu i ponavljanu procenu strukturnih i funkcijskih promena na mrežnjači, ali i za dalja istraživanja mehanizama koja dovode do takvih promena. Takođe, unutrašnji pleksiformni makularni sloj mrežnjače bi mogao biti naročito osetljiv i vredan biomarker u praćenju patoloških promena kod bolesnika sa MS, a moguće i kod drugih neurodegenerativnih oboljenja.

Ključne reči:
angiografija; dijagnoza; multipla skleroza; tomografija, optička, koherentna; n.opticus, neuritis.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). It is one of the most common neurological diseases of the young population, affecting over two million people worldwide [1, 2]. Optic neuritis (ON) is a presenting manifestation of MS in around 25% of cases and occurs at some point in the disease in over half of the patient population [3, 4].

Optic nerve atrophy and thinning of the retinal nerve fiber layer (RNFL) are typical pathological features in patients with MS. Demyelinating retrobulbar optic neuritis (RBN) refers to inflammation and demyelination affecting the optic nerve. In the absence of pathological features in the ocular fundus, these inflammatory and demyelinating processes in the optic nerve are thought to underlie the production of common symptoms of ON, such as acute unilateral loss of vision, relative afferent pupillary defect, and pain during eye movements [5–8]. Dysfunction and degeneration of the optic nerve axon fibers in the optic nerve are early features of the disease; however, the relationship between inflammation, demyelination, and axon loss is unclear. It has been suggested that axon loss may be a result of the delayed type IV hypersensitivity reaction mediated via cytokines and other inflammatory mediators secreted by infiltrating pathogenic T cells. Namely, during ON, acute inflammation is thought to damage axons within the retrobulbar area of the optic nerve. Degeneration initiated at this area progresses retrogradely (dye back) towards the ganglion cell layer, which manifests as residual paleness of the optic nerve head during routine ophthalmoscopy [9–11].

Indeed, axon loss is considered a major underlying mechanism of disability in MS. However, axon loss is hard to quantify. Modern technologies of optical imaging, such as optical coherence tomography (OCT), offer opportunities for such quantification, at least in the retina and optic nerve [12–15]. Namely, OCT, discovered in 1991 (*in vitro* HUANG, *in vivo* HEE 1995), enables examination of a cross-section of inner ocular structures in high resolution, reaching 1–15 μm. This resolution is higher than that which can be currently achieved using ultrasound, magnetic resonance imaging (MRI), and computed tomography. Furthermore, this technique enables visualization *in situ* and in real-time. Importantly, OCT uses a continual, low-frequency beam (infrared spectrum, 800 nm) delivered at less than 1 mW power, making even repeated exposure safe for both general and patient populations [16, 17].

Thus far, the use of OCT has revealed a decrease in the thickness of peripapillary RNFL (pRNFL) in MS patients who had not suffered from ON compared with controls. This finding is in agreement with a large body of literature describing the association of MS with loss of retinal ganglion cells, reduced thickness of RNFL, loss of metabolic activity, and reduced density of retinal vascular plexus [18, 19]. Furthermore, using an adaptation of OCT, the so-called OCT with angiography (OCTA), a decreased vascular perfusion in the region of the optic nerve head (optic disc) in patients with MS, particularly MS patients with a history of ON, was found [20, 21]. Indeed, combining OCTA with other OCT methods vastly improves the diagnostic accuracy of changes in retinal structures in patients with MS.

In recent years, techniques of digital imaging have been further advanced. The latest improvement in the examination of retinal and choroid structures utilizes the so-called swept-source OCT (SS-OCT) beam technology which uses a longer wavelength (1,050 nm) and minimal reflection of choroid plexus than its predecessor, spectral domain (SD-OCT, 840 nm). Hence, SS-OCT enables visualization of the deepest layers of the eye, even through physical obstacles such as cataract, hemorrhage, etc. In addition, the scan speed of SS-OCT is twice that of SD-OCT devices (100,000 A-scans/s compared with 50,000 A-scans/s), enabling faster acquisition and more accurate 3-D imaging of the retina and choroid [22, 23].

In this study, we measured the thickness of the retina and perfusion of the choroid plexus in patients with MS who experienced a previous episode of ON, in both the affected and nonaffected eye and in healthy controls, using a new, deep-range SS-OCT. Our aim was to establish the diagnostic and prognostic value of structural and functional examination using SS-OCT and OCTA techniques in patients with MS who experienced RBN.

Methods

Study design and study subjects

An observational case study was conducted in patients with relapsing-remitting MS and a history of ON and healthy controls, in the period between 2020 and 2022, at the Department of Functional Ophthalmology of the Eye Clinic at the Military Medical Academy, Belgrade, Serbia. The study protocol was was carried out according to the Declaration of Helsinki. All the participants were informed about the

research protocol before giving their written consent to participate in the study.

We examined 15 patients (4 males and 11 females) with confirmed MS according to the McDonald criteria, who had a history of RBN. The average age of the MS patients was 32 years (range 25–43 years). In the control group, we examined 30 healthy volunteers (7 males and 23 females) chosen by a random sample. The average age in this group was 42.5 years (range 36.5–48 years).

Clinical procedures

All patients were examined using head MRI and visual evoked potentials (VEP) to confirm the diagnosis of MS and ON. Patients with a history of ON were assessed using a standardized ophthalmological examination battery: visual acuity, fundus examination, and visual fields examination.

OCT and OCTA were performed using the Topcon-Triton apparatus (Topcon DRI OCT Triton Swept-source OCT, Topcon, Japan), with a 1,050 nm wavelength beam and a scan speed of 100,000 A-scans/s. We measured pRNFL thickness, macular ganglion cell layer (GCL) thickness, and superficial macular perfusion. The thickness of pRNFL was obtained using circular scanning of the optic disc, whereas the macular ganglion cell-inner plexiform layer (mGCIPL) thickness was obtained by scanning the macular volume in the center of the fovea. Images, maps, and data were displayed directly in Topcon software (Figures 1–3). Numerical data were exported to Microsoft Excel.

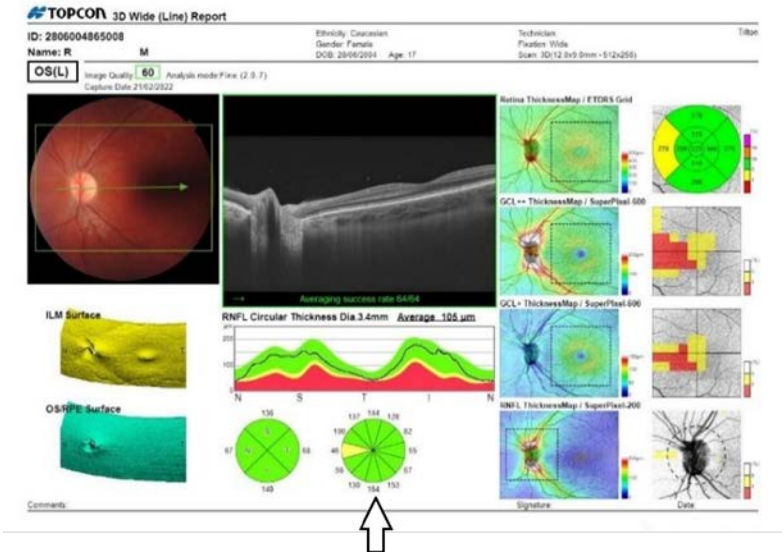

Fig. 1 − Representative images of the retinal nerve fiber layer (RNFL) of the papillary area in the affected eye obtained using Topcon optical coherence tomography (OCT).
Note: The atrophy of papillary retinal nerve fibers in the nasal quadrant.

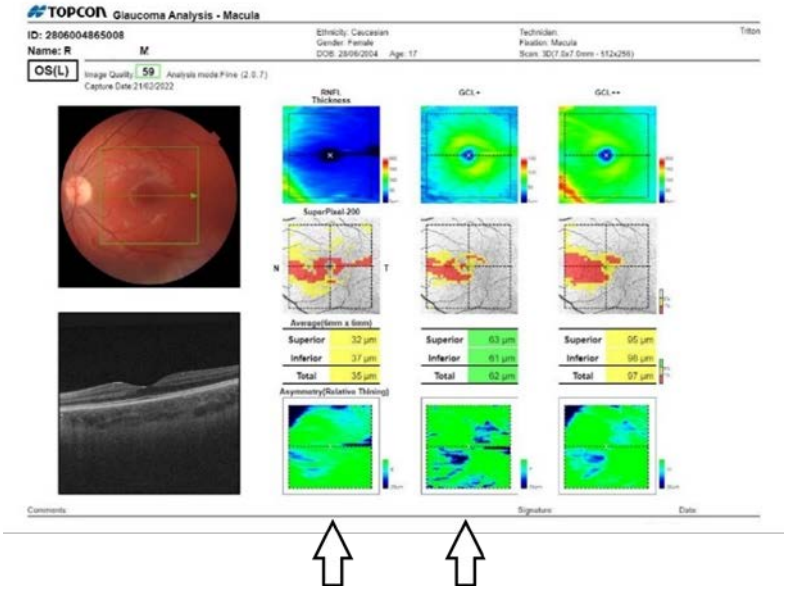

Fig. 2 − Representative images of the ganglion cell layer (GCL) and inner plexiform layer (IPL) in the macula of the affected eye. Bottom left panel shows a cross-section view through the *fovea centralis*, with distinguishable retinal layers.
Note: The GCL and IPL images show a loss of thickness.

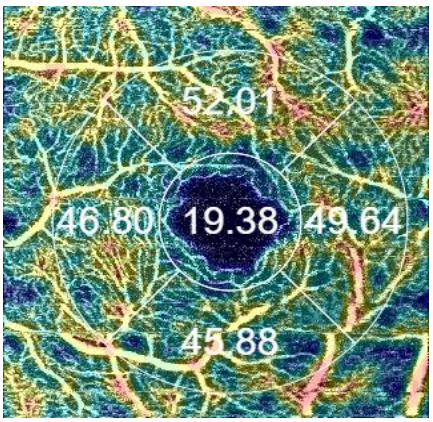

Fig. 3 − Representative image of foveolar and parafoveolar quadrants of the superficial layer of macula examined using optical coherence tomography angiography.

Statistical analysis

The normality of data distribution was tested using the Kolmogorov-Smirnov test. Categorical variables are presented as frequencies and analyzed using the χ^2 test. Continually data are presented as means and standard deviation and analyzed using the matched and nonmatched Student *t*-test. Results are considered statistically significant for a *p*-value lower than 0.05. All analyses were performed using the statistical package IBM SSPS 26.0.

Results

Control group

There were 30 healthy persons in total in the control group (23 females and 7 males), with a mean age of 42.50 years (range 36.50–48.00 years). In order to determine the reference values, we performed OCT and OCTA measurements in healthy controls and compared the values obtained in the left and right eye, as detailed in Table 1. All measurements were within the physiological range. As expected, we found no significant differences between the eyes, apart from a marginal difference in the choroid plexus density in the nasal (N) quadrant (45.97 ± 1.82 vs. 47.922 ± 2.44, right vs. left eye, respectively; $p = 0.028$).

MS patients with RBN

Next, we performed an identical examination in patients with MS, confirmed with the 2017 McDonald criteria, and a loss of vision in one eye, and compared the affected with the nonaffected eye. The average age of these 15 patients (4 males and 11 females) was 32 years. The characteristics of patients with RBN are summarized in Table 2. In

Table 1

Results of OCT and OCTA examination in healthy subjects

Parameter	Right eye	Left eye	*p*-value*
Visual acuity	0.98 ± 0.06	0.99 ± 0.06	0.833
RNFL thickness (μm)	110.27 ± 9.77	110.30 ± 9.61	0.989
superior quadrant	132.73 ± 12.36	134.77 ± 13.82	0.550
inferior quadrant	141.40 ± 16.66	141.70 ± 17.00	0.945
nasal quadrant	85.87 ± 13.68	84.10 ± 13.89	0.622
temporal quadrant	80.33 ± 9.62	78.77 ± 10.04	0.540
mGCIPL (μm)	67.37 ± 4.66	67.70 ± 4.55	0.780
superior	68.10 ± 4.84	68.10 ± 4.78	1.000
inferior	66.63 ± 4.58	67.17 ± 4.42	0.648
Total mRNFL (μm)	41.10 ± 4.54	41.30 ± 5.27	0.875
superior quadrant	39.43 ± 4.69	39.43 ± 5.01	1.000
inferior quadrant	42.70 ± 5.12	43.17 ± 5.74	0.741
OCTA	42.72 ± 1.54	42.87 ± 1.46	0.691
superior quadrant	50.28 ± 2.92	50.42 ± 2.82	0.855
inferior quadrant	49.75 ± 4.40	49.57 ± 3.38	0.859
temporal quadrant	47.65 ± 2.29	46.81 ± 2.42	0.171
nasal quadrant	45.97 ± 1.82	47.22 ± 2.44	0.028
central quadrant	20.05 ± 3.79	20.24 ± 4.17	0.857

OCT – optical coherence tomography; RNFL – retinal nerve fiber layer; mGCIPL − macular ganglion cell-inner plexiform layer; mRNFL – macular RNFL; OCTA – OCT angiography. All values are expressed as mean ± standard deviation.*Independent samples test.

Table 2

General characteristics of patients with RBN

Parameter	Values
Subjects, n	15
Gender, male/female, n (%)	4 (26.7)/11 (73.3)
Age, mean (min-max)	32.00 (25.00–43.00)
Number of patients with loss of VA	15
VA, mean (min-max)	5.00 (1.00–10.00)
Affected eye, left/right, n (%)	10 (66.7)/5 (33.3)

RBN − retrobulbar neuritis; VA – visual acuity; min – minimum; max – maximum; n – number.

these patients, we found that visual acuity was significantly lower before (0.28 ± 0.21) the administration of 1,000 mg of methylprednisolone *iv* as a five-day pulsed treatment than after the treatment was concluded (0.85 ± 0.24).

We found a statistically significant difference in VEP parameters between the affected and nonaffected eye. Namely, the latency was significantly increased in the affected eye (147.20 ± 26.20) compared with the nonaffected eye (119.20 ± 9.61; $p = 0.001$). However, the amplitude of VEP was significantly lower in the affected eye (6.21 ± 3.50) than in the nonaffected eye (9.95 ± 2.78; $p = 0.003$), suggesting neuronal and axonal loss on the affected side. Indeed, further analysis showed a significant loss of RNFL on the affected side compared with the nonaffected side (83.73 ± 18.36 vs. 98.67 ± 11.84; $p = 0.013$). Detailed analysis by quadrants showed that the loss affected the temporal (50.93 ± 13.18 vs. 68.53 ± 14.78; $p = 0.002$), superior (105.13 ± 22.69 vs. 121.87 ± 16.76; $p = 0.029$), and inferior (108.87 ± 27.79 vs. 131.20 ± 19.67; $p = 0.017$) quadrants, but not the nasal quadrant (67.20 ± 14.18 vs. 73.47 ± 14.07; $p = 0.235$).

In addition, we found significant differences in the total thickness of mGCIPL. Namely, total thickness of mGCIPL layers was significantly lower in the affected than the nonaffected eye (54.73 ± 6.06 vs. 61.07 ± 5.04; $p = 0.004$), including that of superior (55.80 ± 6.13 vs. 62.20 ± 5.24; $p = 0.005$) and inferior quadrants (53.47 ± 6.00 vs. 60.20 ± 4.93; $p = 0.002$). Furthermore, the total thickness of macular RNFL was significantly thinner in the affected (31.20 ± 7.64) compared with the nonaffected eye (38.20 ± 5.00; $p = 0.006$). Interestingly, no differences were found in OTCA measurements. Detailed findings in both eyes are presented in Table 3.

Pathological changes within the affected eye in patients with MS compared with the healthy control group

We compared the OCT data (RNFL, mGCIPL, macular RNFL) of the affected eye in MS patients with the healthy controls (both eyes). Indeed, we found that all OCT-derived parameters were significantly lower in the patients than in the healthy controls (Table 4). In addition, the thickness of RNFL and mGCIPL in the nonaffected eye in the patient population was also significantly lower than in healthy controls. Similarly, in the affected eye of patients with RBN, the measurements of the density of choroid plexus obtained using OCTA were significantly lower in the affected eye of the patients compared with the healthy controls (41.86 ± 1.52 vs. 42.80 ± 1.49, respectively; $p = 0.034$), with the single exception for the temporal quadrant.

mGCIPL in the nonaffected eye in patients with RBN compared with the healthy control group

While comparing the nonaffected eye of MS patients with healthy controls, the thickness of RNFL and, in particular, the mGCIPL were significantly reduced (RNFL: 98.67 ± 11.84 for the nonaffected eye in RNB vs. 110.28 ± 9.61 for the healthy controls; $p < 0.001$; mGCIPL 61.07 ± 5.04 for the nonaffected eye vs. 67.53 ± 4.57 for controls; $p < 0.001$). Indeed, the statistically significant reduction between the thickness in all examined quadrants in MS patients vs. controls was replicated (Table 5).

Interestingly, however, the density of the choroid plexus of the nonaffected eye in the patient population was very similar to that of healthy controls (41.86 ± 1.52 vs. 42.52 ±

Table 3

Results of OCT and OCTA examination in MS patients with RBN

Parameter	Affected eye	Nonaffected eye	*p*-value*
VA before methylprednisolone therapy	0.28 ± 0.21	0.99 ± 0.05	< 0.001
VA after methylprednisolone therapy	0.85 ± 0.24	0.99 ± 0.05	0.041
VEP latency	147.20 ± 26.20	119.20 ± 9.61	0.001
VEP amplitude	6.21 ± 3.50	9.95 ± 2.78	0.003
Visual field quadrant degree	3.01 ± 1.08	1.85 ± 1.61	0.028
RNFL (μm)	83.73 ± 18.36	98.67 ± 11.84	0.013
superior quadrant	105.13 ± 22.69	121.87 ± 16.76	0.029
inferior quadrant	108.87 ± 27.79	131.20 ± 19.67	0.017
nasal quadrant	67.20 ± 14.18	73.47 ± 14.07	0.235
temporal quadrant	50.93 ± 13.18	68.53 ± 14.78	0.002
mGCIPL (μm)	54.73 ± 6.06	61.07 ± 5.04	0.004
superior quadrant	55.80 ± 6.13	62.20 ± 5.24	0.005
inferior quadrant	53.47 ± 6.00	60.20 ± 4.93	0.002
Total mRNFL (μm)	31.20 ± 7.64	38.20 ± 5.00	0.006
superior quadrant	29.73 ± 7.55	36.27 ± 4.06	0.006
inferior quadrant	32.53 ± 8.40	39.80 ± 6.55	0.013
OCTA	41.86 ± 1.52	42.52 ± 1.40	0.228
superior	48.43 ± 3.53	49.58 ± 2.33	0.300
inferior	46.84 ± 3.56	48.69 ± 3.14	0.144
temporal	46.77 ± 2.67	46.25 ± 1.98	0.546
nasal	45.18 ± 2.97	46.56 ± 1.68	0.128
central	22.73 ± 4.52	21.64 ± 3.72	0.475

MS – multiple sclerosis; RBN − retrobulbar neuritis; VA – visual acuity; VEP − visual evoked potential. For the abbreviations of other terms see Table 1. All values are expressed as mean ± standard deviation. *Independent samples test.

Table 4

OCT and OCTA findings in the affected eye in patients with RBN and healthy controls (both eyes)

Parameter	Affected eye in RBN	Healthy controls – both eyes	*p*-value*
Visual acuity	0.28 ± 0.21	0.99 ± 0.06	< 0.001
RNFL (μm)	83.73 ± 18.36	110.28 ± 9.61	< 0.001
superior quadrant	105.13 ± 22.69	133.75 ± 13.04	< 0.001
inferior quadrant	108.87 ± 27.79	141.55 ± 16.69	< 0.001
nasal quadrant	67.20 ± 14.18	84.98 ± 13.70	< 0.001
temporal quadrant	50.93 ± 13.18	79.55 ± 9.78	< 0.001
mGCIPL (μm)	54.73 ± 6.06	67.53 ± 4.57	< 0.001
superior quadrant	55.80 ± 6.13	68.10 ± 4.77	< 0.001
inferior quadrant	53.47 ± 6.00	66.90 ± 4.47	< 0.001
Total mRNFL(μm)	31.20 ± 7.64	41.20 ± 4.87	< 0.001
superior quadrant	29.73 ± 7.55	39.43 ± 4.81	< 0.001
inferior quadrant	32.53 ± 8.40	42.93 ± 5.40	< 0.001
OCTA	41.86 ± 1.52	42.80 ± 1.49	0.034
superior quadrant	48.43 ± 3.53	50.35 ± 2.85	0.029
inferior quadrant	46.84 ± 3.56	49.66 ± 3.89	0.013
nasal quadrant	46.77 ± 2.67	47.23 ± 2.38	0.518
temporal quadrant	45.18 ± 2.97	46.59 ± 2.22	0.043
central quadrant	22.73 ± 4.52	20.14 ± 3.95	0.031

RBN − retrobulbar neuritis. For the abbreviations of other terms see Table 1. All values are expressed as mean ± standard deviation. *Independent samples test.

Table 5

OCT and OCTA findings in the nonaffected eye in patients with RBN and healthy controls (both eyes)

Parameter	Nonaffected eye in RNB	Healthy controls – both eyes	*p*-value*
Visual acuity	0.99 ± 0.05	0.99 ± 0.06	0.922
RNFL (μm)	98.67 ± 11.84	110.28 ± 9.61	< 0.001
superior quadrant	121.87 ± 16.76	133.75 ± 13.04	0.004
inferior quadrant	131.20 ± 19.67	141.55 ± 16.69	0.042
nasal quadrant	73.47 ± 14.07	84.98 ± 13.70	0.005
temporal quadrant	68.53 ± 14.78	79.55 ± 9.78	0.001
mGCIPL (μm)	61.07 ± 5.04	67.53 ± 4.57	< 0.001
superior quadrant	62.20 ± 5.24	68.10 ± 4.77	0.001
inferior quadrant	60.20 ± 4.93	66.90 ± 4.47	< 0.001
Total mRNFL (μm)	38.20 ± 5.00	41.20 ± 4.87	0.037
superior quadrant	36.27 ± 4.06	39.43 ± 4.81	0.022
inferior quadrant	39.80 ± 6.55	42.93 ± 5.40	0.058
OCTA	42.52 ± 1.40	42.80 ± 1.49	0.519
superior quadrant	49.58 ± 2.33	50.35 ± 2.85	0.336
inferior quadrant	48.69 ± 3.14	49.66 ± 3.89	0.374
nasal quadrant	46.25 ± 1.98	47.23 ± 2.38	0.144
temporal quadrant	46.56 ± 1.68	46.59 ± 2.22	0.959
central quadrant	21.64 ± 3.72	20.14 ± 3.95	0.189

RBN − retrobulbar neuritis. For the abbreviations of other terms see Table 1. All values are expressed as mean ± standard deviation. *Independent samples test.

1.40, respectively; $p = 0.228$), suggesting that vascular plexus density is a less sensitive parameter than inner ocular layer thickness in the assessment of ocular changes in MS.

Correlation analysis

In order to examine the relationship between the outcome measures obtained using OCT and OCTA, we performed correlation analyses. Indeed, in the affected eye of patients with RBN, we found a highly significant, positive correlation between the RNFL and mGCIPL [Pearson Correlation (r) = 0.833; $p < 0.001$], the tRNFL and macular RNFL ($r = 0.860$; $p < 0.001$), and the mGCIPL and macular RNFL ($r = 0.783$; $p = 0.001$). On the other hand, parameters measured using OCTA did not show a correlation between any variables either in the affected or in the nonaffected eye. In contrast, in healthy controls, a positive correlation between values of OCTA in both the left and right eye was found ($r = 0.491$; $p = 0.006$).

Discussion

Two retinal layers are of considerable importance in MS: the pRNFL, comprised of unmyelinated axons which

form the optic nerve, and the combined mGCIPL which contains the retinal ganglion cells [22, 23]. RNFL is the most proximal part of the afferent optic nerve pathway and a unique pathway in the CNS completely devoid of myelin. Therefore, it is considered one of the most vulnerable regions to pathological and metabolic challenges.

Given that structural changes in these two layers have been associated with visual symptoms and that the retina may be considered an extension of the CNS ("window into the brain"), a high-resolution quantitative assessment of these structures offers an unprecedented opportunity to examine and predict functional optic and wider CNS damage. Indeed, in recent years, the changes affecting the macular inner nuclear layer (INL) have become a major subject of interest as a potential biomarker for CNS inflammation [24–26].

In this study, we examined the thickness of the retina and choroid in both eyes of patients with MS who suffered an episode of ON using a novel SS-deep range imaging OCT technology and compared the results with the healthy control group. Our results show a considerable loss of RNFL in both the affected and nonaffected eye in patients with RBN, compared with a healthy population. Moreover, we show that similar structural changes can be detected in the nonaffected eye of these patients. Although such a finding is not entirely surprising, neurodegeneration is an established and early pathogenic feature of MS. This loss of nerve fibers can be assessed and indirectly quantified by a range of indirect techniques, such as quantification of clinical neurological deficit using the Expanded Disability Status Scale – a gold standard measure of clinical disability in MS patients, MRI, VEP, questionnaires assessing the quality of life, and cognitive ability tests [27–29]. However, precise longitudinal and quantitative measures of axon and neuron loss in MS, with a view to following disease development, progression, and response to therapy and providing prognostic values, is still impractical for most patients and unattainable for the majority of clinics.

Atrophy in pRNFL in MS patients was first described by Parisi et al. [30] in 1999. Since then, several studies have examined the links between the atrophy of inner retinal layers, pRNFL and mGCIPL in particular, and disease characteristics in MS. In patients with ON and confirmed diagnosis of MS, the thickness of pRNFL and mGCIPL was shown to result from retrograde axonal degeneration and retinal ganglion cell loss, which seems to advance up to the INL. On average, the level of atrophy following an episode of ON in MS patients was reported to be 20.1 μm for pRNFL and 16.4 μm for mGCIPL. As the disease progresses, the thinning of these retinal layers increases, and similar degenerative changes have been described in the eye which had not been affected by ON [25, 26].

The ability of OCT to detect structural changes in the retina of patients with MS with a high degree of accuracy and reproducibility propelled this methodology into an irreplaceable tool in differential diagnosis, as well as a reliable high-quality approach to the interpretation of disease progression and therapeutic effects. More specifically, the high precision of OCT in quantifying the loss of thickness of retinal layers within the macula and in peripapillary retinal areas marks a significant advancement in determining the stage of the disease. Moreover, the high safety factor of the technique enabling multiple examinations and longitudinal follow-up has the potential to transform our ability to determine the rate of disease progression in a patient-centered manner. Our results showed that this assessment of the structural integrity of the retina could be refined further using fine subsegmentation of retinal areas, namely, superior, inferior, temporal, and nasal segment assessment. Indeed, our sub-segmental analysis showed that significant loss of retinal thickness occurs in all these segments. Furthermore, we showed that the most severe loss of RNFL thickness affects the temporal segment in both the eye affected by ON in MS patients and the nonaffected eye. This finding is in agreement with results found in other studies [31, 32]. Perhaps surprisingly, the measurements of mGCIPL showed a significant loss of thickness not only in the affected eye of the MS patients but also in the nonaffected eye compared with healthy controls. The latest advancements in the literature confirm our results, suggesting that the thinning in mGCIPL may constitute a particularly sensitive marker of axonal degeneration in MS, indeed more sensitive than the thinning of RNFL [33, 34]. Nonetheless, as the OCT techniques have become more prominent in the clinic, the SS-OCT in particular, further studies are warranted to confirm the exact role of this methodology in the diagnosis and management of neurodegenerative changes [33, 34].

The second part of our study focused on the use of OCTA in the analysis of vascular density within superficial macular layers. Similar to OCT, OCTA is a new, noninvasive method for examination of the vascular perfusion of the macula and optic disc (head). As such, it is of particular importance in the diagnostic assessment of patients with neurodegenerative diseases, particularly of vascular origin, e.g., the small vessel disease of the CNS and its most common consequence, vascular dementia [35]. Indeed, we found a significant reduction in macular perfusion in the eye affected by ON in MS patients, compared with healthy controls. This reduction was particularly pronounced in the temporal segment, which is in agreement with previous studies by Lanzillo et al. [36] and Murphy et al. [37]. However, in contrast with the previous studies, we found no reduction in macular perfusion in the nasal segment of the nonaffected eye in MS patients. This discrepancy is likely to be a result of a relatively small number of participants in our study, a limiting factor in the interpretation of our data. Our correlation analysis showed a strong positive correlation between the analyzed variables, but there was no strong association with OCTA. It can be expected that if we have an increase in one of the indicators, we can expect a jump in other indicators as well because these variables are strongly positively correlated [37, 38].

However, it should be taken into account that the literature describing the use of OCTA in this particular indication is quite scarce. Furthermore, there is a degree of variability in the published literature regarding the dominant location of perfusion deficit in the ON, as well as individual differences in the capillary network, both of which add to the variability encountered in the published work. Nonetheless, the consen-

sus in the literature exists on the fact that macular perfusion deficit, seen in ON and MS, results from pathophysiological characteristics of MS, as opposed to being a consequence of generalized vascular conditions [15, 38]. Of note, however, are epidemiological data suggesting a certain degree of comorbidity between vascular conditions (cardiogenic, cerebrovascular, and peripheral vascular conditions) and MS. Therefore, these systemic conditions may underlie some degree of perfusion changes seen in the macula of MS patients. Further studies to clarify the link between systemic and MS-specific vascular changes are warranted. Importantly, designing such studies should not present a scientific problem in light of the recent advancements in OCT, whereby every patient could be examined for both systemic and macular changes using state-of-the-art techniques. The OCT is perfectly poised in this context, enabling noninvasive, reliable, and reproducible functional measurements of changes in the macular vascular bed. Furthermore, owing to its highly targeted nature of assessment, combined with the ability for frequent reassessment, OCT and OCTA lend themselves to studies where rapid changes in pathogenic and clinical features require techniques with a high degree of sensitivity and specificity in order to provide insight into mechanisms underlying the disease progression.

The limitation of the study was the relatively small number of participants. In addition, the type of study (observational case study) can also be considered a limiting factor.

Conclusion

In this study, we showed that the thinning of the RNFL and GCL layers, as assessed using OCT, represents an accurate and sensitive method for assessing the damage affecting the optic pathway in patients with MS. In addition, we confirmed that, in MS patients, retinal atrophy is present at a certain degree, even in the absence of the previous symptomatic ON. Furthermore, we showed a reduction in vascular plexus density in patients with ON compared with healthy controls. We conclude that OCT represents a novel, noninvasive, reliable, reproducible, and convenient diagnostic tool in general clinical practice, which can be applied to all patients suffering from demyelinating diseases of the CNS. Using this technique, the thickness of retinal layers is quantified in real-time, *in situ*, representing a direct insight into the magnitude of axon and neuron loss in the proximal optic pathway. In addition to the expected thinning of the mGCIPL in the eye affected by ON, we have also detected a thinning in this layer in the nonaffected eye in patients with RBN, compared with healthy controls, which is of great importance. This finding strongly suggests that comparative analysis of RNFL and GCL represents an invaluable biomarker in future research and clinical practice in ON and MS, but also in other vascular and neurodegenerative conditions. Finally, given the well-known neurovascular coupling mechanisms present in the nervous system, the combination of OCT and OCTA promises to lead to a step-change advancement of our understanding and treatment of a wide range of conditions in the eye and CNS as well.

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Influence of solvent use on apical extrusion during removal of Resilon™ from root canals

Uticaj rastvarača na apikalnu ekstruziju tokom uklanjanja *Resilon*™ iz kanala korena zuba

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Abstract

Background/Aim. During retreatment, filling material and debris may extrude and trigger an inflammatory reaction of periapical tissues. Resilon™ has not been investigated in terms of solvent use and influence on apical extrusion during retreatment. The aim of the study was to evaluate the amount of apically extruded debris during Resilon™ removal using ProTaper (PT), Twisted File (TF), and Hedstrom instruments, with and without solvent. **Methods.** In total, 72 extracted teeth with single canals were used. Canals were prepared with PT Universal (F2) and filled with Resilon™ and RealSeal sealer before being assigned randomly to 6 groups (n = 12 in each group). Retreatment in Groups 1–3 was done with PT, TF, or Hedstrom instruments, without solvent. In Groups 4–6, the same instruments were used with chloroform. Apically extruded debris was collected in a simulated periapical environment and assessed visually. Additionally, the time required for retreatment was recorded. Data were analyzed statistically using the Mann-Whitney *U* test, with a significance level of 0.05. **Results.** There were no significant differences in apical extrusion debris between groups ($p > 0.05$) regarding solvent use. Rotary instruments, without solvent, were associated with significantly less debris extrusion when compared with hand files ($p < 0.05$). When solvent was used, rotary instruments caused a higher degree of extruded material, which was similar to the results of hand instruments. **Conclusion.** The use of rotary instruments without solvent resulted in a lower degree extrusion of Resilon ™ material compared to hand instruments, while greater caution is advised in the presence of solvent when rotary instruments are used to remove this material.

Key words:
dental instruments; resilon sealer; root canal filling materials; solvents.

Apstrakt

Uvod/Cilj. Tokom endodontskog retretmana, ostaci materijala za punjenje mogu biti istisnuti kroz apikalni otvor zuba i izazvati inflamacijsku reakciju periapikalnih tkiva. Upotreba rastvarača i njegov uticaj na apikalnu ekstruziju tokom uklanjanja *Resilon*™ nisu ispitivani. Cilj rada bio je da se proceni apikalna ekstruzija ostataka tokom uklanjanja *Resilon*™, primenom instrumenata *ProTaper* (PT), *Twisted File* (TF) i *Hedstrom*, sa i bez upotrebe rastvarača. **Metode.** Korišćena su 72 izvađena zuba sa po jednim kanalom. Kanali su obrađeni instrumentima PT *Universal* (F2) i opturisani *Resilon*™ i *RealSeal* silerom, pre nego što su nasumično podeljeni u 6 grupa (n = 12 u svakoj grupi). Retretman u grupama 1–3 urađen je PT, TF ili *Hedstrom* instrumentima, bez rastvarača. U grupama 4–6 korišćeni su isti instrumenti sa hloroformom. Apikalno istisnuti ostaci sakupljeni su u simuliranom periapikalnom okruženju i procenjeni vizuelno. Pored toga, zabeleženo je vreme potrebno za retretman. Podaci su statistički analizirani korišćenjem *Mann-Whitney U* testa, sa nivoom značajnosti $p < 0,05$. **Rezultati.** Nije bilo značajnih razlika u rezultatima apikalne ekstruzije ($p > 0,05$) u zavisnosti od upotrebe rastvarača. Rotirajući instrumenti, primenjeni bez rastvarača, doveli su do značajno manje ekstruzije u poređenju sa ručnim turpijama ($p < 0,05$). Kada je korišćen rastvarač, rotirajući instrumenti, slično ručnim instrumentima, doveli su do većeg stepena ekstruzije materijala. **Zaključak.** Upotreba rotirajućih instrumenata bez rastvarača dovela je do manjeg stepena ekstruzije *Resilon*™ materijala u poređenju sa ručnim instrumentima, dok se u prisustvu rastvarača savetuje veći oprez pri upotrebi rotirajućih instrumenata za uklanjanje ovog materijala.

Ključne reči:
stomatološki instrumenti; resilon, materijal za punjenje korenskog kanala; zub, materijali za punjenje korenskog kanala; rastvarači.

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Introduction

When endodontic therapy fails and retreatment is required, the removal of filling material can be a challenging procedure [1]. During root canal retreatment, filling material, necrotic pulp tissue, bacteria, irrigants, and solvents may extrude beyond the apical foramen and trigger an inflammatory reaction of the periapical tissues [2, 3]. As an undesirable consequence, postoperative pain, swelling and inter-appointment flare-up, delayed healing, or even treatment failure may occur [4, 5]. Therefore, reducing the risk of debris extrusion into the periradicular tissues would be beneficial for both the patient and the clinician [6].

The most widely used material for root canal obturation is gutta-percha. However, since 2004, a new thermoplastic synthetic polymer material – Resilon™ (Resilon™ Research LLC, Madison, CT) has been available. The material behaves like gutta-percha and adheres to the root canal walls, forming a "monoblock" and reducing microleakage [7–9]. Although canal wall cleanliness after Resilon™ removal was examined in several studies, there are only a few studies in the current literature that evaluate the apical extrusion (AE) of this material during retreatment in terms of different instruments or different obturation techniques used [10–14]. Furthermore, to the best of our knowledge, the effect of solvent use on AE during Resilon™ removal has not been assessed until now. Likewise, these studies only measure the amount of apically extruded material without any simulation of the resistance that the periapical tissues offer in clinical *in vivo* conditions.

Another very recent and important finding should be emphasized. The latest long-term clinical studies indicate that, compared with gutta-percha, teeth obturated with Resilon™ have greater odds of failure, most probably due to the susceptibility of this material to degradation [15–17]. These data suggest that, in the years to come, a greater need for retreatment of teeth obturated with Resilon™ may appear. In light of these findings, the results of this study that investigated the AE of Resilon™ during retreatment and the use of solvent could be found useful by clinicians.

It is generally accepted that none of the instruments or techniques used can prepare root canals or remove obturation material without producing some apically extruded debris. However, the amount of apically extruded debris might vary according to the technique used and the design of the root canal instrument [18, 19]. Instrument systems have been developed specifically for retreatment procedures. ProTaper (PT) Universal Retreatment (Dentsply Maillefer, Ballaigues, Switzerland) is a rotary system made of nickel-titanium (Ni-Ti) and consists of three instruments (D1, D2, D3) used for the removal of filling material from each third of the root canal, respectively. Likewise, a new type of instrument – Twisted File (TF) (SybronEndo, Orange, CA, USA) has become available but has not been specifically designed for retreatment. These files have a twisted design, a triangular cross-section, variable pitch, a safe-ended tip, and no ground surface treatment [20]. According to the manufacturer, this design allows their use in retreatment cases. Only a few studies evaluate the cleaning efficacy of TF instruments in retreatment procedures [21, 22] and the influence of this instrument on AE during retreatment [23].

The aim of the study was to evaluate *in vitro* the amount of apically extruded debris during retreatment of Resilon™-filled root canals, using PT, TF, and Hedstrom instruments, with or without solvent.

Methods

Specimen selection

In total, 72 freshly extracted human mandibular single-rooted incisor teeth with one straight canal (curvatures < 10°), with mature apices, were used. Radiographs were taken to confirm that there was no previous root canal treatment, internal resorption, or root canal calcification. To standardize specimen lengths, teeth were shortened to 16 mm by removing the crown.

Root canal preparation and obturation

Canal patency was confirmed with a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until it was visible at the apical foramen. The working length (WL) was 1 mm short from the observed length. All teeth were prepared with a rotary PT Universal system (Dentsply Maillefer, Ballaigues, Switzerland) to size #25 (F2). Irrigation with 2 mL of 5.25% sodium hypochlorite (NaOCl-Chloraxid, Cerkamed Company, Stalowa Wola, Poland) was used between each instrument. The smear layer was removed with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) (Endo Solution, Cerkamed Company), followed by a rinse with 10 mL of distilled water. After drying with paper points (PT F2, Dentsply Maillefer), master Resilon™ cone size #25.06 (RealSeal 0.06 taper points, SybronEndo, Kerr Corporation, USA) was fitted to the WL to check tug-back. The root canal walls were coated with a self-etching primer using a microbrush, and the excess was removed with paper points. The master cone was coated with an adhesive, methacrylate sealer (RealSeal Root Canal Sealant, SybronEndo Kerr Corporation, USA) and obturated using cold lateral condensation and accessory Resilon™ cones. The excess cones coronally were removed with a heated instrument, and additional vertical condensation was done using pluggers (Dentsply Maillefer). The coronal surface was then light cured with Woodpecker LED.H curing light (Guilin Woodpecker Medical Instruments, China; 12,000 mW/cm^2, S/N:L1390416H) for 40 sec, according to the manufacturer's instruction. Access openings were sealed (Cavit; 3M ESPE, Seefeld, Germany), and obturation quality was confirmed by radiographs from two directions. All teeth were stored at 37 °C in 100% humidity for six weeks to allow the complete setting of the sealer and the aging of the material to some extent. The temporary filling material was replaced every two weeks to maintain a good seal throughout the material seating procedure.

The teeth were coded and randomly assigned into six groups (n = 12). In each group, Gates-Glidden drills (#3) were used to remove 2 mm of material from the coronal portion.

Retreatment techniques

Group 1: ProTaper

PT Universal Retreatment (Dentsply, Maillefer) instruments were applied sequentially, using the D1 file (#30/.09) to remove filling material from the coronal third, whereas D2 (#25/.08) and D3 files (# 20/.07) were used in the middle and the apical third, respectively. The instruments were used in a brushing action with lateral pressing movements, with a rotational speed of 600 rpm (X-Smart, Dentsply Maillefer), according to the manufacturer's instructions. Additional apical preparation was performed with PT Universal instruments F3 (#30) and F4 (#40) at 300 rpm.

Group 2: Twisted File

TF (SybronEndo) instruments were used in the following sequence: TF 35 (0.06 taper), followed by TF 30 and 25 (0.08 taper), until reaching the WL. The rotational speed of the X-Smart motor was set at a maximum of 800 rpm. Finally, TF 35 (0.06 taper) and 40 (0.04 taper) were used to enlarge the apical preparation and additionally clean the canal walls, with a rotational speed of 500 rpm. All the instruments were used with a gentle, in-and-out motion and without pressure, according to the manufacturer's instructions.

Group 3: Hedstrom

Retreatment was performed with Hedstrom files (Dentsply Maillefer) sequentially from #40–20 in a circumferential quarter-turn, push-pull filing motion until the WL was reached. Re-preparation of the canal apical part was carried out till size 40.

Group 4: ProTaper + solvent

The same instrumentation protocol as in Group 1 was used. Resilon™ was previously softened using 0.05 mL of chloroform (Merck, Darmstadt, Germany) for 1 min. This procedure was repeated also in the middle and apical portion of the canal (0.15 mL in total for each specimen). Instruments during material removal were used in the presence of a solvent, except in the apical third, where the excess solvent was collected with paper points before instrumentation. When the WL was reached, the use of solvent was discontinued during re-preparation and apical enlargement.

Group 5: Twisted File + solvent

The same instrumentation protocol as in Group 2 was used, and the solvent was used in the same manner as explained in Group 4.

Group 6: Hedstrom + solvent

The same instrumentation protocol as in Group 3 was used, and the solvent was used in the same manner as explained in Group 4.

Each set of instruments was used to retreat maximally four root canals and then discarded. All instruments were used according to the manufacturer's instructions. During retreatment, the flutes of the instruments were frequently cleaned, and 2 mL of 5.25% NaOCl was used for irrigation after each instrument. Retreatment was considered complete when the WL was reached, and no more material was observed on the instrument or in the irrigating solution. After re-preparation, the canals were irrigated with 5 ml of 17% EDTA for 1 min and then flushed with 10 mL of distilled water. A single experienced operator performed all the root canal procedures to reduce inter-operator variability.

Measurement of collected debris and time for retreatment

Prior to retreatment, the apex of the teeth was covered with Teflon foil, and a ball of soft blue wax was pushed over it. The Teflon served to prevent the wax from being pushed into the apical foramen. This setup was done in order to simulate the resistance offered by the periapical tissues and secure the material extruded through the apical foramen to be flushed away with irrigating solutions used during retreatment. Extrusion of root canal filling material through the apical foramen was detected visually after removal of the debris collection apparatus using loupes with x3 magnification. Scoring was carried out by a second examiner, who was blinded to the group assignment, according to the following system [10, 24]: 0 = no extrusion of filling material through the foramen; 1 = minimal extrusion of filling material, barely detectable; 2 = moderate extrusion of filling material, easily detectable; 3 = extrusion of a considerable amount of filling material.

Additionally, the time required for the retreatment procedures was recorded (in sec) for each sample. The time for irrigation, solvent application, cleaning, and changing of instruments was not recorded.

Statistical analysis

Mean ranks of scores for apically extruded material were calculated and analyzed statistically using the Mann-Whitney *U* test. Data for the retreatment time were analyzed with one-way ANOVA. Analysis was performed with SPSS (version 20.0) at a significance level of $p < 0.05$.

Results

The mean rank of scores for AE and the mean retreatment time data are presented in Tables 1 and 2. The use of solvent had no statistically significant effect on the results of AE between the groups ($p = 0.691$; Table 1). When no solvent was used, the difference between tested instruments was statistically significant ($p = 0.013$), and Hedstrom files extruded more debris when compared with PT ($p = 0.023$) and TF rotary systems ($p = 0.011$; Table 2). When solvent was used, all three tested instruments caused a similar degree of apically extruded material ($p = 0.974$; Table 2).

The time for retreatment decreased significantly when the solvent was used compared to the removal of Resilon™ without solvent ($p < 0.01$; Table 1). The time needed for retreatment with the same type of instrument was significantly shorter when the solvent was used. Ro-

Table 1

Mean rank of scores for apical extrusion during Resilon™ removal and mean retreatment time

Group	Mean rank	Sum of ranks	Mean time (s) ± SD
Resilon™ – without solvent (Groups 1–3)	29.65	889.50	168.37 ± 121.48
Resilon™ – with solvent (Groups 4–6)	31.35	940.50	61.60 ± 34.81*

Number of participants = 72 (six groups of 12 participants each); SD – standard deviation.
*** $p < 0.01$ compared to groups 1–3 (without solvent).**

Table 2

Mean rank of scores for apical extrusion and mean retreatment time for each group

Group		Mean rank	Mean time (s) ± SD
number	name		
1	ProTaper without solvent	12.95[a]	124.10 ± 22.92 [a,b,d]
2	Twisted File without solvent	11.80[a]	188.40 ± 50.22 [a,c,d]
3	Hedstrom without solvent	21.75[A]	485.90 ± 103.53 [A,b,c]
4	ProTaper with solvent	15.05	85.60 ± 13.70 [a,b,D]
5	Twisted File with solvent	15.55	95.80 ± 18.46 [b,C]
6	Hedstrom with solvent	15.90	253.10 ± 39.51 [B]

Number of participants = 72 (six groups of 12 participants each); SD – standard deviation.
*** The difference is statistically significant ($p < 0.05$) between results marked with the same pairs of uppercase and lowercase letters (A-a, B-b, C-c, or D-d).**

tary instruments required significantly less time for retreatment than Hedstrom files, regardless of the solvent use ($p < 0.05$; Table 2).

Discussion

As clinical assessment of AE is not viable, laboratory studies are necessary as a helpful approximation to clinical reality [25]. However, caution should be taken during the interpretation of the results because, in the *in vitro* setup, there are no periapical tissues present that may act as a natural barrier [5]. This study used an innovative experimental setup, incorporating a debris collection apparatus that closely covers the root apices and offers some resistance to AE. Similar strategies were employed in other studies [26, 27]. Laboratory studies of AE during retreatment were most often conducted with a quantitative method, which involves the use of a special apparatus for the collection of apically extruded material and debris, and measuring their amount in grams [2, 19, 28–30]. In some studies, the amount of apically extruded filling material during retreatment was detected visually and evaluated with a scoring system [10, 24], as in the presented study. This kind of evaluation methodology can be criticized due to a certain degree of subjectivity and less precision in assessing the extruded material amount. However, the precision of material extrusion measured in grams may be of limited relevance because extrusion may occur more easily and frequently if there is no periapical barrier that would limit the extrusion to some degree, as in clinical reality [27]. Furthermore, in their study, Alves et al. [27] found no correlation between extruded bacterial counts and the volume of debris.

The number of studies evaluating apically extruded debris during Resilon™ retreatment is limited [10–14], and regardless of the methodology used, all can contribute to the clarification of the AE of the Resilon™ material. AE studies during the removal of gutta-percha are numerous, but a comparison of different obturation materials was made only in a few studies, and no statistically significant difference was observed between the tested materials [10, 12, 13]. A study that also used the visual technique [10] and compared gutta-percha, Resilon™, and EndoRez, found no difference between materials in terms of AE. In the study by Çanakçi et al. [11], a different obturation technique (warm vertical condensation) did cause a statistically significant difference for AE; however, in the groups obturated with cold lateral condensation (CLC), there was no difference between AE of gutta-percha and Resilon™. According to the information available to the authors, no study has assessed the impact of solvent use on debris extrusion during Resilon™ removal. Therefore, this study aimed to test only Resilon™ material and evaluate the influence of solvent use and different instruments used for the retreatment protocol (Hedstrom, PT, and TF) when CLC was used as an obturation method.

The results of the current study showed that chloroform use did not have a statistically significant influence on AE during Resilon™ removal. As recommended by the manufacturer for the retreatment of the Resilon™ system, chloroform was used in this study. Moreover, studies confirmed that this material could be effectively removed with chloroform and rotary instruments [31, 32]. In a recent study, the authors concluded that the use of a solvent specific to the sealer during retreatment decreased the amount of apically extruded debris [33]. Studies evaluating the solubility of the Resilon™ system with different solvents, such as xylol [34], and its impact on AE during retreatment, should be conducted.

Other than the type of solvent, the method of use and the quantity of solvent can also influence the AE of the filling material. In the present study, the amount of chloroform

was the same in all groups where the solvent was used (0.15 mL in total for each sample). Retreatment instruments were used in the presence of a solvent, except in the apical third, where the excess solvent was previously collected with paper points. That was done as a precaution that is also recommended in clinical conditions to prevent additional extrusion into periapical tissues and possible solvent toxicity.

The presented results for AE in terms of different instruments used for retreatment showed that rotary instruments caused significantly less AE than Hedstrom files when no solvent was used. There was no difference regarding the amount of AE between the two rotary instruments, which is in accordance with other studies [12, 23]. However, in the present study, during retreatment with solvent, rotary instruments caused a higher degree of material extrusion, which was similar to the extrusion caused by Hedstrom hand files. In most studies, a common finding is that manual instrumentation causes greater extrusion when compared with engine-driven preparation [12, 19, 23, 28, 29]. That is partially in accordance with the results of the presented study when no solvent was used during retreatment. Most studies have concluded that rotary instruments produce less debris extrusion than hand-filing techniques because they tend to pull the debris into the flutes of the instrument and in a coronal direction [2, 10]. However, in this study, regardless of the convenient design, rotary instruments caused similar AE as hand files when the solvent was used. That may be explained by the fact that Resilon™ is a very thermoplastic material [35], and when additionally softened with chloroform and heat generated by the friction of rotary instruments, it can be easily pushed through the apical foramen. This study can add this observation when Resilon™ retreatment is performed and emphasize that rotary instruments should be used with more care in the presence of a solvent to minimize apically extruded debris.

Not many studies measured retreatment time during the use of different instruments, and mainly these studies evaluated gutta-percha removal. The results of this study showed that retreatment time was significantly reduced with chloroform use. Because of the limited number of studies that evaluated Resilon™ retreatment and measured working time [13], other studies investigating these factors and different types of solvents should be conducted.

The results also showed that the longest retreatment time was needed with hand instruments, regardless of the solvent use, while rotary instruments required significantly less time for retreatment. Somma et al. [10] compared three types of obturating material and Mtwo instruments and also PT rotary instruments with Hedstrom files and concluded that rotary instruments and Resilon™ filling material had a positive impact on reducing the time for retreatment. The study that also evaluated TF for retreatment of Resilon™ reported that Mtwo Retreatment instruments were faster than PT and TF instruments [21]. Another study evaluated the effectiveness of the newer TF Adaptive instruments, but only for gutta-percha removal, and reported that Reciproc and PT Retreatment instruments were more efficient than TF Adaptive instruments and hand files and that the TF Adaptive system was advantageous over hand files only with regard to operating time [22].

Conclusion

Under the experimental conditions of the present study, all tested retreatment systems caused AE. The use of chloroform during Resilon™ removal did not have a significant effect on the results of AE; however, it significantly reduced retreatment time. Rotary instruments caused less AE than Hedstrom files when no solvent was used. Nevertheless, the use of solvent caused a higher degree of extruded material when rotary instruments were used compared to their use without solvent. In accordance with these findings, rotary instruments should be used with precaution for the removal of Resilon™ in the presence of a solvent.

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Assessment of the impact of orthodontic-surgical treatment on the quality of life of patients with mandibular prognathism

Procena kvaliteta života pacijenata posle ortodontsko-hirurškog lečenja mandibularnog prognatizma

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Abstract

Background/Aim. Mandibular prognathism, as one of the more severe forms of dentofacial deformities, impairs the oral functions and appearance of the patient's face and represents a psychosocial handicap with a negative impact on the quality of life (QoL). The aim of the study was to assess the impact of orthodontic-surgical (OS) treatment on the QoL of patients with mandibular prognathism. **Methods.** The study involved 40 patients (19 men and 21 women, with a mean age of 24.1 ± 4.1 years) who underwent the OS treatment for mandibular prognathism. All patients completed two questionnaires – the Oral Health Impact Profile (OHIP-14) and the Orthognathic Quality of Life Questionnaire (OQLQ), before the start of treatment and 12 months after the completion of treatment. **Results.** The results of both questionnaires showed an improvement in the QoL compared to the condition before the treatment. According to the OQLQ questionnaire, there was a postoperative improvement in the QoL (score 24.8 ± 12.9) compared to the preoperative period (score 51.3 ± 15.2). According to the results of the OHIP-14 questionnaire, there was a postoperative improvement in the QoL (score 11.3 ± 2.9) compared to the preoperative period (score 20.8 ± 6.9). The improvement of the QoL, 12 months after the treatment, occurred in all life segments, measured by both questionnaires. **Conclusion.** OS treatment of mandibular prognathism improves all oral functions, including the appearance of the patient's face, thus improving the QoL.

Key words:
mandible; orthognathic surgical procedures; prognathism; quality of life; surveys and questionnaires; treatment outcome.

Apstrakt

Uvod/Cilj. Mandibularni prognatizam predstavlja težak oblik dentofacijalnog deformiteta koji narušava oralne funkcije i izgled lica pacijenta, i predstavlja psihosocijalni hendikep sa negativnim uticajem na kvalitet života (KŽ). Cilj rada bio je da se proceni uticaj ortodontsko-hirurškog (OH) lečenja pacijenata sa mandibularnim prognatizmom na KŽ. **Metode.** U istraživanju je učestvovalo 40 pacijenata (19 muškaraca i 21 žena, prosečne starosti 24,1 ± 4,1 godina) kod kojih je izvršeno OH lečenje mandibularnog prognatizma. Svi ispitanici su pre početka lečenja i 12 meseci nakon završenog lečenja odgovorili na pitanja upitnika o uticaju oralnog zdravlja na KŽ (*Oral Health Impact Profile* – OHIP-14) i upitnika o uticaju dentofacijalnog deformiteta na KŽ (*Orthognathic Quality of Life Questionnaire* – OQLQ). **Rezultati.** Rezultati oba upitnika pokazali su poboljšanje KŽ u odnosu na stanje pre početka lečenja. Prema rezultatu OQLQ upitnika došlo je do postoperativnog poboljšanja KŽ (skor 24,8 ± 12,9) u odnosu na preoperativni period (skor 51,3 ± 15,2). Prema rezultatu OHIP-14 upitnika, došlo je do postoperativnog poboljšanja KŽ (skor 11,3 ± 2,9) u odnosu na preoperativni period (skor 20,8 ± 6,9). Do poboljšanja KŽ, 12 meseci posle lečenja, došlo je u svim segmentima merenim putem oba upitnika. **Zaključak.** OH lečenjem mandibularnog prognatizma postiže se poboljšanje svih oralnih funkcija, uključujući izgled lica pacijenta, a samim tim i poboljšanje KŽ.

Ključne reči:
mandibula; hirurgija, ortognatska, procedure; prognatizam; kvalitet života; ankete i upitnici; lečenje, ishod.

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Introduction

Mandibular prognathism (MP) is a developmental skeletal irregularity with a predominantly developed lower jaw and impaired appearance of teeth and face, which, in addition to impaired oral function, leads to psychosocial problems that significantly reduce the quality of life (QoL) of the patient [1].

The most common treatment for MP is orthodontic-surgical (OS) treatment, which improves the appearance of the face, oral functions, and the patient's QoL [2, 3]. The outcome of the OS treatment can be objectively confirmed by analysis of postoperative dental occlusion as well as by measuring cephalometric parameters; however, in modern dentistry, an important role in determining the effectiveness of treatment results is the patient's assessment of the QoL [2, 4]. Increased understanding of the patient's perception, expectations, and views on the overall treatment is necessary to achieve a successful treatment outcome [4].

According to the definition by the World Health Organization, the QoL is an individual's perception of their life position depending on the culture and value system in which they live and is related to their goals, expectations, standards, and interests. It is a broad concept that consists of the physical health of an individual, psychological status, material independence, social relations, and their relations according to the significant characteristics of the external environment [5]. Quality of life is assessed using general health questionnaires and disease-specific questionnaires.

Numerous studies that have been conducted in recent years confirmed that MP is a significant psychosocial deficiency for patients and negatively affects their QoL [6–8]. Many studies have shown that surgical treatment of deformities contributes to good aesthetic results, significantly changing the psychological status of these patients, with a positive impact on their self-confidence and awareness of their values, thus improving their QoL [6, 7]. For some patients, after the OS treatment of MP, the domain of the social aspect was more important than the improved facial appearance and oral function [9]. In general, OS treatment had a positive effect on the QoL of patients with dentofacial deformities [10–13].

The aim of our study was to assess the impact of OS treatment on the QoL of patients with MP.

Methods

This study included 40 patients with an age range of 19–34 years. All patients were diagnosed with MP and underwent the OS treatment of the deformity. The patients were treated at the Department of Orthodontics and the Department of Maxillofacial Surgery of the Dental Clinic of the Military Medical Academy in Belgrade, Serbia. All activities and procedures applied in this study were approved by the Ethics Committee of the Military Medical Academy in Belgrade, on December 13, 2018 according to the Declaration of Helsinki.

The study involved patients with good mental health and psychological status with no previous history of orthodontic treatment (OT). Patients with cleft lip and palate and all other craniofacial deformities, patients with a history of facial trauma or some orthognathic surgery, patients with temporomandibular joint disease, facial asymmetries, etc., were excluded from the study.

Patients in the study were divided into two groups – patients who underwent surgery on one jaw (monomaxillary group) and patients who underwent surgery on both jaws (bimaxillary group).

All patients underwent preoperative OT with the same protocol to achieve adequate and stable postoperative occlusion. After the orthodontic preparation, surgical repositioning of the jaws (mono or bimaxillary type) with rigid fixation was performed. A standard sagittal step osteotomy was performed in the area of the lower jaw ramus, while a Le Fort osteotomy I of the middle facial mass was performed in the area of the upper jaw.

In this study, patients completed two questionnaires to assess the QoL before (T1) and 12 months after OS treatment of MP (T2). One questionnaire contained questions on oral health in general – Oral Health Impact Profile (OHIP-14) [14], and another questionnaire, the Orthognathic Quality of Life Questionnaire (OQLQ) [15, 16], was specifically formulated for orthognathic patients.

The OHIP-14 contained 14 questions that assess the impact of oral health on patients' QoL through seven areas: functional limitations, physical pain, psychological discomfort, physical, psychological, and social disabilities, and handicap. Patients were asked to complete the questionnaire expressing the degree of agreement on a five-point scale, from 0 to 4, where a higher score represents a more frequent presence of functioning problems. The total possible number of points was 56. Moreover, a higher score indicated a poorer QoL. The Serbian version of the OHIP-14 questionnaire was validated by Stančić et al. [17], and the reliability and validity of this questionnaire were confirmed in a study by Lekić et al. [18, 19].

The OQLQ contained 22 questions that assess the impact of dentofacial deformities on a patient's QoL through four areas: social aspects, facial aesthetics, oral function, and awareness of facial deformity. The answers to the questions are ranked on a scale from 1 to 4, and thus a subjective view is expressed in the extent to which each of the claims relates to the patient. Answer ranked as number 1 means that the patient is a little bothered by this condition, while 4 means that it bothers the patient a lot. Answers ranked with numbers 2 and 3 belong between these two statements. There was also a N/A answer, which means that the condition does not apply to the patient or does not bother the patient at all. The result of the questionnaire represented the total sum of rounded numerical options by claims and can be expressed comprehensively or by domains. The total score of the answers to all questions can range from 0 to 88. A score defined by a larger number indicates a poorer QoL. Cunningham et al. [15, 16] developed the OQLQ, which was used in numerous studies, and Vučić et al. [20] validated it in Serbian.

Statistical analysis

The Kolmogorov-Smirnov test was used to examine the layout of a statistical series. Pearson's chi-squared (χ^2) test was

used to test the relationship between the two qualitative variables. Differences in numerical variables were examined using One-Factor Analysis of Variance (ANOVA) and/or *t*-test for large independent samples. To examine the relationship between the two continuous variables, Pearson's correlation coefficient was used as a parametric test, and Spearman's correlation coefficient as a nonparametric substitution. Differences in the values of numerical variables, measured in several time intervals, were tested by the repeated measure ANOVA test. Statistical significance was defined at the level of probability of the null hypothesis of $p < 0.05$. Statistical processing and analysis were done in the computer program Statistical Package for the Social Sciences 24 (SPSS 24), and graphical and tabular presentation in the software package Microsoft Office (Excel and Word).

Results

The study included 40 patients, of which 19 (47.5%) were men and 21 (52.5%) were women. The mean age ± standard deviation (SD) of the patients was 24.1 ± 4.1 years. Out of the total number of patients, surgery on one jaw was performed in 16 (40%) respondents (monomaxillary group), while surgery on both jaws (bimaxillary group) was performed in 24 (60%) respondents. These two groups of patients did not differ significantly concerning gender, age, and type of surgery performed.

Descriptive indicators of QoL measured by the OHIP-14 questionnaire

The results of the OHIP-14 questionnaire, which measured the difference between the QoL of patients before (T1) and 12 months after the OS treatment of MP (T2), showed postoperative improvement of the QoL (11.3 ± 2.9) compared to the preoperative period (20.8 ± 6.9) (OHIP index). There was an improvement in the QoL after completion of treatment in all life segments measured by the questionnaire scale (T2 vs. T1): functional limitation 0.8 ± 1.1 vs. 1.6 ± 1.2; physical pain 1.8 ± 1.5 vs. 3.2 ± 1.2; psychological discomfort 4.3 ± 2.3 vs. 7.2 ± 2.1; physical disability 2.2 ± 2.8 vs. 3.3 ± 2.7; psychological disability 0.5 ± 0.7 vs. 1.1 ± 1.2; social disability 0.9 ± 1.1 vs. 2.3 ± 1.3; handicap 0.9 ± 0.8 vs. 2.1 ± 0.9. The highest average score before and after treatment was recorded on the question: *"Have you been self-conscious because of your teeth, mouth, or dentures?"* (3.1 ± 0.9 vs. 2.5 ± 1.2), which represents the biggest preoperative and postoperative problem for patients. The lowest average result before and after treatment and, at the same time, the smallest problem were recorded on the question: *"The change in the sense of taste because of problems with the teeth, mouth, or dentures."* (0.1 ± 0.3 vs. 0.2 ± 0.5).

Descriptive indicators of the QoL measured by the OQLQ questionnaire

The results of the OQLQ questionnaire, which measured the difference between the QoL of patients before (T1) and 12 months after the OS treatment of MP (T2), showed postoperative improvement of the QoL (24.8 ± 12.9) compared to the preoperative period (51.3 ± 15.2). Improvement in the QoL after completion of treatment occurred in all segments measured by the questionnaire scale (T2 vs. T1): awareness of facial deformity 6.9 ± 4.2 vs. 10.4 ± 4.3; oral function 5.4 ± 2.9 vs. 12.5 ± 3.9; facial aesthetics 6.7 ± 3.8 vs. 14.9 ± 3.7; social aspects 5.7 ± 4.5 vs. 13.5 ± 7.4. Our study showed that the highest average result before treatment was recorded on the question: *"I don't like seeing a side view of my face (profile)."* (3.6 ± 0.7), which is the biggest preoperative problem for patients. The highest average result after the end of treatment was recorded on the question: *"I'm self-conscious about my facial appearance."* (2.0 ± 1.4), which was the biggest postoperative problem for patients. The lowest average result and, at the same time, the littlest problem for the patients, both before and after the treatment, was the question: *"I worry about meeting people for the first time."* (1.1 ± 1.3 vs. 0.6 ± 0.7).

Characteristics of patients and QoL measured by OQLQ and OHIP questionnaires

Table 1 shows that gender did not have a statistically significant effect on the change in QoL ($p > 0.05$). The interaction of gender, age, and type of surgery did not affect the change in subscale values ($p > 0.05$).

Based on the split-plot ANOVA (SPANOVA) test, it was proven that there is a statistically significant effect of the intervention on the change in the QoL measured before and 12

Table 1

Impact of gender, age, and type of surgery on the change in the quality of life measured by the OQLQ and OHIP-14 questionnaires before (T1) and 12 months after (T2) the orthodontic-surgical treatment of mandibular prognathism

Parameter	OQLQ				OHIP-14			
	Wilks' lambda	F	*p*	η^2	Wilks' lambda	F	*p*	η^2
Difference between T1 and T2	0.132	209.639	**0.000**	0.868	0.290	78.375	**0.000**	0.710
Difference between T1 and T2 *gender	0.913	3.045	0.091	0.087	1.000	0.000	0.987	0.000
Difference between T1 and T2 *age	0.967	1.094	0.303	0.033	0.974	0.850	0.363	0.026
Difference between T1 and T2 *group	0.987	0.434	0.515	0.013	0.962	1.266	0.269	0.038
Difference between T1 and T2 *gender*age	0.998	0.060	0.808	0.002	0.907	3.265	0.080	0.093
Difference between T1 and T2 *gender*group	0.971	0.966	0.333	0.029	0.984	0.536	0.470	0.016
Difference between T1 and T2 *age*group	0.992	0.273	0.605	0.008	0.993	0.215	0.646	0.007
Difference between T1 and T2 *gender*age*group	0.942	1.971	0.170	0.058	1.000	0.007	0.932	0.000

OQLQ – Orthognathic Quality of Life Questionnaire; OHIP – Oral Health Impact Profile; F – split-plot analysis of variance (SPANOVA); *p* – statistical significance; η^2 – squared Eta. Bolded values are statistically significant.

months after treatment (p = 0.00). The value of Eta² measured with the OQLQ questionnaire was 0.868, while the value measured with the OHIP questionnaire was 0.710, which indicated that the impact of the intervention was large (0.01= small impact, 0.06 = moderate impact, 0.14 = large impact).

Differences in the QoL of patients measured by the OQLQ questionnaire before and 12 months after the OS treatment of MP in patients with surgery on one jaw (monomaxillary group) and patients with surgery on both jaws (bimaxillary group)

Before and after the OS treatment, no intergroup differences were observed in the recorded values of the OQLQ scale, as well as on the subscales of the questionnaire: awareness of facial deformity, oral function, facial aesthetics, and social aspects (Table 2).

Differences in the QoL measured by the OHIP questionnaire before and 12 months after the OS treatment of MP in patients with surgery on one jaw (monomaxillary group) and patients with surgery on both jaws (bimaxillary group)

Before the start of treatment, intergroup differences were noted on the subscale: physical pain (-2.135, p = 0.039). Patients from the bimaxillary group felt preoperatively greater pain (1.8 ± 0.5) compared to patients from the monomaxillary group (1.4 ± 0.6). After the completion of the OS treatment, intergroup differences were observed on the same subscale of the OHIP questionnaire as at the beginning of the treatment (Table 3).

Discussion

Patients with dentofacial deformities, including MP, have a lack of self-confidence due to their appearance, which negatively affects their social relationships, employment, making emotional relationships, and QoL. In patients with MP, OS treatment is becoming increasingly important, which improves the oral functions and facial appearance of the patients [21].

The impact of malocclusions, including MP, on the QoL of patients was the subject of numerous studies, which confirm that malocclusions cause a higher degree of dissatisfaction with the appearance of the face. Most of these studies found that the QoL of patients with MP significantly improved after OS treatment and that most patients were satisfied with the outcome of treatment [3, 12].

In recent years, many studies have examined the impact of oral health problems on patients' QoL, using general and specific types of questionnaires such as the 36-item Short-Form Health Survey (SF-36), OHIP-14, and OQLQ [12, 22–24]. The questionnaire OHIP-14, developed by Slade [14], is most often used to assess the impact of oral health on the QoL of patients.

To assess the QoL, we used two questionnaires in our study, the OHIP-14 and the OQLQ. The results of our study showed that the biggest problem for patients before treatment

Table 2

Differences in the quality of life of patients before (T1) and 12 months after (T2) the orthodontic-surgical treatment of mandibular prognathism inside of monomaxillary and bimaxillary groups, according to the results of the OQLQ questionnaire

Parameter	Monomaxillary			Bimaxillary		
	T1	T2	*p*	T1	T2	*p*
Awareness of facial deformity	2.7 ± 0.9	1.8 ± 0.9	**0.004**	2.5 ± 1.2	1.7 ± 1.1	**0.001**
Oral function	2.7 ± 0.5	1.1 ± 0.4	**0.000**	2.4 ± 0.9	1.0 ± 0.7	**0.000**
Facial aesthetics	3.4 ± 0.3	1.5 ± 0.6	**0.000**	2.9 ± 0.8	1.3 ± 0.8	**0.000**
Social aspects	1.6 ± 1.0	0.6 ± 0.5	**0.000**	1.7 ± 0.9	0.8 ± 0.6	**0.000**
Overall score	2.4 ± 0.6	1.1 ± 0.5	**0.000**	2.3 ± 0.7	1.1 ± 0.7	**0.000**

OQLQ – Orthognathic Quality of Life Questionnaire. The *t*-test of repeated measurements was applied. Bolded values are statistically significant. The results are presented as mean ± SD.

Table 3

Differences in the quality of life of patients before (T1) and 12 months after (T2) the orthodontic-surgical treatment of mandibular prognathism inside of monomaxillary and bimaxillary groups, according to the results of the OHIP-14 questionnaire

Parameter	Monomaxillary			Bimaxillary		
	T1	T2	*p*	T1	T2	*p*
Functional limitation	0.8 ± 0.6	0.3 ± 0.4	**0.000**	0.8 ± 0.6	0.5 ± 0.6	**0.000**
Physical pain	1.4 ± 0.6	0.8 ± 0.8	**0.004**	1.8 ± 0.5	0.8 ± 0.7	**0.000**
Psychological discomfort	2.5 ±0.7	1.3 ± 0.9	**0.000**	2.3 ± 0.7	1.5 ± 0.7	**0.000**
Physical disability	1.2 ± 1.1	0.6 ± 0.9	0.067	1.1 ± 0.8	0.9 ± 1.0	0.244
Psychological disability	1.3 ± 1.2	0.6 ± 0.7	**0.044**	1.0 ± 1.2	0.5 ± 0.7	**0.043**
Social disability	1.2 ± 0.6	0.4 ± 0.5	**0.002**	1.2 ± 0.7	0.5 ± 0.6	**0.000**
Handicap	2.2 ± 0.8	0.8 ± 0.8	**0.000**	2.0 ± 0.9	0.9 ± 0.9	**0.000**
Overall score	1.5 ± 0.6	1.8 ± 0.7	0.212	1.5 ± 0.5	1.8 ± 1.1	0.129

OHIP – Oral Health Impact Profile. The *t*-test of repeated measurements was applied. Bolded values are statistically significant. The results are presented as mean ± SD.

was facial appearance because the highest result was recorded on the following questions: *"Have you been self-conscious because of your teeth, mouth, or dentures?"* (according to the OHIP questionnaire) and *"I don't like seeing a side view of my face (profile)."* (according to the OQLQ questionnaire). Based on the results of both questionnaires, patients had the littlest problems with their sense of taste. These results justify the fact that the main motive for treating patients is a change in facial appearance due to the present dentofacial deformity.

Based on the results of the OHIP questionnaire, we concluded that worse results were observed in patients before the start of treatment, while better results were recorded after the end of treatment. That shows that patient satisfaction with the QoL is significantly improved after completion of treatment, which is confirmed by a study by Kilinc and Ertas [2] with similar results.

Joachim et al. [8] examined the impact of OS treatment on the QoL of patients with MP and, similar to our results, concluded that this type of treatment has a positive effect on the QoL of both men and women in the physical and social domains.

Some studies have examined the impact of OS treatment on QoL at shorter time intervals after the surgery. In the Eslamipour et al. [21] study, after six months, there was a significant improvement in the QoL compared to the first three months after treatment. In our study, we examined the QoL 12 months after the completion of treatment, after the removal of orthodontic braces, and after the completion of the post-surgical phase of OT. During this period, there were no postoperative problems such as swelling, pain, bleeding, or neurosensitivity disorders which can negatively affect the QoL and temporarily cause its deterioration. Lee et al. [25], also assessing the QoL six weeks and six months after surgery, concluded that there was a deterioration in the QoL in the early postoperative phase compared to the period of six months after treatment. This gradual post-surgical improvement is supported by a study by Choi et al. [26], who reported moderate to vast improvements in the time interval of three to six months after completion of treatment.

In terms of different domains of QoL, when it comes to the analysis of our OQLQ questionnaire, the biggest changes occurred in the emotional domain, followed by psychological and functional aspects. The smallest changes occurred in the social domain, with similar results before and after treatment. According to the analysis of the OHIP-14 questionnaire, in terms of different domains, the littlest problem for patients was the domain of functional limitation both before and after treatment.

Eslamipour et al. [21] and Choi et al. [26] came to similar results, with the difference that the smallest changes occurred in the functional domain, and there were no significant changes in the first three months after the end of treatment. That is also in accordance with the study by Choi et al. [26], in which it was found that there was no significant improvement in the QoL; there was even a short-term deterioration in the QoL of the patients immediately after surgery. Both of these studies assessed the QoL for a limited period of six months after completion of treatment.

Desforges et al. [27] proved that improvement in the functional domain occurs later than the changes in other domains measured by the QoL questionnaires. This finding is to be expected because the surgery itself brings certain inconveniences for the patient, such as pain, swelling, neurosensitivity disorders, limited mouth opening, and reduced muscle efficiency [28].

For some patients, the social aspect was more important than improved facial appearance and oral function after treatment [9]. According to the results of our study, patients had the littlest problems in the social domain before and after the OS treatment. The changes that most affected the QoL were in the domains of facial aesthetics, awareness about deformity, and oral function in the last place.

After completing the treatment, patients have numerous psychological benefits, such as improving their body image, facial appearance, and better interpersonal relationships. Azuma et al. [29] examined changes in QoL about psychological status in patients with malocclusions after combined OS treatment. They concluded that patients after the OS treatment, regardless of the severity of malocclusion, had a lower degree of anxiety and improved QoL, measured by specific questionnaires for oral health and malocclusions, compared to the same parameters before treatment.

When it comes to the differences between men and women, the results of our study showed that there are no significant gender differences in overall QoL as well as in the domains measured by the patient QoL questionnaires after the OS treatment of MP.

Eslamipour et al. [21] noted that the overall QoL outcome for women in all four domains (especially in the emotional and social domains) shows poorer status compared to men before surgery. However, the QoL in women achieved remarkable improvement in all four domains to the same extent as in men after surgery. That shows that the QoL in both women and men has changed for the better, with women having bigger improvements. The results of the study by Rezaei et al. [3] showed that there were no differences between men and women regarding the OHIP questionnaire. When it comes to the OQLQ questionnaire, in all domains measured by the questionnaire, women were more dissatisfied before surgical treatment than men. That means that men had a better QoL before treatment compared to women and that women are more sensitive when it comes to facial appearance.

Our study did not confirm the difference in the QoL between the monomaxillary and bimaxillary groups of patients. Patients from both groups, those who underwent surgery on one and those who underwent surgery on both jaws, showed the same improvement in QoL after treatment. These findings support the fact that OS treatment leads to remarkable improvements in various aspects of the psychological, functional, social, emotional, and physical well-being of the patient [12, 22, 30, 31].

Our study has certain limitations such as the fact that the OS treatment lasts a long time and includes

many phases. A larger number of patients who would follow through all the phases of the OS treatment, including a longer period of time after completion of the treatment, would contribute to a better understanding of the impact of overall treatment on the QoL of patients with MP.

Conclusion

The OS treatment of patients with MP improves all oral functions, the appearance of the patient's face, and self-confidence. All of that leads to a significant improvement in the QoL.

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Gingival crevicular glucose estimation and patient's perception of pain during routine dental examination – a concept based on a novel patented periodontal device

Procena nivoa glukoze u gingivalnom sulkusu i percepcija bola kod pacijenata tokom rutinskog stomatološkog pregleda – koncept zasnovan na novopatentiranom parodontalnom uređaju

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Abstract

Background/Aim. Diabetes mellitus (DM) is a common metabolic disease that causes high mortality and morbidity worldwide. Therefore, in order to implement prevention strategies, physicians need to identify this condition as early as possible. The aim of the study was to test the concept of a novel periodontal device that can be attached to a glucose monitoring device as an innovative tool to screen for periodontitis and DM simultaneously during a routine dental examination. Hence, the correlation of blood glucose between the conventional finger-prick blood glucose (FPBG) and gingival crevicular blood glucose (GCBG) method, along with an estimation of the patient's pain perception by visual analog scale (VAS) was examined. **Methods.** A cross-sectional comparative study was conducted among 250 participants whose GCBG and FPBG were estimated. The VAS score scale was recorded for each patient immediately after the procedure. **Results.** The mean GCBG value was 151.19 ± 42.64 mg/dL, while the mean FPBG was 150.48 ± 42.95 mg/dL, showing a high Pearson's correlation ($r = 0.9932$; $p < 0.00001$). The Mann-Whitney *U* test for VAS score between both groups showed a statistically significant difference ($p < 0.00001$). **Conclusion.** The GCBG method was well tolerated by patients, and highly correlated with peripheral blood glucose levels. The proposed concept of the novel periodontal device appeared to be a feasible option for examining periodontium and screening DM simultaneously in dental clinics.

Key words:
blood glucose; dental instruments; diabetes mellitus; fingers; gingival crevicular fluids; pain measurement; periodontitis.

Apstrakt

Uvod/Cilj. Dijabetes melitus (DM) je česta metabolička bolest, koja izaziva visok morbiditet i mortalitet širom sveta. Zbog toga, kako bi primenili strategije prevencije, lekari moraju što ranije da identifikuju to stanje. Cilj rada bio je da se testira novi parodontalni uređaj koji se može povezati sa uređajem za praćenje nivoa glukoze u krvi kako bi tokom rutinskog stomatološkog pregleda istovremeno vršio skrining parodontitisa i DM. Stoga, ispitana je korelacija nivoa glukoze u krvi izmerenih primenom konvencionalne metode merenja glukoze u krvi iz prsta (KMIP) i metode merenja glukoza u krvi iz gingivalnog sulkusa (UKGS), uz istovremenu procenu percepcije bola pacijenata vizuelnom analognom skalom (VAS). **Metode**. Komparativna studija preseka sprovedena je među 250 ispitanika kojma su procenjeni UKGS i KMIP. Bodovna skala VAS zabeležena je za svakog pacijenta odmah nakon postupka. **Rezultati.** Prosečna vrednost glukoze UKGS iznosila je 151,19 ± 42,64 mg/dL, dok je KMIP vrednost glukoze bila 150,48 ± 42,95 mg/dL, što je pokazalo visoku *Pearson*-ovu korelaciju ($r = 0,9932$; $p < 0,00001$). Mann-Whitney *U* test za VAS skor između obe grupe pokazao je statistički značajnu razliku ($p < 0,00001$). **Zaključak**. Metodu UKGS pacijenti dobro tolerišu, a njene vrednosti značajno korelišu sa nivoom glukoze u perifernoj krvi. Predloženi koncept korišćenja novog parodontalnog uređaja pokazao se kao pogodan izbor za ispitivanje parodoncijuma i istovremeni skrining DM u stomatološkim klinikama.

Ključne reči:
glukoza u krvi; stomatološki instrumenti; dijabetes melitus; prsti; gingivalna sulkusna tečnost; bol, merenje; periodontitis.

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Introduction

Diabetes mellitus (DM) is a common metabolic disease that causes high mortality and morbidity worldwide. There has been a steady increase in its prevalence, as a recent report shows that it has increased from 4.7% to 9.3% over the last few decades. Researchers expect the numbers to rise from 463 million to 578 million cases in the next decade [1]. The rising medical expenses associated with DM begin much before its diagnosis. Therefore, physicians need to identify this condition early to implement prevention strategies [1]. DM causes periodontal breakdown [2] and peri-implantitis [3]. Observational [4] and longitudinal studies [5, 6] demonstrate that DM increases the risk of periodontitis in adults [7, 8]. The role of uncontrolled DM as a risk factor for periodontal disease and tooth loss has been widely studied [9, 10]. Moreover, persons with DM also have delayed wound healing [11]. Hence, screening for elevated blood glucose (BG) levels prior to any dental procedure is crucial for achieving adequate glycemic control before any surgical procedure or dental implant placement [10].

In our body, glucose is found in blood and other secretions such as intracellular fluids, tears, saliva, and urine. Its concentration is highest in the arterial circulation [7]. However, in the laboratory, venous blood samples are usually taken to diagnose DM using the glucose oxidase method. Nevertheless, if the tourniquet is left for a long time, BG concentrations can fluctuate as much as 25 mg/dL [12]. The capillary BG levels used in the finger-prick blood glucose (FPBG) estimation are found to be between venous and arterial concentrations. The chairside glucometer used to monitor BG levels typically uses glucose oxidase biosensors to determine BG concentration from the FPBG method using a lancet. This technique is a direct and accurate method for assessing BG concentrations. Yet, it has been found that patients are often distressed while doing this test due to pain [13, 14].

Recent studies show that gingival crevicular blood glucose (GCBG) is emerging as an alternative source of blood for determining BG concentration [15–19]. Bleeding on probing (BOP) occurring in the gingival crevice during routine dental examination is an objective sign of periodontal breakdown. Apparently, this bleeding during routine periodontal probing can be used to estimate the BG level instead of the traditional FPBG [20–23]. Many invasive dental procedures require persons with DM to first screen for their current blood sugar status since hyperglycemia and hypoglycemia can be detrimental [4, 16]. For most persons with DM, especially the elderly who report to dental procedures, BG levels should be regularly monitored [4, 16]. That is not feasible with traditional BG detection methods; thus, continuous glucose monitoring in these persons using the GCBG method during lengthy dental procedures may be of great clinical application value and more in line with market trends. Although there are many glucometers in the global market, none of these have the mechanism to measure periodontitis and screen for DM simultaneously. Therefore, this study aimed to test the concept of a novel patented periodontal device that can be attached to a glucose monitoring device as an innovative tool to screen for periodontitis and DM simultaneously during a routine dental examination. Accordingly, the correlation of BG between the conventional FPBG method and the GCBG method was assessed.

Methods

Clinical protocol

We conducted a cross-sectional comparative study among 180 participants who reported to the Outpatient Department, College of Dentistry, King Khalid University, Saudi Arabia, using a randomized sampling technique from April to November 2021. Ethical Approval was obtained from the Institutional Review Board of King Khalid University, Saudi Arabia (IRB/KKUCOD/ETH/2020-21/053). The study followed the code of ethics in the Declaration of Helsinki (version 17c, 2004). Complete information about the study was given to the participants in their language, and informed consent was obtained before the commencement of the study.

Participants

Patients older than 18 years who had chronic periodontitis (with at least one bleeding site) were recruited for the study. Patients with the following conditions were excluded: (1) recent use of antibiotics; (2) patients with hematological disorders; (3) use of medications that interfere with coagulation; (4) presence of any systemic disorder.

Sources of data and details of methods of assessment

Patients who met the inclusion criteria were taken for the study after obtaining informed consent. They reported to the clinic at 8 am to have their glucose levels recorded. At first, the patients underwent periodontal probing using the University of Michigan “O” periodontal probe with Williams graduations at six sites on each tooth. A single examiner carried out these examinations to detect each patient’s bleeding gingival site. A GCBG sample was collected from the site with maximum inflammation and bleeding [16]. Removal of a piece of supragingival calculus was needed in some cases to collect blood from the gingival sulcus. About 10 to 15 μL of blood sample was collected after isolating the area with cotton rolls to prevent saliva contamination and drying with compressed air. Glass capillary tubes were used to transfer the blood to the test strip that was already loaded into the glucometer following the manufacturer’s instructions.

Patented device

A device for simultaneous measuring of periodontitis (in terms of clinical attachment level, probing depth, and bleeding on probing) and DM (in terms of peripheral BG levels) has been recently granted a patent (Patent Number: SA 6757 B – Saudi Arabia; patented February 2020). The patented glass periodontal probe with markings that can be

attached to a glucose monitoring device is an innovative tool to screen for peripheral BG levels and periodontitis simultaneously during a routine dental examination. It is a manual periodontal probe with 1 mm graduations, a small, battery-powered glucometer, and a display screen attached to its handle. The probe tip is a small glass capillary tube of 0.5 mm that collects blood oozing from the gingival sulcus/pocket following routine periodontal pocket probing and transfers it to the test strip of a glucose self-monitoring device attached to the handle of the probe. Graphical presentations of the device are presented in Figure 1 (a–d).

Visual Analog Scale

After blood sugar analysis using both methods, the patients were asked about their perception of pain during each method, which was marked as Visual Analog Scale (VAS) pain [24] using a 10 mm VAS scale within 10 min of the procedure. This scale has a straight line, and the left end of the scale was marked as "no pain" and the right end as "worst imaginable pain".

Statistical analysis

A subject-level analysis was performed statistically for variables using SPSS software for Windows, Version 22.0. (SPSS Inc., Chicago, IL, USA). The mean value and standard deviation (SD) were calculated for each variable measured. Karl Pearson's product-moment correlation was done to determine associations between the two techniques of determining BG levels. A Scatter plot of the linear relationship between the two techniques of BG determination was drawn. Differences in mean values between the pain scores were assessed using the Mann-Whitney *U* test. All statistical procedures were performed at a significance level of 5% ($p < 0.05$).

Results

All 180 participants (88 males and 92 females) completed this study. The mean age of the participants was 41.65 ± 9.68 years. The mean GCBG value was 151.19 ± 42.64 mg/dL, while the FPBG value was 150.48 ± 42.95 mg/dL

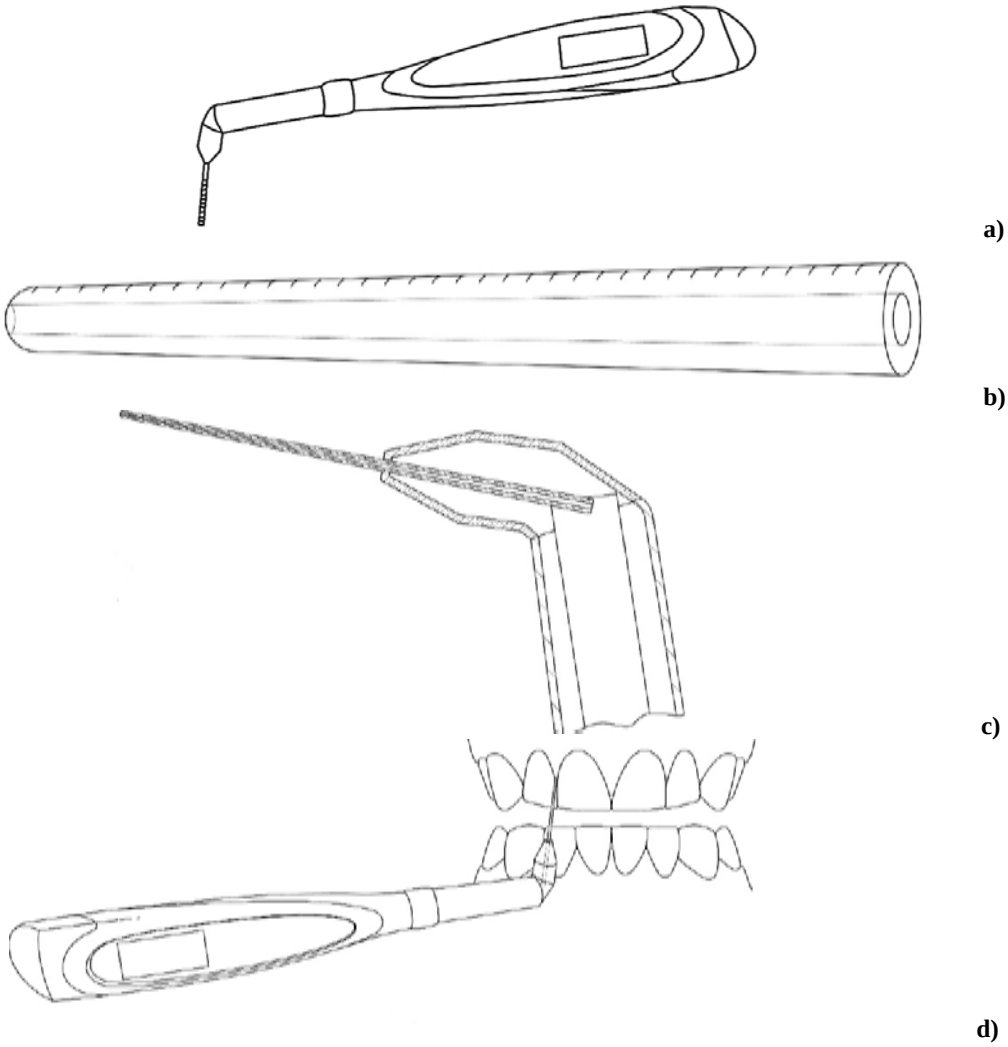

Fig. 1 – a) Graphical representation of the unique patented periodontal device; b) The proposed glass probe of the patented periodontal device; c) Attachment of the proposed glass probe to the self-monitoring glucometer; d) Proposed mode of application of the patented device.

Table 1

The mean and standard deviation of blood glucose using GCBG and FPBG methods

GCBG (mg/dL)	FPBG (mg/dL)	*p*-value	*t*-value
151.19 ± 42.64	150.48 ± 42.95	0.437	0.158

GCBG – gingival crevicular blood glucose; FPBG – finger-prick blood glucose. The results are expressed as mean ± standard deviation.

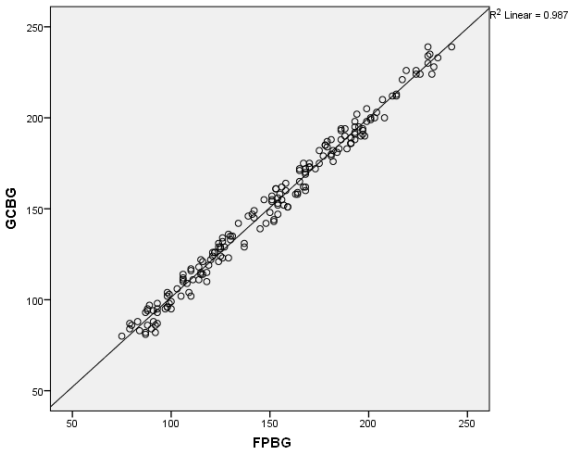

Fig. 2 − Scatter plot of the linear relationship between gingival crevicular blood glucose (GCBG) and finger-prick blood glucose (FPBG).

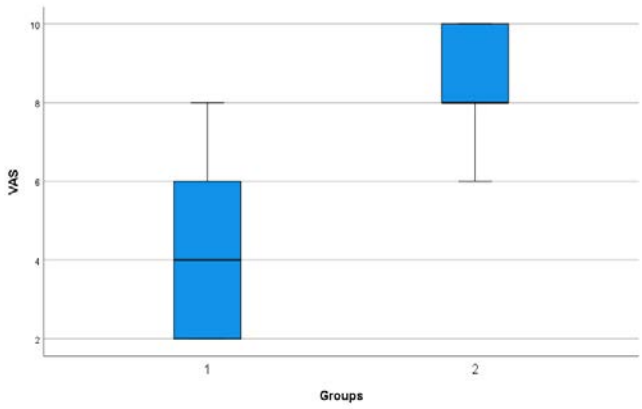

Fig. 3 − Box plot for the mean of visual analog scale (VAS) gingival crevicular (GC) group (VAS-GC) and VAS finger-prick (FP) group (VAS-FP). Group 1 – VAS-GC; Group 2 – VAS-FP.

(Table 1). This difference was not statistically significant ($p = 0.437$).

In order to understand the correlation between these two techniques, Karl Pearson's product-moment correlation was done, which showed a very high coefficient (r = 0.9932) between the two methods ($p < 0.00001$).

Comparison between the difference in the mean VAS score was tested using the Mann-Whitney *U* test for VAS in the gingival crevicular (GC) group (VAS-GC) (4.71) and VAS in the finger-prick (FP) group (VAS-FP) (8.63); it showed a statistically significant difference ($p < 0.00001$).

Figure 2 shows the linear relationship between GCBG and FPBG. The regression equation for *Y* was 1.00043*X* - 0.77668. The box plot representation for the mean of VAS-GC and VAS-FP is shown in Figure 3.

Discussion

The concept described in this study was tested by a study that checked the correlation of BG between the conventional FPBG and GCBG methods. The mean GCBG value was 151.19 ± 42.64 mg/dL, while the mean FPBG value was 150.48 ± 42.95 mg/dL. A dental surgeon can actively screen patients for BG levels during a routine periodontal examination. Early detection of DM can also reduce the financial burden and deterioration of oral and periodontal health. That is even more relevant in the case of undiagnosed DM, which can be referred to the physician for appropriate management of the condition. Poorly controlled DM risks developing periodontal breakdown and adversely affects treatment outcomes. Therefore, this study tested the concept of a novel patented periodontal device that can be attached to a glucose monitoring device to screen for periodontitis and DM simultaneously during routine dental examinations. Several studies report a strong positive correlation between gingival BG levels and peripheral BG levels [12–14, 16, 17, 19, 23]. Many authors have used this technique to detect BG levels and screen for persons with DM in the dental office during periodontal treatment [16, 17, 19, 25–28]. The present study showed an almost perfect correlation (r = 0.9932) between GCBG and FPBG methods ($p < 0.00001$) which was in accordance with the findings of previous studies that show a good correlation between crevicular and peripheral BG levels [14, 17–19, 26, 29]. Contrary to these results, a report by Muller and Behbhani [25] in a Kuwaiti population did not demonstrate a correlation between gingival crevice blood (GCB) and capillary finger-stick BG levels. GCB samples from sites that showed sufficient BOP were useful for screening BG levels [29]; participants with sufficient BOP had a higher correlation coefficient (r = 0.89) and acceptable limits of agreement (-27.1 to 29.7).

Variations in the number of blood samples used in different studies raised questions about the feasibility of the GCBG method during routine dental examinations [30]. Parker et al. [30] used 10–15 µL gingival blood samples, a large volume present only in sites with periodontal inflammation and not always seen. On the other hand, Beikler et al. [20] used only 3 µL of blood in a self-monitoring device in periodontitis patients. Muller and Behbhani [25] reported using only 0.3 µL of a blood sample, although sufficient bleeding was not seen in

some cases. This study also reports low agreement and broader limits of repeatability when GCBG was considered.

The present study is one of those that propose a noninvasive/minimally invasive method to monitor BG levels and, at the same time, record periodontal pocket depth. Therefore, this device can simultaneously screen DM while detecting periodontal disease. Moreover, the safety features of this design include the rounded tip of the probe so that it does not cause any injury to the gingival tissue during insertion. It is also important that the probe attached to the model is disposable in order to maintain infection control protocol.

Although some studies mention a GCBG as a painless technique [20, 30], none of these have measured pain using any pain scale. We chose to report on patients' perceptions as understanding that is known to enhance the effectiveness of care provided [31]. Periodontitis patients and practitioners [32] report dental visits suitable for screening BG, and patients preferred the GCBG method to the FPBG method.

Several reports indicate that gingival crevicular blood is a feasible and quick method for screening BG levels as it can be performed as a chairside screening test. However, the application of this technique is limited to a routine test because gingival crevicular blood depends on the presence of inflammation and can be available only when there is periodontal inflammation. In fact, the ability of this technique to screen with high sensitivity needs to be further tested in the presence of lower periodontal inflammation. As a result, when periodontal inflammation subsides following periodontal therapy treatment, minimal or no bleeding (less than 4 μL of blood) can limit the utility of this technique. The possible contamination or dilution of crevicular blood following periodontal probing by gingival crevice fluid needs to be explored further. Furthermore, the probability of increased glucose levels in gingival crevice fluid from sites of periodontal inflammation compared to healthy sites also needs to be considered [33]. Our team plans future studies to evaluate the sensitivity, specificity, and predictive values of the GCBG method to explore its applicability in screening persons with DM in a larger population sample before and after periodontal therapy.

Conclusion

Within the limitations of the study, a significant difference was seen in the pain score between the VAS-GC group and the VAS-FP group. The use of the presented proposed concept of the unique patented periodontal device appears to be a feasible option for simultaneously examining the state of periodontium and screening for DM in dental clinics. Additional longitudinal studies should be done in a larger population sample to understand the clinical applicability and diagnostic accuracy of the presented device.

Conflicts of interest

The authors declare no conflict of interest.

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Application value of bedside ultrasound for assessing volume responsiveness in patients with septic shock

Korist od primene ultrazvuka za procenu odgovora bolesnika sa septičkim šokom na nadoknadu volumena

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Abstract

Background/Aim. Septic shock (SS) is a complication that can occur as a consequence of an infection. As the effective circulating blood volume is of great importance in these cases, keeping constant track of the blood volume parameter is essential. The aim of this study was to explore the application value of bedside ultrasound for assessing volume responsiveness (VR) in patients with SS. **Methods.** A total of 102 patients with SS were selected. The volume load (VL) test was performed, and based on the results of the test, the patients were divided into two groups. The first group was the response (R) group, which had an increase in stroke volume (ΔSV) ≥ 15% after the VL test, and the second was the non-response (NR) group, with ΔSV < 15% after the VL test. There were 54 patients in the R group and 48 in the NR group. Hemodynamic parameters were compared before and after the VL test. The correlation between ΔSV and each hemodynamic index was explored by Pearson's analysis. The receiver operating characteristic (ROC) curves were plotted for some of the parameters. **Results.** Before the VL test, retrohepatic (RH) *inferior vena cava* (IVC) (RHIVC) distensibility ($\Delta_{RH}IVC_1$) index, respiratory variation in RHIVC ($\Delta_{RH}IVC_2$) index, respiratory variation in aortic (AO) blood flow peak velocity ($\Delta Vpeak_{AO}$) index, respiratory variation in brachial artery (BA) blood flow peak velocity ($\Delta Vpeak_{BA}$) index, and respiratory variation in common femoral artery (CFA) blood flow peak velocity ($\Delta Vpeak_{CFA}$) index were all higher in the R group than those in the NR group ($p < 0.05$), while heart rate (HR), mean arterial pressure (MAP), and central venous pressure (CVP) were similar in both groups ($p > 0.05$). After the VL test, the R group had significantly decreased values of HR and the $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ indices, while the MAP and CVP values ($p < 0.05$) were increased. The NR group had a significantly decreased value of CVP ($p < 0.05$), while no significant changes were noticed in the values of other indices. The indices $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ significantly correlated with ΔSV ($r = 0.589$, $r = 0.647$, $r = 0.697$, $r = 0.621$, $r = 0.766$, respectively; $p < 0.05$), but there was no correlation between CVP and ΔSV ($r = -0.345$; $p > 0.05$). The areas under the curve (AUC) of ROC graphics for $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ indices, used for the prediction of VR, were 0.839, 0.858, 0.878, 0.916, and 0.921, respectively, and were significantly larger than the AUC of ROC graphic for CVP (0.691), indicating higher sensitivity and specificity of the $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ indices compared to CVP. **Conclusion.** Bedside ultrasound monitoring of the $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ indices can assess the VR in patients with SS more precisely.

Key words:
blood volume; hemodynamic monitoring; infusions, intravenous; saline solution; shock, septic; ultrasonography.

Apstrakt

Uvod/Cilj. Septički šok (SŠ) je komplikacija koja može nastati kao posledica infekcije. S obzirom na to da je efektivni cirkulatorni volumen krvi od velike važnosti u ovim slučajevima, kontinuirano praćenje parametara volumena krvi je ključno. Cilj ovog rada je bio da se istraži značaj primene ultrazvuka za procenu odgovora na

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nadokandu volumena (*volume responsiveness* – VR) kod bolesnika sa SŠ. **Metode.** Odabrano je ukupno 102 bolesnika sa SŠ. Urađen je test volumenskog opterećenja (*volume load* – VL), i na osnovu rezultata testa, bolesnici su bili podeljeni u dve grupe. Jednu grupu činili su bolesnici koji su pokazali odgovor (*response* - R) na VL testu (grupa R); kod njih je povećanje udarnog volumena (*stroke volume*-SV) (ΔSV) bilo ≥ 15% posle VL testa. Drugu grupu (*non-response* – NR) činili su bolesnici kod kojih je ΔSV bio < 15% posle VL testa. U grupama je bilo 54 bolesnika (R) i 48 bolesnika (NR). Ispitivani hemodinamički parametri upoređivani su pre i posle VL testa. Korelacija između ΔSV i svakog pojedinačnog hemodinamičkog indeksa ispitivana je Pirsonovom analizom, a za određene parametre korišćene su ROC krive. **Rezultati.** Pre VL testa, veće vrednosti u grupi R u odnosu na grupu NR ($p < 0,05$) imali su sledeći indeksi: rastegljivost retro-hepatične ($_{RH}$) donje šuplje vene [*inferior vena cava* (IVC)] ($\Delta_{RH}IVC_1$), respiratorna varijacija $_{RH}IVC$ ($\Delta_{RH}IVC_2$), respiratorna varijacija najveće brzine protoka krvi u aorti ($\Delta Vpeak_{AO}$), respiratorna varijacija najveće brzine protoka krvi u brahijalnoj arteriji ($_{BA}$) ($\Delta Vpeak_{BA}$) i respiratorna varijacija najveće brzine protoka krvi u zajedničkoj femoralnoj arteriji [*common femoral artery* ($_{CFA}$)]($\Delta Vpeak_{CFA}$). Frekvencija srca (FS), srednji arterijski pritisak (SAP) i centralni venski pritisak (CVP) su imali slične vrednosti ($p > 0,05$) u obe grupe. Nakon VL testa, grupa R imala je značajno smanjenje vrednosti FS i indeksa $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$ i $\Delta Vpeak_{CFA}$, a povećanje vrednosti SAP i CVP ($p < 0,05$). Grupa NR imala je značajno smanjen CVP ($p < 0,05$), a nisu primećene značajne promene u vrednostima ostalih indeksa. Indeksi $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$ i $\Delta Vpeak_{CFA}$ bili su u značajnoj korelaciji sa ΔSV ($r = 0,589$, $r = 0,647$, $r = 0,697$, $r = 0,621$, $r = 0,766$, redom; $p < 0.05$), ali nije bilo korelacije između CVP i ΔSV ($r = -0,345$; $p > 0,05$). Površine ispod ROC krive [*areas under the curve* (AUC)] za indekse $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$ i $\Delta Vpeak_{CFA}$, koji su korišćeni za predviđanje VR, iznosile su 0,839, 0,858, 0,878, 0,916 i 0,921 redom, i bile su značajno veće od AUC za CVP (0,691), što je ukazivalo da su indeksi $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$ i $\Delta Vpeak_{CFA}$ imali viši nivo osetljivosti i specifičnosti u poređenju sa CVP. **Zaključak**. Ultrazvučnim praćenjem indeksa $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$ i $\Delta Vpeak_{CFA}$ može se preciznije proceniti VR kod bolesnika sa SŠ.

Ključne reči:
krv, volumen; hemodinamika, monitoring; infuzije, intravenske; rastvor, fiziološki; šok, septički; ultrasonografija.

Introduction

Septic shock (SS) is primarily caused by hemodynamic instability due to various pathogen infections, and it is characterized by high cardiac output (CO), low peripheral vascular resistance, and the resulting sepsis-induced tissue hypoperfusion. SS has a high mortality rate and many complications, which are more common in the Intensive Care Unit (ICU) [1–3]. At present, fluid resuscitation (FR) is an important strategy for the clinical treatment of SS, which can supplement the effective circulating blood volume, improve tissue perfusion, and correct cellular hypoxia, thereby lowering the mortality rate [4, 5]. According to the Frank-Starling Law of the heart, the cardiac reserve is sufficient among patients in the ascending branch of the curve, and the increase in cardiac preload within a certain range can increase CO and fully achieve FR. However, when the left or right ventricle is at the plateau of the Frank-Starling curve, excessive FR will worsen the cardiac volume load (VL) in patients, raise the risk of pulmonary edema and decreased oxygenation, and aggravate shock [6]. After early rapid FR, however, it is difficult to assess the patient's blood volume status. Excessive FR will induce complications such as heart failure and pulmonary edema, while insufficient FR will increase the risk of organ dysfunction in patients [7, 8]. Therefore, it is essential to accurately assess the volume responsiveness (VR) in patients with SS for FR therapy. Bedside ultrasound can dynamically predict and evaluate the responsiveness of the circulatory system to the VL in convenient, non-invasive, and real-time manners. Full attention has been given to it in hemodynamic assessment in patients with SS. In this study, the hemodynamic indices of VR in patients with SS were observed by bedside ultrasound, and the value of ultrasound hemodynamic indices in assessing the VR was explored to provide a theoretical basis for fluid therapy in patients with SS.

Methods

A total of 102 patients with SS who received mechanical ventilation (mode: AC/PC; PS: 15 cmH_2O; PEEP: 4 cmH_2O; FIO_2: 45%) in Jinshan Hospital, China, from April 2018 to February 2021 were selected, including 55 males and 47 females aged 23–75 years. The patients had an average age of 56.4 ± 12.4 years. Inclusion criteria were as follows: 1) patients meeting the diagnostic criteria for SS in the "International Guidelines for Management of Sepsis and Septic Shock: 2016" [9]; 2) patients with any of the following clinical manifestations of tissue hypoperfusion – a) systolic blood pressure (SBP) ≤ 90 mmHg (decline in SBP > 50 mmHg in hypertensive patients); b) heart rate (HR) >100 bpm; c) urine volume < 0.5 mL/kg for two consecutive hours; d) piebald skin; 3) patients with sinus HR.

Exclusion criteria were as follows: 1) patients with intra-abdominal hypertension; 2) patients with congenital heart disease, severe cardiac insufficiency, severe arrhythmia or pulmonary arterial hypertension; 3) patients with severe obesity, i.e., body mass index (BMI) > 40 kg/m^2; 4) patients with complications such as cerebrovascular accident, neurogenic shock, coronary heart disease or intra-aortic balloon counterpulsation; 5) pregnant women; 6) those with contraindications for fluid infusion (left ventricular ejection fraction – LVEF ≤ 40%, lower limb vein thrombosis, aortic valve or pulmonary valve disease, mitral valve stenosis or insufficiency > degree 2, or volume overload).

General clinical data of patients (gender, age, BMI, and infection site), acute physiology and chronic health evaluation II (APACHE II) score, and sequential organ failure assessment (SOFA) score were recorded within 24 hrs after entering the ICU.

The patients were given effective analgesia and sedation in a supine position, and 200 mL of normal saline was quickly infused *via* the central vein within ten minutes. Blood volume supplementation was terminated when the patient's central venous pressure (CVP) reached the value of more than 5.0 cm H_2O (1 cm H_2O = 0.098 kPa), and the increase in stroke volume (ΔSV) was less than 10% of the basal level, or when obvious pulmonary edema occurred. Before and after the VL test, hemodynamic monitoring was performed. Transthoracic echocardiography was conducted using a Zonare color Doppler ultrasound diagnostic apparatus (P4-1C probe, frequency: 3.5 MHz). Left ventricular outflow tract dimension was measured in the left ventricle long-axis view, and one complete respiration cycle was monitored in the apical five-chamber view in order to obtain the left ventricular outflow tract velocity-time integral (VTI) and the maximum ($Vpeak_{max}$) and minimum value ($Vpeak_{min}$) of the aortic ($_{AO}$) blood flow peak velocity with respiratory motion. The retro-hepatic ($_{RH}$) *inferior vena cava* (IVC) ($_{RH}$IVC) was explored by the probe longitudinally below the right rib; the minimum end-inspiratory dimension (D_{min}) and the maximum end-expiratory dimension (D_{max}) of IVC were monitored in the subxiphoid IVC long-axis view during one complete respiration cycle. Then the brachial artery ($_{BA}$) blood flow velocity was measured at the cubital *fossa* using the L10-5 probe (frequency 8 MHz), and the $Vpeak_{max}$ and $Vpeak_{min}$ of the ($_{BA}$) blood flow peak velocity with respiratory motion were monitored during one complete respiration cycle. The same parameters were measured for the common femoral artery ($_{CFA}$).

The above indices were measured three times, and the average value was taken. Moreover, CO, ΔSV, distensibility of $_{RH}$IVC ($\Delta\ _{RH}IVC_1$) index, respiratory variation in $_{RH}$IVC ($\Delta_{RH}IVC_2$) index, respiratory variation in ($_{AO}$) blood flow peak velocity ($\Delta Vpeak_{AO}$) index, respiratory variation in ($_{BA}$) blood flow peak velocity ($\Delta Vpeak_{BA}$) index, and respiratory variation in ($_{CFA}$) blood flow peak velocity ($\Delta Vpeak_{CFA}$) index were calculated using the following formulas:

$$SV = (D/2)^2 \times \pi \times VTI$$

$$\Delta SV = (SV_{load\ value} - SV_{basal\ value}) / SV_{basal\ value}$$

$$\Delta_{RH}IVC_1 = (D_{max} - D_{min}) / D_{min} \times 100\%$$

$$\Delta_{RH}IVC_2 = 2 \times (D_{max} - D_{min}) / (D_{max} + D_{min}) \times 100\%$$

$$\Delta Vpeak_{AO} = 2 \times (Vpeak_{max} - Vpeak_{min}) / (Vpeak_{max} + Vpeak_{min}) \times 100\%$$

$$\Delta Vpeak_{BA} = 2 \times (Vpeak_{max} - Vpeak_{min}) / (Vpeak_{max} + Vpeak_{min}) \times 100\%$$

$$\Delta Vpeak_{CFA} = 2 \times (Vpeak_{max} - Vpeak_{min}) / (Vpeak_{max} + Vpeak_{min}) \times 100\%$$

where the $Vpeak_{max}$ is the maximum value of blood flow peak velocity during one respiration cycle for each of the mentioned blood vessels, and $Vpeak_{min}$ is the minimum value of blood flow peak velocity during one respiration cycle for each of the mentioned blood vessels.

Based on ΔSV after fluid infusion, the patients were divided into the positive volume response (R) group ($\Delta SV \geq 15\%$) and the non-response (NR) group ($\Delta SV < 15\%$).

SPSS 22.0 software was used for statistical analysis. Normally distributed measurement data were expressed as mean ± standard deviation and compared between two groups by independent-samples *t*-test. Numerical data were expressed as a percent and compared between two groups by χ^2 test. The correlation between ΔSV and each hemodynamic index was explored by Pearson's analysis. The receiver operating characteristic (ROC) curve was plotted; the value of the hemodynamic index for assessing the VR in patients with SS was analyzed. The value of $p < 0.05$ was considered statistically significant.

Results

Among the 102 patients with SS, there were 40 cases of pulmonary infection, 10 cases of intracranial infection, 18 cases of abdominal infection, 16 cases of urinary system infection, 14 cases of blood-borne infection, and four cases of other types of infection. Fifty-four (52.9%) patients had a positive volume response. At the baseline, the patient's gender, age, BMI, tidal volume, respiratory rate, LVEF, SBP, lactic acid level, APACHE II score, SOFA score, shock index, and infection site had no significant differences between positive volume response (R) patients, and negative volume response (NR) patients ($p > 0.05$), indicating that the baseline data were comparable (Table 1).

There were no significant differences in HR, CO, mean arterial pressure (MAP), and CVP between the two groups before the VL test ($p > 0.05$). After the VL test, HR declined, while CO, MAP, and CVP rose in R patients ($p < 0.05$). After the VL test, CO and CVP rose in NR patients ($p < 0.05$), while HR and MAP had no significant changes. After the VL test, the R group had lower HR and higher CO and MAP than the NR group ($p < 0.05$) (Table 2).

Before the VL test, the $\Delta\ _{RH}IVC_1$, $\Delta\ _{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ indices were all higher in the R group compared to the NR group ($p < 0.05$). After the VL test, the $\Delta_{RH}IVC_1$, $\Delta\ _{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ indices declined to different levels in the two groups. There were significant differences in the values of the parameters before and after the VL test in R patients ($p < 0.05$), while there were no significant changes before and after the VL test in NR patients ($p > 0.05$) (Table 3).

CO and the $\Delta\ _{RH}IVC_1$, $\Delta\ _{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ indices were significantly correlated with ΔSV before the VL test ($r = -0.672$, $r = 0.589$, $r = 0.647$, $r = 0.697$, $r = 0.621$, $r = 0.766$, respectively; $p < 0.05$), but there was no correlation between CVP and ΔSV ($r = -0.345$; $p > 0.05$). The results showed that ultrasonically measured hemodynamic indices before the VL test could be used to assess VR in patients with SS (Table 4).

The area under the curve (AUC) of ROC graphics for CO and the $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and

Table 1

Baseline clinical data of patients

Parameter	Response group (n = 54)	Non-response group (n = 48)	t/χ^2	*p*-value
Age (years)	54.9 ± 10.3	57.8 ± 11.9	0.724	0.468
BMI (kg/m^2)	23.3 ± 3.7	23.7 ± 3.6	0.468	0.635
TV (mL)	556.9 ± 87.9	528.9 ± 72.8	1.162	0.258
RR (time/min)	19.1 ± 3.4	18.9 ± 3.2	0.697	0.502
LVEF (%)	58.7 ± 10.3	59.2 ± 11.0	0.236	0.805
SBP (mmHg)	115.3 ± 21.1	113.0 ± 22.3	0.413	0.681
Lactic acid (mmol/L)	2.5 ± 0.6	2.7 ± 0.6	1.470	0.146
APACHE II score	27.6 ± 9.4	26.3 ± 10.2	0.320	0.742
SOFA score	10.4 ± 3.1	10.0 ± 1.1	0.285	0.821
Shock index	1.4 ± 0.4	1.3 ± 0.5	1.086	0.267
Infection site				
pulmonary infection	21 (38.9)	19 (39.6)	0.299	0.765
intracranial infection	4 (7.4)	6 (12.5)	2.668	0.258
abdominal infection	10 (18.5)	8 (16.7)	1.963	0.167
urinary system infection	9 (16.7)	7 (14.6)	0.049	0.976
blood-borne infection	7 (12.9)	7 (14.7)	0.946	0.514
other	3 (5.6)	1 (2.1)	0.422	0.685

BMI − body mass index; TV − tidal volume; RR − respiratory rate; LVEF − left ventricular ejection fraction; SBP − systolic blood pressure; APACHE II − acute physiology and chronic health evaluation II; SOFA − score and sequential organ failure assessment.
Results are shown as mean ± standard deviation except infection sites which are shown as numbers (percentages).

Table 2

General hemodynamic indices before and after the volume load test

Parameter	Before	After	*t*	*p*-value
Response group (n = 54)				
HR (beats/min)	118.7 ± 9.2	113.0 ± 8.9	4.534	**0.001**
MAP (mmHg)	67.2 ± 7.8	73.0 ± 7.2	-3.845	**0.003**
CVP (cmH_2O)	6.9 ± 1.4	9.3 ± 1.5	-7.034	**< 0.001**
CO (L/min)	6.2 ± 0.7	8.3 ± 1.0	12.465	**< 0.001**
Non-response group (n = 48)				
HR (beats/min)	118.7 ± 9.2	117.0 ± 8.5*	-1.021	0.284
MAP (mmHg)	70.4 ±7.4	71.1 ± 7.8*	-1.883	0.125
CVP (cm H_2O)	7.1 ± 1.4	9.2 ± 1.8	-5.969	**0.001**
CO (L/min)	6.2 ± 0.6	6.2 ± 0.7*	0.219	0.827

HR − heart rate; MAP − mean arterial pressure; CVP − central venous pressure; CO − cardiac output.
Bolded values are statistically significant; $^*p < 0.05$ vs. response group.
Results are shown as mean ± standard deviation.

Table 3

Ultrasound hemodynamic indices before and after the volume load (VL) test

Parameter	$\Delta_{RH}IVC_1$	$\Delta_{RH}IVC_2$	$\Delta Vpeak_{AO}$	$\Delta Vpeak_{BA}$	$\Delta Vpeak_{CFA}$
Response group (n = 54)					
before the VL test	19.1 ± 3.4	17.4 ± 3.4	14.9 ± 1.4	16.2 ± 1.4	17.0 ± 2.4
after the VL test	16.0 ± 4.0	15.0 ± 3.1	12.1 ± 1.2	13.2 ± 1.3	16.1 ± 2.2
t	5.109	7.568	10.645	8.174	4.730
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Non-response group (n = 48)					
before the VL test	14.4 ± 2.6*	13.6 ± 2.2*	12.6 ± 1.5*	13.4 ± 1.9*	11.9 ± 2.6*
after the VL test	14.2 ± 2.6	13.2 ± 1.8	12.5 ± 1.3	13.2 ± 1.9	11.8 ± 2.8
t	0.998	1.789	1.146	1.523	1.702
p-value	0.328	0.089	0.253	0.145	0.106

$\Delta_{RH}IVC_1$ − index of distensibility of retro-hepatic ($_{RH}$) *inferior vena cava* (IVC)($_{RH}$IVC); $\Delta_{RH}IVC_2$ − index of respiratory variation in $_{RH}$IVC; $\Delta Vpeak_{AO}$ − index of respiratory variation in aortic ($_{AO}$) blood flow peak velocity; $\Delta Vpeak_{BA}$− index of respiratory variation in brachial artery ($_{BA}$) blood flow peak velocity; $\Delta Vpeak_{CFA}$ − index of respiratory variation in common femoral artery blood flow peak velocity. Results are shown as mean ± standard deviation. Bolded values are statistically significant; $^*p < 0.05$ vs. response group.

Table 4

Correlation between the increase in stroke volume and each hemodynamic index before the volume load test

ΔSV	CVP	$\Delta_{RH}IVC_1$	$\Delta_{RH}IVC_2$	$\Delta Vpeak_{AO}$	$\Delta Vpeak_{BA}$	$\Delta Vpeak_{CFA}$
r	-0.345	0.589	0.647	0.697	0.621	0.766
p-value	0.135	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

ΔSV − increase in stroke volume; CVP − central venous pressure. For the abbreviations of other indices see Table 3. Bolded values are statistically significant.

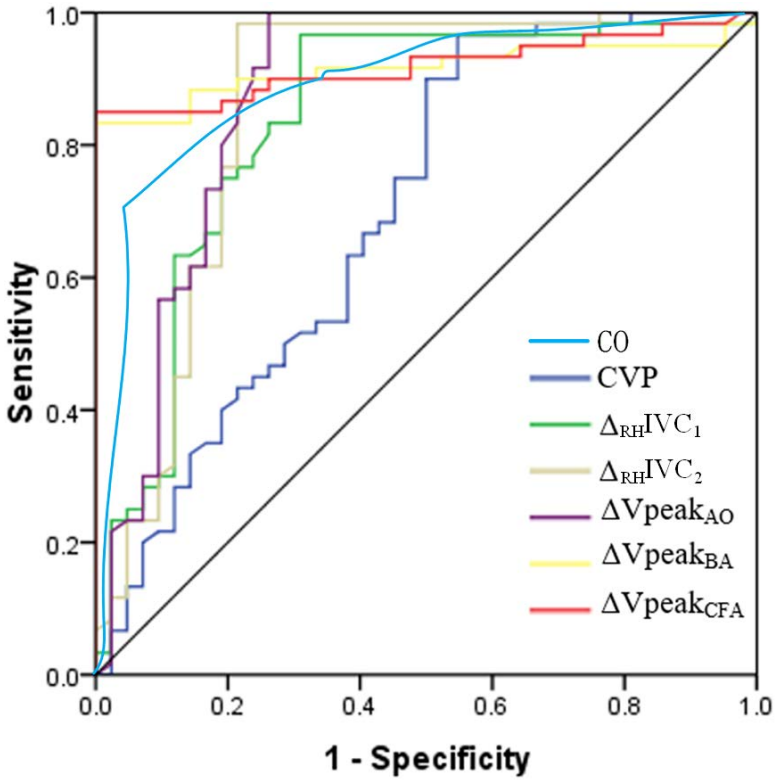

Fig. 1 – Receiver operating characteristic curves of hemodynamic indices for assessing VR in patients with septic shock. CO – cardiac output. For the abbreviations see Table 3.

Table 5

Receiver operating characteristic curve analysis results of each hemodynamic index for assessing volume responsiveness before the volume load test

Index	CVP	$\Delta_{RH}IVC_1$	$\Delta_{RH}IVC_2$	$\Delta Vpeak_{AO}$	$\Delta Vpeak_{BA}$	$\Delta Vpeak_{CFA}$
AUC	0.691	0.839	0.858	0.878	0.916	0.921
95% CI	0.431~0.792	0.705~0.973	0.796~0.923	0.820~0.952	0.785~0.997	0.866~0.978
p-value	0.229	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Optimal cutoff value (%)	6.2	17.5	16.5	13.5	14.7	15.5
Sensitivity (%)	55.2	65.4	65.4	84.4	70.4	75.2
Specificity (%)	74.8	84.9	84.9	72.7	81.8	85.3
Positive predictive value (%)	76.8	77.8	77.8	93.2	88.8	93.3
Negative predictive value (%)	71.7	72.7	72.7	79.2	68.9	76.0

AUC − area under the curve; CI − confidence interval. Bolded values are statistically significant. For the abbreviations see Table 3.

$\Delta Vpeak_{CFA}$ indices before the VL test, intended for prediction of the VR, all exceeded the value of 0.8, which was significantly higher than the AUC of ROC graphic for CVP, indicating higher sensitivity and specificity of the mentioned indices (Figure 1; Table 5).

Discussion

At present, FR is one of the most effective methods for managing shock in the clinic, but it is also necessary to determine the VR in patients. There is a study showing that on-

ly 50% of patients with hemodynamic instability had VR. Among them, VR was found in only 43.5% of septic patients. Therefore, assessing the volume status and responsiveness of patients with SS is the key to determining whether further FR can be performed [6]. Bedside, ultrasound can reflect the volume status of patients with SS, which has recently been paid extensive attention to in the field of critical care medicine in China and foreign countries [10]. Currently, VR is predicted mainly through the assessment of SV by the VL test and mini rehydration test combined with ultrasound. These methods are not affected by the patient's ventilation mode and cardiac rhythm, with high sensitivity and specificity, which can assess the VR well.

The blood volume status of patients was mainly determined by HR, blood pressure, and urine volume previously, but the effect was unsatisfactory. CVP is close to the right atrial pressure and can be monitored easily in a highly operable manner, which can be used to determine the VR indirectly. However, CVP may be affected by the positive end-expiratory pressure during mechanical ventilation, leading to distorted results, so there are certain limitations [11, 12]. In this study, CVP had a significant difference before and after the VL test in R and NR patients, indicating that CVP has a certain value in assessing the VR in patients with SS.

It has been found that there is a high consistency between continuous monitoring of CO and measurement of SV by transthoracic echocardiography in predicting fluid responsiveness [13]. The IVC is a capacity vessel characterized by a large inner diameter and good compliance, and its lumen diameter changes with respiration. The blood volume in patients with SS declines considerably, which leads to a decrease in IVC lumen diameter and an increase in variation during respiration. The thoracic pressure of critically ill patients who cannot breathe spontaneously and receive mechanical ventilation increases during inhalation, and the IVC blood backflow to the right atrium decreases, expanding the IVC inner diameter; in contrast, the IVC inner diameter decreases during exhalation [14]. Therefore, VR in patients is often assessed by ultrasonic measurement of $\Delta_{RH}IVC_1$ and $\Delta_{RH}IVC_2$ in clinical conditions. It has been confirmed that the respiratory variation in IVC diameter is an accurate predictor for the VR under the ventilation mode of tidal volume ≥ 8 mL/kg and positive end-expiratory pressure ≤ 5 cm H_2O (1 cm H_2O = 0.098 kPa), with sensitivity and specificity of 80% and 94%, respectively [15]. In this study, the results revealed that both $\Delta_{RH}IVC_1$ and $\Delta_{RH}IVC_2$ in R patients were significantly higher than those in NR patients before the VL test. After the VL test, $\Delta_{RH}IVC_1$ and $\Delta_{RH}IVC_2$ significantly declined in the R group, while they had no significant changes in the NR group. $\Delta_{RH}IVC_1$ and $\Delta_{RH}IVC_2$ were significantly correlated with ΔSV before the VL test. The results of ROC curve analysis showed that $\Delta_{RH}IVC_1$ and $\Delta_{RH}IVC_2$ had higher sensitivity and specificity in predicting the VR in patients, which is consistent with the findings of Huan et al. [16]. To sum up, $\Delta_{RH}IVC_1$ and $\Delta_{RH}IVC_2$ can be used to guide the FR therapy in the clinical treatment. $\Delta Vpeak_{AO}$ can reflect the degree of dependence of the patient's circulatory system on cardiac preload, which has the closest correlation with left ventricular SV, but its transesophageal monitoring has a certain technical difficulty [17]. The ultrasonic image of the $_{BA}$ with a large inner diameter and superficial location is clear, and its monitoring is highly reliable, so the sensitivity and specificity of $\Delta Vpeak_{BA}$ in predicting VR are higher than 90% [18]. $\Delta Vpeak_{CFA}$ is prone to disturbance by intra-abdominal arterial pressure, but its accuracy in predicting the VR of mechanically ventilated patients is higher if its value changes ≥ 12% during deep inspiration [19]. In this study, the results showed that $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ in the R group were significantly higher than those in the NR group before the VL test. After the VL test, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ declined in the R group, while they had no significant changes in the NR group. According to correlation analysis, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ were significantly correlated with ΔSV before the VL test, suggesting that cardiac indices and peripheral artery indices can better predict the VR in patients and provide references for clinical volume therapy. Besides, the results of ROC curve analysis showed that both sensitivity and specificity of $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ were higher in predicting the VR under a cutoff value of 13.5, 14.7, and 15.5, respectively, which is consistent with the research results of Seif et al. [20].

Conclusion

In conclusion, bedside ultrasound monitoring of $\Delta_{RH}IVC_1$, $\Delta_{RH}IVC_2$, $\Delta Vpeak_{AO}$, $\Delta Vpeak_{BA}$, and $\Delta Vpeak_{CFA}$ can better assess the VR in patients with SS and provide a basis for clinical FR therapy. Regardless, this study is limited since it is a single-center study with a small sample size, so the results may be biased. Further multicenter studies with larger sample sizes are ongoing in our group.

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Conflict of interest

The authors declare no conflict of interest.

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CASE REPORTS

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Severe hemophagocytic syndrome after intravesical BCG instillation with a fatal outcome

Težak hemofagocitni sindrom nakon intravezikalne instilacije BCG sa fatalnim ishodom

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Abstract

Introduction. Hemophagocytic syndrome (HS) after *Bacillus Calmette-Guérin* (BCG) immunotherapy is extremely rare in everyday practice. Only three cases of HS have been reported in the world until now. BCG is used for preventing the recurrence of superficial tumors of the urinary bladder. Severe complications after BCG immunotherapy are rarely seen. **Case report.** A 55-year-old patient was transferred to the Clinic for Urology after the second round of BCG immunotherapy, in bad condition, after transurethral resection of a bladder tumor. Computed tomography of the abdomen and lesser pelvis was performed, which did not indicate any clear signs of organ failure or disease. Antitubercular, antibiotic, corticosteroid, and symptomatic therapies were applied. The achieved effect of therapy was not satisfactory. HS after BCG immunotherapy was suspected. During further hospitalization, the patient's already severe condition further deteriorated and became more complicated in the form of multiorgan dysfunction syndrome. Death occurred on the sixth day of hospitalization. A urine culture test was performed *post-mortem* and three months later, it was positive for *Mycobacterium xenopi*. **Conclusion.** Secondary HS after BCG immunotherapy is an extremely rare disease accompanied by a severe general condition of the patient, with many life-threatening complications that can lead to death. We have presented a case of severe HS after BCG immunotherapy that caused the death of the patient. This case was unique because, for the first time, the possible causative agent was isolated – *Mycobacteria*.

Key words:
bcg vaccine; lymphohistiocytosis, hemophagocytic; multiple organ failure; mycobacterium xenopi; urologic surgical procedures.

Apstrakt

Uvod. Hemofagocitni sindrom (HS) nakon *Bacillus Calmette-Guérin* (BCG) imunoterapije se u svakodnevnoj praksi sreće izuzetno retko. Do sada su opisana samo tri slučaja HS u svetu. BCG imunoterapija se koristi u prevenciji recidiva površinskih tumora mokraćne bešike. Teške komplikacije nakon BCG imunoterapije su izuzetno retke. **Prikaz bolesnika.** Bolesnik star 55 godina premešten je na Kliniku za urologiju u lošem stanju nakon druge doze BCG imunoterapije, nakon transuretralne resekcije tumora mokraćne bešike. Načinjena je kompjuterizovana tomografija abdomena i male karlice, koja nije ukazivala na jasne znake oboljenja ili zatajenja organa. Primenjena je antituberkulozna, antibiotska, kortikosteroidna i simptomatska terapija kojom nije postignut zadovoljavajući efekat. Postavljena je sumnja na postojanje HS uzrokovanog primenom BCG imunoterapije. U daljoj hospitalizaciji došlo je do produbljivanja bolesti i razvoja komplikacija u vidu multiorganskog disfunkcionalnog sindroma. Smrt je nastupila šestog dana hospitalizacije. Posle smrti bolesnika, urađena je urinokultura koja je tri meseca nakon zasejavanja bila pozitivna na *Mycobacterium xenopi*. **Zaključak.** Sekundarni HS nakon BCG imunoterapije je izuzetno retko oboljenje, praćeno teškim opštim stanjem bolesnika, uz mnoštvo pretećih komplikacija koje mogu dovesti do smrtnog ishoda. Prikazali smo bolesnika kod koga se HS nakon BCG imunoterapije završio smrtnim ishodom. Ovo je jedinstven slučaj, jer je prvi put izolovan mogući uzročnik – mikobakterija.

Ključne reči:
bacillus calmette-guerin; limfohistiocitoza, hemofagocitna; insuficijencija više organa; mycobacterium xenopi; hirurgija, urološka, procedure.

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Introduction

Hemophagocytic syndrome (HS) is an extremely severe, life-threatening condition. HS is the result of an excessive immune response of the organism, and it can be primary and secondary. In 1976, Morales et al. [1] devised a treatment protocol that is still used successfully today. Until now, no severe or fatal outcomes have been reported after BCG immunotherapy instillation. The clinical symptoms of HS are not easily recognizable because they are usually accompanied by general symptoms in the form of fever. Unfortunately, this disease is recognized at an advanced stage after a series of extensive clinical and laboratory examinations. There is no specific therapy. Only three cases of HS have been reported after *Bacillus Calmette-Guérin* (BCG) immunotherapy so far. The aim of this paper was to present a case of secondary HS after BCG immunotherapy, which ended in death due to complications. For the first time, a possible causative agent was isolated. The question is whether there is any possibility that *Mycobacteria* can cause this type of immune response. There is a hope that further research will determine the exact cause of HS, as well as specific therapy.

We present a patient with HS caused after immunotherapy with BCG performed after surgical treatment, transurethral resection of bladder tumor (TURBT), for superficial bladder tumor.

Case report

A 55-year-old patient was transferred to our Clinic in bad condition characterized by fever symptoms, initial signs of liver and kidney failure, and abdominal pain under the right rib cage. From the anamnestic data, the patient did not take any drugs and did not suffer from other chronic diseases. The patient was hospitalized in the General Hospital a week after the second round of instillation of BCG immunotherapy into the urinary bladder. A cystoscopy and surgical treatment were performed before the transfer to our Institution. Cystoscopy revealed two tumors about 2 × 1 cm large on the right bladder wall. That was the first presentation of the tumor. TURBT surgery was performed. The pathohistological examination indicated papillary transitional cell carcinoma (TCC), pT1-high grade. The Morales treatment was applied 30 days after the TURBT surgery. After the first dose of BCG immunotherapy, the patient did not feel any signs of local or general side effects. However, after the second dose, the patient started shivering with fever and displaying general weakness and fatigue. The patient was prescribed the antituberculosis drug rifampicin and dual antibiotic therapy with amikacin and ceftriaxone. The treatment worsened the patient's condition, causing abdominal pain and pain under the right rib cage, after which the patient was transferred to our Clinic.

The latest laboratory results before being transferred to our Clinic were as follows: total bilirubin 103.9 μmol/L [reference range (RR) 3.0–21.0 μmol/L], direct bilirubin 81.4 umol/L (RR 0.1–4.2 μmol/L), amylases 36 U/L (RR 20–102 U/L), alkaline phosphatase (ALP) 557 U/L (RR 43–115 U/L), alanine aminotransferase (ALT) 515 U/L (RR 5–63 U/L), aspartate aminotransferase (AST) 747 U/L (RR 5–37 U/L), gamma-glutamyl transferase (GGT) 1.181 U/L (RR 3–55 U/L), C-reactive protein (CRP) 111.1 mg/L [normal values (NV) < 5.0 mg/L].

New laboratory analysis indicated leukopenia 3.2 × 10^9/L (RR of white blood cells is 4.0–10.0 ×10^9/L) and hemostasis disorder. Activated partial thromboplastin time (aPTT) was 2.45 ratio (R) (NV < 1.30 R), prothrombin time (PT) was 1.37 R (NV < 1.30 R), fibrinogen was 0.89 g/L (RR 2.20–4.96 g/L), a slight rise in nitrogens – urea 10.7 mmol/L (RR 2.2–7.1 mmol/L), creatinine 126 mmol/L (RR 49–115 mmol/L), potassium 4.4 mmol/L (RR 3.5–5.1 mmol/L), and a slight decrease in ALT, AST, ALP, GGT, and CRP values were found. Values of the other tested laboratory parameters were as follows: procalcitonin (PCT) 1.15 ng/mL (NV < 0.05 ng/mL), beta 2 microglobulin > 15.99 mg/L (RR 0.97–2.64 mg/L), total cholesterol 2.74 mmol/L (NV < 5.21 mmol/L), triglycerides 2.38 mmol/L (NV < 1.71 mmol/L), D-dimer > 10,000 ng/mL (NV < 500 ng/mL), ferritin 3,224 ug/L (RR 15–30 ug/L), albumins 27 g/L (RR 35–55 g/L), lactate dehydrogenase 591 U/L (RR 120–246 U/L), cholinesterase 3,640 U/L (RR 4,389–10,928 U/L). Reverse transcription polymerase chain reaction (RT-PCR) for cytomegalovirus, hepatitis C virus (HCV), hepatitis B virus, human immunodeficiency virus as well as latex-RF test, Waller-Rose test, hemoculture and urine culture were negative. Antistreptolysin O test was < 200 u/mL (NV up to 200 u/mL). A computed tomography (CT) scan of the abdomen and lesser pelvis showed liver enlargement with signs of periportal edema and an edematous gallbladder wall without intraluminal contents. CT scan also showed an enlarged spleen with slight perisplenic effusion and slight effusion in the lesser pelvis. Antibiotic ceftriaxone and amikacin and antituberculotic drug rifampicin treatments were stopped. Triple antibiotic treatment was prescribed: linezolid 600 mg/12 hrs, meropenem 1g/8 hrs, and metronidazole 500 mg/8 hrs.

Due to the deterioration of the patient's condition on the second day of hospitalization, he was transferred to the intensive care unit. HS caused by BCG immunotherapy was suspected. The treatment was changed, by adding the following: immunoglobulin, in doses of 10 g per 8 hrs; methylprednisolone sodium succinate, in doses of 40 mg per 8 hrs, according to the following schedule: 80 mg + 40 mg + 40 mg; one dose of cryoprecipitate, a second-generation fluoroquinolone 100 mg/8 hrs, was included as an additional antibiotic; metronidazole treatment was stopped, and hemodialysis was conducted. On the third day of hospitalization, the patient's breathing deteriorated, and hemoptysis occurred; thus, the patient received mechanical ventilation. Laboratory values were in decline: aPTT 9.36 R, PT 2.37 R, urea 23.2 mmol/L, creatinine 282 mmol/L, direct bilirubin 107 μmol/L, total bilirubin 148.6 μmol/L, ALP 542 U/L, AST 641 U/L, GGT 1.247 U/L, ALT 222 U/L, potassium 5.5 mmol/L, PCT 21.32 ng/mL. The peripheral blood film indicated anisopoikilocytosis. Results of chemiluminescent immunoassay (CLIA) and fluorescence immunoassay (ELFA) VIDAS anti-HCV assays were as follows: CLIA, anti-HCV

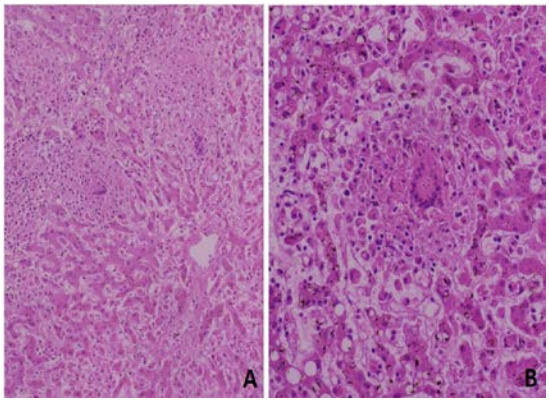

Fig. 1 − Granulomatous hepatitis:
A) hematoxylin-eosin (HE), ×100; B) HE, ×200.

– reactive 8.4 (normal < 1, reactive/positive ≥ 1) and ELFA VIDAS, anti-HCV – reactive 17.51 (normal < 1, reactive/positive ≥ 1). Antinuclear antibodies (ANA), antimitochondrial antibodies, antiparietal cell antibodies, anti-smooth muscle antibodies, and ANA HeP-2 cells screening were negative. A repeated CT scan of the thorax and abdomen showed consolidation of both sides of the lung parenchyma (of inflammatory etiology) and bronchiectasis in the inferior lobes on both sides with minor pleural effusion, hepatosplenomegaly, ascites, and portal hypertension. A bronchoscopy was done on the fourth day.

Due to a severely bad general condition, sepsis, suspicions of secondary HS caused by BCG immunotherapy, and the consequential multiple organ dysfunction syndrome (MODS), death occurred on the sixth day of hospitalization.

The patient's body was sent for autopsy. The autopsy confirmed a severe case of HS with signs of hepatorenal syndrome, pulmonary edema, ascites, endogenous intoxication, and consequential MODS, while histopathological analysis proved the existence of granulomatous hepatitis with granulomas composed of lymphocytes and individual Langhans multinucleated giant cells, with smaller focused areas of necrosis (Figure 1). Liver weight was 3,400 g. The Ziehl-Neelsen stain was used for liver tissue, and granulomatous structures were negative for the presence of acid-alcohol-resistant bacillus. The urinary bladder was without signs of residual tumor tissue and no granulomas, but granulation tissue had been found as the result of previous transurethral resection. Spleen weight was 1,300 g with a very soft cut surface, and pathohistological findings showed slight atrophy of white pulp and histiocytic infiltration of the spleen parenchyma with some erythrophagocytosis and no granulomas.

Tests of blood and urine culture for *Mycobacterium* that were done *post-mortem* were negative. After three months of cultivation, urine culture microscopically proved positive for *Mycobacterium xenopi (M. xenopi).*

Discussion

BCG immunotherapy for preventing the recurrence of superficial tumors of the urinary bladder is considered a safe treatment [2]. It was first described by Morales et al. [1] and has since been in use in urologic oncology. The most common adverse effects are mild and light, while more severe ones are rarely encountered (< 5%). Severe adverse effects are often localized inflammatory processes combined with an immunological response and happen within the first three months of intravesical BCG instillation [3].

HS is a collection of disorders that includes sepsis and conditions similar to sepsis, cytopenia, hepatosplenomegaly, coagulation disorders, disorders of the immune system, etc. There are two types of HS: hereditary (autosomal recessive, most common in children) and acquired (as a secondary outcome of other diseases in adults). HS was recorded with infectious viral diseases [2], associated with several cases of tuberculosis [4], and after BCG immunotherapy in three recorded cases worldwide. The commonality between all previously recorded HS cases after BCG immunotherapy is a good outcome [5–7]. The patient was transferred to our Institute with a progression of the primary disease and abdominal pain under the right rib cage, mild leukocytopenia, insignificant hemoglobinemia with a preserved hemostatic mechanism, uremia, azotemia, and high CRP. Additional differential diagnostics indicated a possible complicated form of cholecystitis, but laboratory and radiological parameters indicated an immunological disorder. Based on the CT scan (a consolidation of both lungs of inflammatory etiology and bronchiectasis with effusion, portal hypertension, ascites, hepatosplenomegaly), negative RT-PCR tests, anisopoikilocytosis, sepsis, hyperferritinemia, hyperlipidemia, a hemostatic disorder, and hemoptysis, the diagnosis was secondary HS caused by BCG immunotherapy [8, 9]. According to Morales et al. [1], hyperferritinemia with values almost 11 times higher than normal is not a specific HS marker, while a result of over 10,000 mg/L can be considered a pathognomonic sign. Significantly higher values of D-dimer, hypertriglyceridemia, pancytopenia, and hypofibrinogenemia are considered clear signs of HS [10, 14]. Pathophysiological HS is completely unstudied. It is believed that uncontrolled activation of T-lymphocytes starts an immune response by activating macrophages *via* Th_1 cytokines [8]. According to De Kerguenec et al. [15], this process occurs in 34% of patients who receive BCG treatment, leading to liver damage and elevated rates of liver enzymes. In Thevenot et al. [7], the cause of HS and proven granulomatous

hepatitis is a hypersensitive reaction and not BCG-caused HS (*Mycobacterium* was not isolated), as well as a positive response to corticosteroid and antibiotic treatment. In our case, the initially prescribed anti-tuberculosis (and later stopped), antibiotic, symptomatic, and anti-inflammatory treatment did not have a positive effect. Replacement therapy with plasma and immunoglobulin had a short-term positive effect. *M. xenopi* was isolated for the first time in our case after three months of cultivation. A case of isolating any strain of *Mycobacterium* has not been recorded until now [5–7, 14, 16, 17]. We have confirmed by autopsy that the issue was a systemic inflammatory response, HS, and MODS as a consequence.

Conclusion

HS is an immunologic-hematologic disorder that can occur as a secondary effect after BCG immunotherapy and is hard to diagnose. The fact that it has only been recorded a few times as a consequence of BCG immunotherapy makes it more difficult to recognize. Contributing factors are bacterial infections which can lead to death.

M. xenopi can be considered one of the potential factors which contribute to the BCG immunotherapy-caused HS mortality rate. The therapy has not been researched enough, but we can conclude that antibiotic therapy has no beneficial effect. Replacement therapy (immunoglobulin, cryoprecipitates, symptomatic) had a short-term but positive effect. Antituberculosis therapy should be the first choice, considering we are dealing with a *Mycobacterium* strain, as well as systemic therapy with maximal doses of corticosteroids to reduce the systematic inflammatory response.

We stress that it is necessary to recognize the symptoms in time and follow the precise instructions for treating recurring tumors of the urinary bladder, which decreases the possibility of a fatal outcome.

Conflict of interest

The authors declare that they have received no financial support and have no conflict of interest.

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CASE REPORT

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Right heart thrombus in a patient with acute pulmonary embolism – a practice in hide-and-seek with guidelines

Tromb u desnom srcu kod bolesnika sa akutnom embolijom pluća – „igra žmurke" sa smernicama

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Abstract

Introduction. Right heart thrombus (RHT) is a relatively rare phenomenon found in 4% of patients with acute pulmonary embolism (PE), and some reports show that the presence of RHT is associated with poor outcomes. The optimal treatment of patients with PE and RHT is still controversial, with no clear consensus. **Case report.** A 38-year-old woman with repeated chest pain and worsening dyspnea was admitted to the hospital. The echocardiographical exam showed a large thrombus floating in the right atrium, protruding through the tricuspid valve into the right ventricle. Massive bilateral pulmonary embolism was confirmed by computed tomography pulmonary angiogram. Bearing these findings in mind and due to the clinical and hemodynamic instability of the patient, thrombolytic therapy was immediately initiated (alteplase), which resulted in excellent clinical outcomes with no adverse events at follow-up. **Conclusion.** Treating patients with PE and RHT is very difficult. While waiting for the results of some future randomized clinical trials on this topic, the treatment of these patients should be based on current guidelines while also considering the patient's hemodynamic stability.

Key words:
computed tomography angiography; diagnosis; echocardiography; heart ventricles; multidetector computed tomography; pulmonary embolism; thrombolytic therapy; treatment outcome.

Apstrakt

Uvod. Tromb u desnom srcu (TDS) je relativno redak fenomen koji se javlja kod 4% bolesnika sa akutnom plućnom embolijom (PE), a pojedine studije ukazuju da je prisustvo TDS povezano sa lošijom prognozom. Optimalna strategija lečenja bolesnika sa PE i TDS je još uvek bez jasnog konsenzusa. **Prikaz bolesnika.** Žena stara 38 godina primljena je u bolnicu zbog bolova u grudima koji su se ponavljali i progresivne dispneje. Ehokardiografskim pregledom uočena je velika fluktuirajuća trombna masa u desnoj pretkomori koja je kroz trikuspidnu valvulu prominirala u desnu komoru. Masivna bilateralna embolija pluća je potvrđena angiografijom pluća primenom kompjuterizovane tomografije. Imajući u vidu ove nalaze, a usled kliničke i hemodinamičke nestabilnosti bolesnice, odmah je primenjena trombolitička terapija (alteplaza), koja je dovela do odličnog kliničkog odgovora bolesnice, bez neželjenih događaja tokom daljeg praćenja. **Zaključak.** Lečenje bolesnika sa PE i TDS predstavlja veliki izazov. Dok se čekaju rezultati budućih randomizovanih kliničkih studija po ovom pitanju, lečenje bi trebalo zasnovati na trenutno važećim preporukama, uzimajući u obzir hemodinamičku stabilnost bolesnika.

Ključne reči:
angiografija, tomografska, kompjuterizovana; dijagnoza; ehokardiografija; srce, komore; tomografija, kompjuterizovana, multidetektor; pluća, embolija; tromboliza, terapijska; lečenje, ishod.

Introduction

Pulmonary embolism (PE) is one of the leading causes of death in most countries [1, 2]. Right heart thrombus (RHT) is detected echocardiographically in 4% of patients with PE [3]. Some reports suggest that in patients with acute PE, the presence of RHT was significantly associated with an increase in 30-day mortality [4]. On the other hand, the international Right Heart Thrombi European Registry (RiHTER) showed that 30-day mortality was correlated with hemodynamic consequences of the PE and not with the RHT characteristics [5]. There are several therapeutic approaches for treating patients

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with PE and RHT, such as thrombolytic therapy, surgical and percutaneous embolectomy, and anticoagulant therapy with heparin [6], but the optimal therapeutic approach for these patients is still subject to debate. Although some studies (e.g., a meta-analysis of 177 cases with RHT and PE) [7] showed a preference for thrombolytic therapy, the potential benefits of thrombolysis in clinically stable patients with RHT and PE are still unclear.

Case report

A 38-year-old woman was admitted to the coronary care unit of the General Hospital Valjevo, Serbia due to symptoms of repeated chest pain and dyspnea. In the patient's medical history, there was information of a deep venous thrombosis (DVT) incident some time ago.

On admission, the patient was hypotensive (blood pressure was 70/50 mmHg) and tachycardic, with signs of peripheral cyanosis, while the other findings were unremarkable. Electrocardiography presented a sinus tachycardia with a heart rate of 132 bpm, slight axis deviation to the right, incomplete right bundle branch block (RBBB), as well as S1Q3T3 (Figure 1). On admission, oxygen saturation (SaO_2) was 86%, and hypoxemia, with the values of pO_2 = 67 mmHg and pCO_2 = 21 mmHg, was observed. Laboratory tests showed increased value of white blood cell count [6.9 × 10^9/L, reference range (RR): 4.0–10.0 × 10^9/L], lower level of hemoglobin (Hgb) (110 g/L, RR: 120–180 g/L), high C-reactive protein (CRP) (113.5 mg/L, RR: 0.1–8.2 mg/L), lower values of total proteins (58 g/L, RR: 65–80 g/L) and albumins (29 g/L, RR: 35–50 g/L), as well as increased values of D-dimer (> 1,050 mcg/L, RR: < 380 mcg/L) and high sensitivity troponin (312.6 pg/mL, RR: < 5.6 pg/mL), while other parameters were unremarkable.

Transthoracic echocardiography was performed, and a large thrombus was discovered floating in the right atrium, protruding through the tricuspid valve into the right ventricle (RV) (Figure 2A). The RV was enlarged [RV outflow tract, (RVOT), was 36 mm], systolic blood pressure in the RV was 55 mmHg, tricuspid annular plane systolic excursion (TAPSE) was 17 mm, *vena cava inferior* was 22 mm, and tricuspid regurgitation (TR) 3+ was registered. The patient

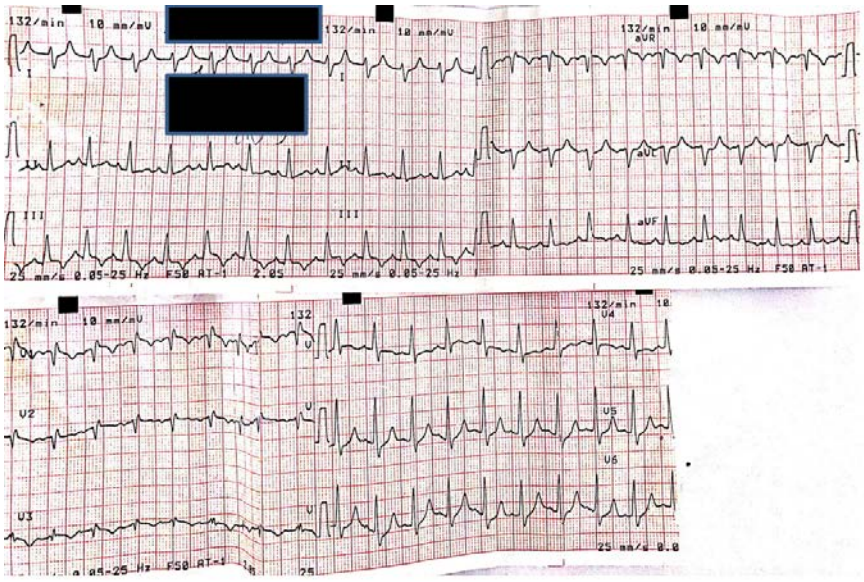

Fig. 1 – Electrocardiographic (ECG) finding of the presented patient on admission to the coronary care unit. ECG registered sinus tachycardia with a heart rate of 132 bpm, slight axis deviation to the right, incomplete right bundle branch block, as well as S1Q3T3. Black blocks overlap the patient's personal data.

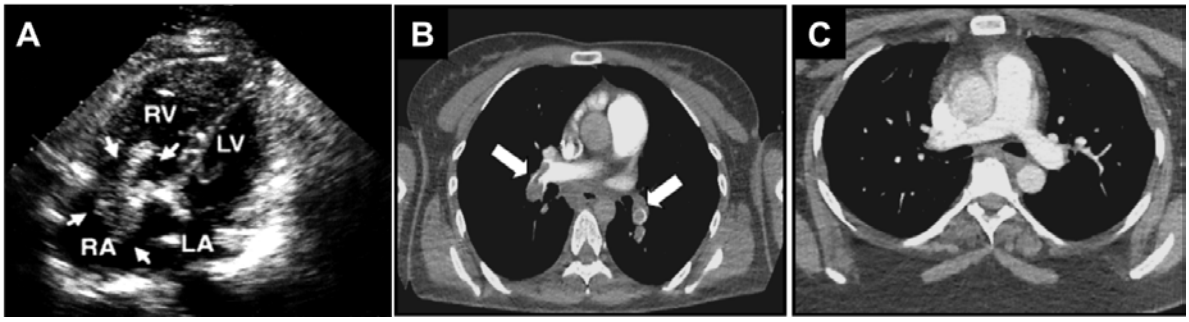

Fig. 2 – A) Transthoracic echocardiography showed a large thrombus floating in the right atrium (RA), protruding through the tricuspid valve into the right ventricle (RV); B) Computed tomography pulmonary angiography demonstrated bilateral pulmonary embolism; C) Follow-up control six months later: multidetector computed tomography showed complete resolution. LV – left ventricle; LA – left atrium.

underwent urgent multi-slice detector computed tomography (CT) pulmonary angiography (CTPA), which revealed bilateral PE and dilatation of the RV (Figure 2B). Bearing in mind the echocardiographic and CTPA findings, we decided to apply thrombolytic therapy since the patient was clinically unstable, with the Pulmonary Embolism Severity Index (PESI) score of 108 and simplified PESI (sPESI) score of 3. After the CTPA procedure, therapy was initiated immediately with intravenous unfractionated heparin 8,000 IU bolus (weight-adjusted bolus), after which thrombolytic therapy (alteplase) was applied, according to the protocol for PE (100 mg of drug-infused peripherally over two hours, with gastroprotection). Thrombolytic therapy was accomplished without complications, and low-molecular-weight heparin was administered for five days. On day six, novel oral anticoagulant therapy (dabigatran 150 mg twice a day) was introduced. During hospitalization, the patient was clinically stable, with mild dyspnea at rest. A high level of CRP and a low levels of total proteins and albumins indicated an infection (urinary tract infection was observed), which was solved by parenteral antibiotics therapy. Analyses carried out in search for an eventual tumor (tumor markers and multi-slice detector CT of abdomen and pelvis) and autoimmune diseases [antinuclear antibodies (ANA) and extractable nuclear antigens antibodies (ENA), anti-neutrophil cytoplasmic antibodies (ANCA), C3, C4, antiphospholipid antibodies, and rheumatoid factors] came out negative. Doppler ultrasound of the lower extremities was also performed, which showed no signs of a new incidence of DVT. The echocardiographic evaluation on the seventh day of hospitalization showed no signs of intracardial thrombus, normal ejection fraction (65%), partial recovery of RV (RV = 34 mm, systolic blood pressure of RV = 43 mmHg), and mild tricuspid regurgitation. After discharge, the patient had the polymerase chain reaction (PCR) assay done for the genes coding the coagulation factor V (Leiden) and the plasminogen activator inhibitor type 1 (PAI-1). The results of the PCR test showed that both genes had mutations that carry a risk of thrombophilia, and according to this, lifelong oral anticoagulant therapy was recommended. On follow-up control, six months later, it was noted that the patient had not suffered any adverse events during that time, including no new episodes of DVT. The control multidetector CT showed complete resolution (Figure 2C).

Discussion

Right heart thrombus is a relatively rare finding in patients with PE, but their prevalence may reach even 22% in high-risk patients [8, 9]. Barrios et al. [9] have shown that RHT is an independent predictor for all-cause of death, PE-related death, and recurrent venous thromboembolism, especially in hemodynamically stable patients. Meta-analyses published so far show that a high mortality rate exists in patients with PE and RHT. In the first meta-analysis published in 2002, the mortality rate was 27% [10], while in a recently published one, the mortality rate was lower (16.7%) [3]. However, it is still unknown whether RHT is the cause or just an indicator of adverse outcomes because the results of the RiHTER trial suggest that RHT characteristics such as size, morphology, or mobility are not correlated with short-term outcomes [5]. Optimal management of patients with PE and RHT is not defined clearly yet, because of a low number of cases and the absence of randomized clinical trials. The benefits of thrombolysis have been shown in high-risk and selected intermediate-high-risk cases according to current guidelines [11]. In reports with small series of patients with PE and RHT, a favorable outcome was reported [5, 9, 12], as well as in the study of Rose et al. [10], who described an improved survival rate in the thrombolytic therapy group compared to anticoagulant therapy and surgery. On the other hand, a study that included 325 patients with PE and RHT suggested no significant difference between reperfusion therapy and anticoagulant therapy regarding mortality and bleeding but that there was a higher risk of recurrences in the reperfusion therapy group [13]. Surgical embolectomy is another treatment option; it should be applied in patients where thrombolysis is contraindicated or ineffective or in patients with *foramen ovale* and potential systemic embolization risk [6, 8]. In patients with PE and RHT, parenteral anticoagulant therapy can be used as first-line therapy or additional therapy following thrombolytic therapy or surgery. Anticoagulant therapy should be used as first-line therapy in clinically stable patients and in cases with a high risk of bleeding [13].

In this case, the choice of thrombolytic therapy based on the hemodynamic instability of the patient resulted in good clinical outcomes, complete thrombus resolution, and RV function recovery.

Conclusion

This case demonstrated the difficulty of managing patients with PE and RHT without hard evidence for optimal treatment. While waiting for more data that would result from conducting more randomized clinical trials on this topic, the treatment of these patients should be based on current guidelines regarding their hemodynamic stability.

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CASE REPORT

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Rapid progression to Richter's syndrome in a patient with chronic lymphocytic leukemia and near-triploid karyotype

Brza progresija hronične limfocitne leukemije u Rihterov sindrom kod bolesnika sa kariotipom blizu triploidnog broja hromozoma

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Abstract

Introduction. The presence of aneuploidy in patients diagnosed with chronic lymphocytic leukemia (CLL), except trisomy 12, is considered quite uncommon. Hyperdiploidy or near-tetraploidy (occurring in 1–3% of all CLL patients) usually confer a poor prognosis. **Case report.** We report a patient in a progressive phase of CLL with near–triploid karyotype. The prognosis of the disease was more precisely determined by applying the cytogenetic analysis of the karyotype and was complemented with molecular methods and pathohistological examination. The complex karyotype was accompanied by the *TP53*, *C-MYC*, and *IGH* gene disruptions, the most probable cause of rapid evolution into Richter's syndrome. **Conclusion.** The use of comprehensive contemporary diagnostic techniques is highly recommended in patients who are in the progressive phase of CLL, primarily for the adequate choice of management strategy. The presented case confirms that aneuploidy in CLL patients indicates poor prognosis, which is in accordance with previous publications reporting on cases of CLL patients with aneuploidy.

Key words:
abnormal karyotype; chronic lymphocytic leukemia; disease progression; karyotyping; richter's syndrome.

Apstrakt

Uvod. Prisustvo aneuploidije kod bolesnika sa dijagnozom hronične limfocitne leukemije (HLL), sa izuzetkom trizomije 12, smatra se retkom pojavom. Pojava hiperdiploidnog ili kariotipa blizu tetraploidnog broja hromozoma (koji se javlja kod 1–3% svih bolesnika sa HLL) smatra se lošim prognostičkim parametrom. **Prikaz bolesnika.** Prikazan je bolesnik u uznapredovaloj fazi HLL sa kariotipom blizu triploidnog broja hromozoma. Prognoza bolesti je preciznije određena citogenetičkom analizom kariotipa bolesnika, i dopunjena molekularnim metodama i patohistološkom analizom. Otkriveno je prisustvo kompleksnog kariotipa udruženog sa poremećajima u genima *TP53, C-MYC* i *IGH*, što je najverovatnije bio uzrok brze progresije u Rihterov sindrom. **Zaključak.** Primena savremenih dijagnostičkih metoda veoma je značajna kod bolesnika u uznapredovaloj fazi HLL, prvenstveno zbog adekvatnog terapijskog pristupa. Prikazani slučaj ukazuje da je prisustvo aneuploidije kod bolesnika sa HLL loš prognostički znak, što je u saglasnosti sa prethodno publikovanim prikazima bolesnika sa HLL i sa aneuploidijom u kariotipu.

Ključne reči:
kariotip, abnormalni; hronična limfocitna leukemija; bolest, progresija; kariotip, određvanje; rihterov sindrom.

Introduction

Chromosomal aberrations in chronic lymphocytic leukemia (CLL) are of key importance for predicting disease outcomes and are often used in therapeutic decisions [1, 2]. These alterations, including trisomy (TS) 12 (+12) and deletion of chromosomes 13q14 (13q-), 11q22 (11q-), and 17p13 (17p-), are detected in more than 80% of the CLL cases by fluorescence *in situ* hybridization (FISH) technique [1]. However, FISH provides information only

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about chromosomal regions covered by the probes used, leaving the majority of the present karyotype changes undetectable.

In recent years, various studies revealed the prognostic impact of chromosomal abnormalities (CAs), which are not covered with FISH probes [3–5]. The International Workshop on CLL [6] proposed conventional karyotype analysis be performed before first-line therapy and any subsequent treatment due to the frequent gain of additional CAs during the course of the disease.

Complex karyotype with three or more abnormalities is present in 14–34% of untreated CLL patients and confers a worse disease outcome [5, 7, 8]. The estimation of karyotype complexity at the time of CLL diagnosis is crucial since its presence has been linked to the evolution of Richter's syndrome (RS) [9]. The most frequent aberrations in complex karyotypes include structural changes in chromosomes 11, 13, 14, and 17. On the contrary, the occurrence of numerical changes (except TS 12) is extremely rare and, when present, can be seen within hyperdiploid or near-tetraploid karyotypes in 1–3% of all CLL cases [4, 10–13].

Herein, we report a CLL patient with a complex, near-triploid karyotype in the progressive phase of CLL, who rapidly evolved to RS.

Case report

A 71-year-old female presented with a six-month history of fatigue, sweating, weight loss, and enlargement of tonsils, peripheral lymph nodes, and spleen. Laboratory findings revealed the following: hemoglobin 108 g/L [reference range (RR) 120–160 g/L], white blood cells 10.7x10^9/L (RR 3.6–10.0 x10^9/L), with neutrophils 12% (RR 42–75%), lymphocytes 21% (RR 20–51%), atypical lymphoid cells 37%, monocytes 27% (RR 2–10%), platelets 202 x10^9/L (RR 150–450 x10^9/L), elevated lactate dehydrogenase (LDH) 735 IU/L (RR 220–460 IU/L), β2 microglobulin 7.5 mg/L (RR 970–2,640 mg/L) and C-reactive protein 1.9 mg/L (RR 0.0–3.0 mg/L). Flow cytometry confirmed score 5 for CLL with CD38 and CD49d positivity [$CD19^+$, $CD20^{low}$, $CD21^{low}$, $CD22^{low}$, $CD2^{interm}$, $CD24^{intermed}$, $CD5^{bright}$, $CD43^{low}$, $CD49d^{low}$, $CD38^*$(*the pattern of CD38 expression profile was characterized as bimodal, with the concomitant presence of one population expressing high levels of CD38 and a second population completely negative), $FMC7^-$, $CD79b^-$].

Conventional cytogenetic analysis was performed on chromosome metaphases prepared from peripheral blood lymphocytes cultured for 72 hrs with lipopolysaccharide using the standard technique. Giemsa-banded metaphases were analyzed, and the findings were reported according to the International System for Human Cytogenetic Nomenclature in 2013 [12].

FISH analysis for common cytogenetic abnormalities associated with CLL was performed on interphase nuclei obtained from the culture of peripheral blood samples and tissue sections prepared from a formalin-fixed, paraffin-embedded tonsilar biopsy. A panel of probes designed to detect TS 12 and deletions of 13q14.3, 17p13.1, and 11q22.3, according to the manufacturer's instructions (Vysis/Abbott Laboratories, Des Plaines, IL), were used. In order to assess the immunoglobulin heavy locus (*IGH*) gene and *C-MYC* proto-oncogene, FISH analysis was performed using dual-color break-apart probes (Vysis/Abbott Laboratories).

For tumor protein 53 (*TP53*) mutational analysis, DNA was isolated from formalin-fixed, paraffin-embedded bone marrow samples using QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The *TP53* mutational status was determined by polymerase chain reaction amplification of coding exons 4–10 and flanking intronic regions, as recommended in Pospisilova et al. [13], followed by direct Sanger sequencing (forward and reverse strand) with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on 3130 Genetic Analyzer (Applied Biosystems, USA). The results were interpreted by GLASS software, a web-based Sanger sequence trace viewer, editor, aligner and variant caller, and the locus-specific International Agency for Research on Cancer (IARC) database.

The computerized tomography scan showed an enlargement of lymph nodes from 14 mm in the mediastinal area and 21 mm in the retroperitoneum to 29 mm in the axillar region. The liver was normal in size and appearance; the spleen was enlarged (150 mm) with the presence of homogenous bilateral pleural effusion. Bone marrow core biopsy revealed diffuse infiltration with small lymphocytes (80%). Due to tonsilar enlargement, a tonsillectomy was performed. Histopathological evaluation of the tonsils, followed by immunohistochemical analysis, revealed signs of initial progression of small lymphocytic lymphoma (SLL)/CLL into diffuse large B-cell lymphoma (DLBCL), the occurrence of single large cells without sheets, with the phenotype PAX-5^+, CD79α$^+$, $CD20^+$, $CD3^-$, $CD5^+$, $CD23^+$, $CD43^+$, Cyclin $D1^-$, $SOX11^-$, bcl-2^+, bcl-6^-, $CD10^-$, $CD38^-$, $CD30^-$, $CD15^-$, $MUM1^-$, C-MYC^-, $ZAP70^-$, $p53^+$, Ki-67^+ in 30-40% of them, approximately three mitoses per proliferation center (Figure 1). Tonsilar tissue FISH analysis confirmed the result obtained from peripheral blood, with an additional chromosome copy number. Finally, the patient was diagnosed with CLL Rai stage III and was initially treated with a high dose of methylprednisolone, followed by rituximab, fludarabine, and cyclophosphamide (R-FC) lite protocol, which she did not tolerate. Febrile neutropenia with prolonged recovery developed, and after improvement, treatment was continued with cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisolone (CHOP) protocol, again with poor response and further lymph node progression. Due to right popliteal phlebothrombosis, anticoagulant treatment was commenced. Because of the substantial toxicity of chemotherapy, additional palliative treatment and extra methylprednisolone cycles were applied. Unfortunately, 11 months after diagnosis, the patient experienced intestinal complications with positive *Clostridium difficile* enterocolitis, leading to the fatal outcome.

Conventional cytogenetic analysis was performed on 20 chromosome metaphases from a peripheral blood sample,

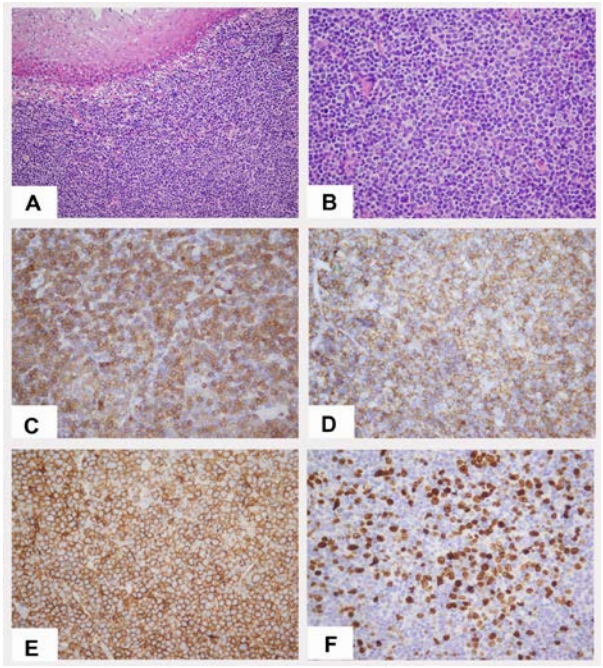

Fig. 1 − Biopsy of tonsilar tumor: A) Increased number of large single cells with immunoblastic features and expanded proliferation centers [hematoxylin and eosin (HE), ×100]; B) Approximately three mitoses per proliferation center (HE, ×400); C-F) Immunohistochemical features of tumor cells (streptavidin-biotin, ×400): C) CD79α staining; D) CD5 staining; E) CD23 staining; F) Ki-67 staining highlights high proliferation rate of the cells in the proliferation centers.

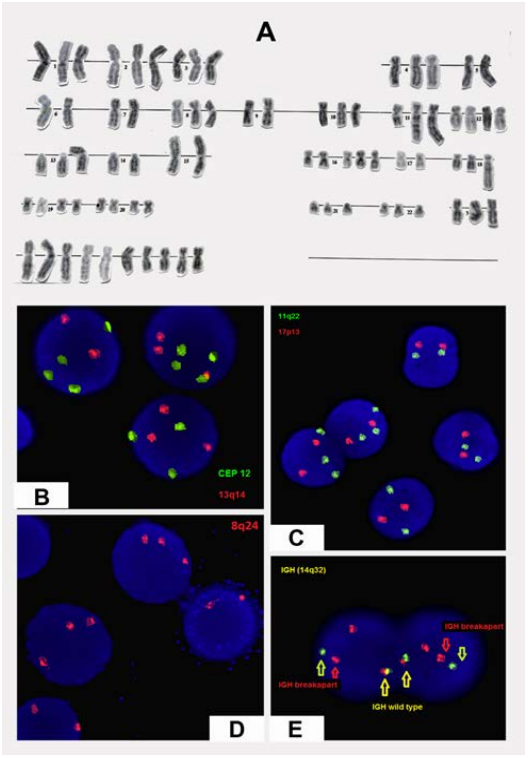

Fig. 2 − A) Near-triploid karyotype with complex aberrations from peripheral blood; B) Trisomy and tetrasomy of CEP12 accompanied with trisomy of 13q14; C) Trisomy of 11q22 with disomy of 17p13; D) Trisomy of 8q24 (*C-MYC*); E) Rearrangement of 14q32 (*IGH*) showing an extra red signal of the 3'*IGH*.

revealing a near-triploid female karyotype with multiple numerical and structural changes in 13 out of 20 metaphases, described as: 70-77,XXX,+1,+2,+3,+4,+8,der(9)add(9)(p24),+10,der(11)add(11)(q25),+der(11)add(11)(q25),+12,+12,+der(13)add(13)(p11),der(15)add(15)(p11),+der(15)add(15)(p11),+16,+16,+16,der(18)add(18)(q22),+19,+19,+20,+20,+21,+22,+10mar[cp13]/46,XX [7] (Figure 2A).

FISH analysis of peripheral blood and paraffin-embedded tonsilar and bone marrow tissue, using the CLL panel, revealed three copies of chromosomes 11q22.3, 8q24, and 13q14.3 in 30%, 50%, and 70% of nuclei, respectively. Moreover, three and four copies of chromosome enumeration probes (CEP) 12 were spotted in a total of 70% of nuclei, while the rearrangement of the *IGH* gene with an extra copy of the 3'*IGH* signal was present in 30% of nuclei. These findings were presented in Figures 2B–E and described as: nuc ish [(D13S319,D12Z3)x(380/200)]/nuc ish (D13S319x3,D12Z3x4)[60/200] (Figure 2B); nuc ish [ataxia telangiectasia mutated (ATM)x3,*TP53*x*2*][60/200] (Figure 2C); nuc ish(*C-MYC* x 3)(5'*C-MYC* con 3'*C-MYC* x3)[100/200] (Figure 2D); nuc ish(3'*IGH*x3,5'*IGH*x2)(3'*IGH* con 5'*IGH*x1)[60/200] (Figure 2E). *TP53* mutational analysis revealed frameshift mutation in exon 6 [c.626_627delGA (p.R209fs*6)].

Discussion

The incidence rate of progression of CLL to DLBCL in newly diagnosed patients is relatively rare; it usually occurs in 0.5–1.1% of patients and develops 1.8–4 years after the initial diagnosis, depending on the duration of clinical follow-up, patient population studied, and diagnostic criteria used to define it [4, 14, 15]. Some of the biological factors predictive of RS development include expression of CD38 and CD49, inactivation of *TP53* or *C-MYC* abnormalities, as well as genomic complexity, which has been considered an adverse prognostic indicator [16–18].

Our patient was diagnosed with SLL/CLL, showing splenomegaly, more than three enlarged lymph node regions, and elevated serum LDH and β2 microglobulin levels at presentation. The presentation of highly progressive disease was established by other diagnostic methods, including immunophenotype (CD38 and CD49d positivity), pointing out that the patient had an increased risk of both short survival and clonal evolution [17, 18].

The rapid disease evolution and the patient's resistance to chemotherapy coincided with the presence of a near-triploid karyotype. The FISH result revealed TS of the 13q14 region and *C-MYC* gene as the most prevalent aberrations,

followed by TS of 11q, CEP12, and tetrasomy of chromosome 12. The karyotype analysis defined trisomic signals seen by FISH more closely: two signals of the 11q22 region marked two derivative chromosomes 11, one of the three signals for 13q14 belonged to one derivative chromosome 13, while three and four signals for 8q24 and CEP12, respectively, presented simple copy number changes of the whole chromosomes 8 and 12. In addition, multiple structural and numerical changes of chromosomes not covered with FISH probes were observed as well.

However, the karyotype itself gave us no information about the sequence of events leading to its development. A relatively short period of time (6 months) from the first disease symptoms and rapid progression to RS can be explained by the early acquisition of some of the high-risk gene mutations, which would favor chromosomal instability and the development of additional chromosomal changes. Indeed, mutational analysis of the *TP53* gene was positive for the frameshift mutation in exon 6, while FISH for *IGH* and *C-MYC* genes showed positivity for both rearrangements. It is worth noting that our findings were in contrast with the recent report of Rossi et al. [19], who found that TS 12 and 17p aberrations seemed mutually exclusive.

In our report, the presence of near-triploid karyotype was designated as an uncommon and very rare event in both CLL and RS. However, in the context of complex karyotype, our results are consistent with similar published cases showing karyotype complexity with *TP53* and/or *C-MYC* rearrangements as particularly adverse prognostic indicators, often seen in CLL patients with the evolution to RS [4, 7].

Conclusion

We can point out that karyotype analysis revealed infrequent cytogenetic aberrations in CLL, contributing to the identification of the CLL patient with a highly aggressive clinical course. The combination of morphologic and immunohistochemical analyses, together with the incorporation of conventional cytogenetics in CLL diagnostics, could provide complementary information to FISH and mutation analyses in defining high-risk patients and facilitating therapy strategy.

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

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Primeri referenci:

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Tabele

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

Ilustracije

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

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

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