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Resistance of microorganisms to antimicrobial drugs (antimicrobial resistance – AMR) occurs when bacteria, viruses, fungi, and parasites no longer respond to the active ingredients in the drugs used to fight them. The main driver of AMR is the misuse and overuse of antimicrobials in human treatment or for food production. Data from the World Health Organization (WHO) indicate that almost 5 million people die each year due to AMR. This is why the WHO has identified AMR as one of the 10 largest public health threats that humanity is facing. World AMR Awareness Week, established by the WHO in 2015, is celebrated from 18 to 24 November every year. This year's slogan remained the same as last year's: "Preventing Antimicrobial Resistance Together".

Otpornost mikroorganizama na antimikrobne lekove (antimikrobna rezistencija – AMR) nastaje kada bakterije, virusi, gljivice i paraziti više ne reaguju na aktivne sastojke u lekovima koji se koriste za borbu protiv njih. Glavni pokretač AMR su zloupotreba i prekomerna upotreba antimikrobnih sredstava u lečenju ljudi ili za proizvodnju hrane. Podaci svetske zdravstvene organizacije (SZO) ukazuju da zbog AMR svake godine umre skoro 5 miliona ljudi. Zbog toga je SZO ukazala na AMR kao jednu od 10 najvećih pretnji po javno zdravlje sa kojima se čovečanstvo suočava. Svetska nedelja podizanja svesti o AMR, koju je SZO ustanovila 2015. godine, obeležava se od 18. do 24. novembra svake godine. Ovogodišnji slogan ostao je isti kao prošlogodišnji: „Zajedno sprečavamo antimikrobnu rezistenciju".

GENERAL REVIEW

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Cellular cryobiology – a review of basic concepts and "operating design" of cryopreserved cells

Celularna kriobiologija – prikaz osnovnih koncepata i „operativnog dizajna" kriokonzervisanih ćelija

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Key words:
cryopreservation; cryoprotective agents; cryoinjury; stem cells; platelets.

Ključne reči:
kriokonzervacija; krioprotektivni agensi; krioоštećenja; matične ćelije; trombociti.

Introduction

Cell preservation systems could be classified into the following three categories: 1) liquid-state conservation (at hypothermic condition, but over 0 °C; 2) frozen-state storage at ultra-low temperatures (cryostorage); 3) cell cultivation in an artificial medium (normothermic storage). Cell or tissue cultures need nutrients, buffers, and other supplements for metabolism to preserve cells in a "near-normal" (comparatively physiological) condition. On the contrary, the purpose of cell cooling (refrigeration) is to reduce cell needs for energy production and its consumption (for protein synthesis, ion transport, and other biochemical activities) at long-term storage [1].

Cryobiology is an innovative scientific discipline that evaluates influences of subzero (≤ 0 C°) and ultra-low (-80 ± 5 °C or lower) temperatures on cell integrity and functionality, as well as determines facts/approaches applicable in cryo-practice. Cryopreservation (CP) is a thermodynamically well-defined operating system, specific for each cell type or "biosubstratum", that protects cellular organelles, isolated cells, cell clusters, or tissues during cooling to ultra-low temperatures. This method is beneficial when cells are biologically and/or thermally unstable using liquid-state cell preservation [2–4].

The key CP requests are to minimize cellular thermal damages (cryoinjury-score) and their consequences after the freezing/thawing procedure for each "cryo-biosystem" (frozen biological systems). Briefly, it is a specific system composed of some cells ("biosubstratum"), medium, and cryoprotective agent (CA) [cryoprotectant (CPt)] [2–5]. Different CP systems have been described over the past decades: 1) ultra-rapid cooling/thawing technique (with no obligatory use of a CPt); 2) freezing by nonpenetrating polymers [cooling rate (CR)] could be slower and relatively uncontrolled; 3) vitrification method (for CP of tissues when even extracellular freezing process induces unacceptable or intolerable cryoinjuries); 4) equilibrium-freezing (the "cryo-biosystem" must contain enough CA to reduce extracellular freezing and avoid osmotic stress, as well as intracellular ice formation until vitrification is developing) [4–11].

This paper is a review of basic events of physicochemical/biophysical phenomena within workable protocols used in both theoretical and practical cell CP approaches. There

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fore, it could be a synopsis of factors relevant for affecting the quantitative/qualitative recovery of various cells exposed to ultra-low temperatures. The efficacy of our original CP protocol, based on research data and clinical evaluation of the cells applied, will also be briefly described.

Initial cellular cryo-investigations

The ever-increasing use of cell-mediated treatments has resulted in increased needs for both stem cells (SCs) and other immunoreactive cells, but also for superior *ex-vivo* manipulative/operating procedures to minimize cell damage during their collection, processing, and storage in liquid or frozen state. The objective of cryo-investigations is to determine critical events in cryo-practice, predict cell response to the cooling/thawing process, and CPt addition/removal, maximizing post-thaw cell quantity/quality [3–5].

The epoch of cryobiology began in 1949 by CP of the fowl spermatozoa using glycerol as a low molecular weight (MW) CPt [6]. Then, methods using glycerol and dimethyl sulfoxide (DMSO) were initiated for freezing human hematopoietic progenitors and blood cells [7–10]. In brief, glycerol is a potent stabilizer of different macromolecules, and it is non-toxic to the majority of cells, even at high-level concentrations. The major inconvenience of this CPt is that glycerol diffuses (penetrates) into a lot of cells just gradually and/or relatively in slow motion. DMSO has the benefit of more rapid (prompt) penetration into the majority of cells, but it is toxic in higher concentrations [4, 5].

Today, several CP protocols are in clinical use, but a universal optimized freezing procedure based on adequate type and concentration of CPt has not yet been achieved. Freezing/thawing systems applicable in medical cryo-practice should be persistently improved to minimize thermal damages and maximize cell recovery and viability. In this context, re-evaluation of biophysical and biochemical factors (including osmotic characteristics, water/CPt permeability coefficients), as well as other cryobiological parameters responsible for cryoinjuries, is still a very popular topic for investigation by researchers and practitioners [5, 11–16].

Cell cryoinjury – initiation and manifestations

Thermal injury can be manifested as partial cellular lesions due to various malfunction(s) or as complete cell destruction or cytolysis. Generally, post-thaw cell recovery/viability is better when correct freezing methods and high-quality CPt are applied. The essential factors that can result in superior cryoprotection during living cell CP are the following: 1) the use of an effective freezing system, such as optimized controlled-rate freezing with compensation of the released fusion heat during the "phase transition" step (from liquid to solid stage); 2) the determination of acceptable cryostorage category and conditions (adequate temperature and length of cryostorage); 3) the selection of practical thawing technique; 4) the choice of an appropriate type and concentration of CA. For SCs, progenitor and mature blood cell CP, glycerol, DMSO, or hydroxyethylstarch (HES) – a high-molecular-mass agent, are regularly used, although in different combinations and concentrations [5, 14–17].

Initially, it was believed that cryoinjuries emerge predominantly because of the consequent occurrence of extracellular ice crystals. It was supposed that the application of a sufficiently high cooling speed (CS) might avoid extracellular ice formation and later cell destruction. However, an extremely high CS (adequate to prevent cell damage) could not be realized in practice because of the heat transfer thermodynamic limitations. Additionally, complete cell destruction was observed using ultra-rapid freezing procedures [2–5].

At present, it is considered that cell thermal damage during the freeze-thaw process may be the result of high-level cell dehydration with subsequent high-level volume reduction ("solution effect") or development of extensive intracellular ice formation followed by resulting organelle and membrane damages ("mechanical damage"). The first mechanism is typically expressed during low-rate freezing as a result of a progressive rise in the osmotic gradient between extracellular and intracellular space. Extracellular hypertonicity is followed by subsequent cell dehydration "triggering" volume decrease, membrane malformations, and, lastly, cytolysis. Extracellular ice crystals do not regularly induce mechanical cell destruction due to membrane penetration despite their physical presence. The second process is characteristic of rapid or high-rate freezing when the intracellular water cannot leak out of the cell (absence of fluid efflux). Intracellular ice formation, followed by cell destruction, is the most critical harmful effect. These phenomena ("solution effect" and "mechanical damage") are independent cryo-events but can sometimes affect and work together and typically result in cell destruction [5, 14–16].

Recent cryo-investigations (including ours) are primarily focused on the evaluation of controlled-rate freezing (microprocessor restricted/programmed cooling) vs. uncontrolled-rate freezing ("dump-freezing" without programmed CR) techniques; for that reason, only those techniques will be discussed in this manuscript. Briefly, the controlled-rate method is a "time-consuming" process that requires specific equipment and high-level technical expertise. The second one is a less expensive freezing technique since it does not require a computer-controlled device. However, there are reports that controlled-rate freezing is more effective (compared to the uncontrolled-rate technique) because of higher post-thaw cellular quantitative/qualitative recovery [5, 16–22].

The "osmotic threshold" vs. crystallization or vitrification

Ice formation (crystallization) is regularly initiated by a nucleation (homogeneous or heterogeneous) process. It is carried out by hydrophilic sites on a particle that "mimics" the water molecule collections on the surface of an ice crystal [4]. Different fluids rarely or almost never freeze spontaneously at the melting temperature due to the lack of sufficiently large "nucleators". Unfrozen liquids at a temperature below their nominal freezing point (such as 0 °C for water) are called "super-cooled" fluids. Regardless of the fact whether

nucleation is homogeneous or heterogeneous, the presence of solutes in each solution decreases the fluid's freezing point/temperature. Otherwise, it is a critical fact that functioning living cells do not naturally have ice nucleators in their intracellular organization or area. Hence, they can get "super-cooled" close to temperatures of -30 ºC or even -40 °C when homogeneous nucleation becomes evident [3–5]. Since cells do not normally contain ice nucleators, initially, extracellular ice develops, especially during relatively slow cooling [4, 5].

Cells have a limit to their stability in isotonic conditions (hyperosmotic or hypoosmotic restriction). Cell exposure to hyperosmotic conditions results in reversible or irreversible alterations of membrane permeability and integrity ("membrane stress") while rarely leading to the extrusion of some membrane components. However, cells have a limit to swelling in a hypoosmotic environment [4, 5].

During freezing by slow CS, significant ice crystals are formed in the extracellular space. These crystals do not regularly initiate/create mechanical cell cryoinjury (transmembrane penetration) despite their physical existence. However, the formation of ice crystals in the extracellular area causes a permanent osmotic gradient rise with subsequent intracellular fluid (water) efflux. As a final consequence, cells become dehydrated (volume reduction), followed by resulting organelle and membrane malformations, as well as complete cell destruction or cytolysis [4–11].

At rapid cell freezing, extracellular vs. intracellular osmotic gradient has no time to arise/intensify; consequently, cell dehydration and volume reduction are minor or not observed. Intracellular ice crystal development and enlargement following mechanical cell injury is the most damaging event. The level of cell damage correlates with the whole intracellular ice mass and the size of solitary ice crystals [5–8]. The process of intracellular ice crystallization occurs in the following situations: 1) when a super-cooled cytoplasm approaches the temperature specific for nucleation and freezes spontaneously; 2) while the diameter of ice particles, which is getting smaller as the medium temperature decreases, becomes so small that it can penetrate membrane pores; 3) even when membrane damages allow ice to grow through cell membrane defects. Once ice forms inside cells by crystallization, it can result in mechanical damage to cellular organelles/structures, as well as cell membranes [4, 5].

As stated, crystallization could be also prevented by lowering the freezing point when raising the solute concentration in a solution. Due to the rapid cooling of liquid solution (in the absence of nucleators and crystallization), a specific process can develop a process known as vitrification. It is a biophysical/biochemical process of transition of some liquid solution into an amorphous glass. As the temperature decreases, solutions gradually become more viscous. Precisely, synchronized co-action of growth of solute concentration and decrease of temperature increases the viscosity of the medium in the unfrozen residual solution and reduces the speed and rate at which water can move from a liquid solution to the structure of ice crystals. Thus, at a sufficiently low temperature and at a high enough solute concentration, the solution becomes a solid glass substance (vitrification). Since the nucleating rate is minor at extremely low temperatures, it is possible to prevent the nucleation process and ice crystallization during an ultra-rapid cooling of solutions, as well as obtain a vitrification process in the absence of solutes [4, 5].

Therefore, the determination of an optimized freezing approach and CR (specific for each "cryo-biosystem") should be considered. It is a sufficiently high CS that prevents cell dehydration, as well as adequately low for the efflux of water from cells (preventing intracellular ice formation). The optimized CR during CP can be defined with the ratio of cell surface/volume by the permeability of the membrane for water and other substances, as well as its specific temperature coefficient; however, it also depends on which freezing technique is applied [4, 5, 11].

Consequently, the basic goal of each freezing protocol is to avoid intracellular crystallization and make real intracellular vitrification. The correlation between cell damage from extracellular vs. intracellular ice crystallization, as a function (among others) of CR, was determined and established in initial cryo-investigations [5–10]. Possible causative mechanisms of non-crystallization-mediated cell thermal damages incorporate the concentration of intracellular solutes (salts and sugars) and the occurrence of the "membrane stress" followed by cell volume reduction. The category and intensity of undesired alterations owing to cell "membrane stress" is dependent on the cell type, the temperature, and the category/concentration of extracellular solutes [5, 11–20].

During the use of controlled-rate freezing, if the released fusion heat is not considered and not compensated, it could result in additional temperature fluctuation in the "cryo-biosystem" with further cellular thermal damage. In other words, most studies recommend 1 °C/min as an optimized CS for SCs and platelet CP, although there are reports that these CRs are perhaps higher (nearly 2–3 °C/min) [5, 16, 22–26]. The "phase transition" step of freezing is also critical because a significant reduction in cell recovery/viability was detected when this step was elongated. Thus, the optimal CR for CP of blood-derived cells mentioned above is 1 ºC/min, with a superior CS (2 ºC/min) at "phase transition" step [5, 17–20, 26]. Finally, there are reports that uncontrolled-rate systems can be also effective for SCs and platelet cryostorage [19, 20, 27–31]. However, this system could generate an unbalanced freezing process (CR $\geq$ 3 ºC/min). Therefore, the configuration of "freezing bags" and the volume of cell suspension ("bag-thickness") are also hazardous parameters, which could significantly change the freezing procedure (the kinetics of programmed CR) [5].

Last but not least, cryoinjuries may also develop due to "dilution shock" or cell "swelling", as well as ice recrystallization during thawing [5, 23–25]. Rapid and massive ice thawing produces extracellular water mass increase with a following hypoosmotic condition. As a result of extracellular hypoosmolality, besides minor effusion of penetrating CPt from cells, a massive water influx into the intracellular space happens following the "dilution shock". Cells are usually more vulnerable to enlargement than to the reduction of their volume; consequently, they can be simply destroyed due to the

"dilution shock" [4, 5]. Throughout extracellular recrystallization, additional cell dehydration could happen. Again, during intracellular recrystallization, further mechanical cell damage can be expressed; small ice crystals could develop into crystal agglomerates or enlarge their mass [4–11].

Cryoprotective agents – types and working options

The choice and use of a high-class freezing technique is essential, but it cannot explain cryoinjury origin nor eliminate it completely. Post-thaw cell recovery/viability is superior merely when effective CPt is added to the "cryobiosystem" to prevent/reduce potential thermal damages. Mechanisms of the action of various CPt are complex and incompletely explained. Due to the differences in their physicochemical/biophysical properties, it is not possible to determine a general protective mechanism for them all [5].

CAs can be categorized into intracellular or penetrating and extracellular or nonpenetrating compounds. The potential/speed for "trans-membrane penetration" of CPt is very important for successful cell protection. However, cryoprotection can be achieved by quickly penetrating agents (DMSO) and slowly penetrating agents (glycerol), as well as by nonpenetrating agents (HES). Typically, intracellular CAs could give cell protection during low-rate freezing by decreasing the intensity of cellular dehydration and volume reduction. Then again, extracellular CPt could protect cells commonly during rapid freezing, reducing the degree of intracellular ice formation [3–5]. The speed and quantity of CPt trans-membrane penetration (diffusion or influx) is, in fact, again a temperature-dependent process. Namely, the temperature of the suspension at which the cell is exposed to a CPt has also an effect on diffusion rate; at lower temperature levels, poorer CPt influx/efflux was observed [1–5].

The use of CAs in sufficiently high concentration but below critical cytotoxicity (especially DMSO) and temperature fall results in intracellular hyperviscosity. Because of that, water molecule mobility and subsequent crystallization are delayed. Penetrating CPt increases intracellular solute concentration, producing a condition with a lower temperature at which ice crystallization will develop. These agents and events also reduce extracellular vs. intracellular osmotic gradient (minimized "solution effect"). Finally, certain CPt can simply modify water trans-membrane penetration rate, thus affecting the level of cell dehydration [5, 7–9, 11–13].

As stated, glycerol and DMSO were discovered in the middle of the last century for CP of blood-derived and other cells. As main penetrating CPt, they have superior molar volume than low MW intracellular salts and sugars (e.g., the volume of one mole of glycerol and NaCl are 40.7 mL and 27 mL, respectively) [4]. Hence, their use could delay and diminish cell dehydration and volume reduction even at low temperatures. Besides the effects on osmotic gradient, glycerol and DMSO have additional effects. Namely, the protective action of these intracellular CAs is achieved because of their colligative (connective) effect, that is, the potential for water binding [4, 5].

Glycerol, DMSO, and HES show particular effectiveness in blood-derived cell CP. They are key hydrogen bond acceptors; consequently, they can effectively connect a high quantity of water molecules, and, as a result, they have an important cryoprotective potential. For that reason, cells can be stored under a nominal freezing point of a specific solution with no extreme intracellular ice crystallization and severe dehydration [5].

Concisely, DMSO could be described as a transparent or colorless fluid with a sulfur-like smell. DMSO is a very polar molecule that dissolves many water-soluble and lipid-soluble substances. It has exothermic properties and should be mixed slowly with the cell suspension to dissipate the generated heat. Furthermore, given intravenously (even in small concentrations), DMSO may cause certain adverse events such as nausea, vomiting, local vasospasm, etc. [7–9, 11–13].

Very important nonpenetrating CA is the HES with average MW ranging from high (≥ 450 kDa), medium (200–400 kDa) to low MW (150–200 kDa). Therefore, it has a larger MW than glycerol or DMSO. Combined with DMSO, HES was originally used for granulocyte CP. As a potent cryoprotector, HES acts predominantly extracellularly during low-rate freezing [5, 21].

The action of cryoprotection using HES is different from that for penetrating cryoprotectors such as glycerol and DMSO, which reduce the solution freezing point and decrease the temperature at which the salt concentration becomes cell-destructive [5, 21]. Namely, the cryoprotective action of HES is, above all, the result of its ability to absorb water molecules (around 0.5 g of water per 1 g of HES) and keep these molecules thermally "inert" in a glassy state missing event of "phase transition" (liquid into solid stage) during (super)cooling. Thus, HES affects the viscosity of the solution and reduces the CR required for cell survival during vitrification, reducing/delaying the ice formation [3–5, 21]. Finally, there is data that CP of blood-derived cells using HES with DMSO is possible (cells frozen by this technique have sufficient post-thaw recovery/viability) [5, 11–13].

In summary, for CP of SCs, progenitors, different white blood cells (lymphocyte and granulocyte freezing systems), and platelets (whose freezing represents a specific challenge due to their limited tolerance to osmotic fluctuations), typically DMSO or DMSO with HES, are used as effective CAs, although in various final concentrations and/or combinations. They can express a protective effect due to the reduction of cellular dehydration and/or a decrease in the number of intracellular ice crystals. However, CAs cannot protect cells from dehydration that already exists or from the effects of earlier-developed ice crystals in the intracellular space [2–5].

From stemness to cryopreserved cell practice – experimental and clinical data

Hematopoietic tissue was the first and most explored cytopoietic or "tissue-generating" system in humans. Stemness is a (hemo)biological molecular process that combines the ability of an immature cell to maintain or perpetuate its lineage (self-renewal or self-maintenance capacity), give rise

to several more developed daughter cells or specialized mature cells (differentiation and proliferation potential), and interact with milieu, extracellular matrix to continue and keep a balance between cellular latency, proliferation, and regeneration. The very primitive SCs compartment could be illustrated as cells with high-pitched expression of developmental pathways and by the important intensity of epigenetic plasticity [5, 32–37]. In the steady state circumstance, the character and actions of SCs are regulated with a set of genes and by well-organized and precisely synchronized signaling systems. Defects in signaling cascade(s) or loss of intercellular balance (cell-cell communication) can initiate uncontrolled cell growth or death, as well as cell malfunctions and/or transformation (development into a variety of diseases, including tissue defects or cancer) [23–25, 35–40]. Together with explained physicochemical/biophysical factors, SC-related events are also regulated and restricted by the mechanical environment in which SCs reside and stay alive. The process of SCs-biology "supervising" through specific mechanical factors remains inadequately understood or still lacking, and it is the strategic target for developing the field of mechanobiology [41, 42].

The existence and functioning of SC partition guarantee steady-state homeostasis in each tissue-generating system. Hematopoietic SCs are capable and competent to provide bone marrow (BM) repopulation following SCs-transplant in patients with partially or completely damaged hematopoiesis and some other disorders. The compartment of SCs and progenitors express a specific CD34 antigen. Thus, they are also called $CD34^+$ cells, a cluster differentiation/designation (CD) marker for a transmembrane glycoprotein. On the cell membrane of more primitive SCs, the CD90 antigen (a specific marker for more immature $CD34^+/CD90^+$ compartment or repopulating SCs) is also inherent. The occurrence of cells expressing this antigen in the graft is essential for complete, stable, and long-term marrow repopulation following SCs-transplant with hematopoietic reconstitution [5, 23, 26].

Generally, SCs can be collected from BM using multiple aspirations or with harvesting from peripheral blood (PB) after mobilization and by processing (purification) of umbilical cord blood. For therapeutic use (SCs-transplants or regenerative medicine), BM was the first SCs source. Cells are collected from the posterior and anterior iliac crest (rarely from the sternum). The optimal timing for allogeneic PB-derived SCs harvesting is on the fifth day (at maximum "$CD34^+$ peak") of recombinant human granulocyte colony-stimulating factor (rHuG-CSF) administration. However, determining the optimal timing for autologous SC harvesting is more complex. These patients are given higher rHuG-CSF doses combined with chemotherapy. The count of circulating $CD34^+$ cells correlates with the superior $CD34^+$ yield in the harvest. When the number of $CD34^+ \geq 40/\mu L$ in PB, the possibility of collecting $CD34^+ \geq 2.5 \times 10^6$/kg/body mass (bm), or more, is approximately 60% [5, 30].

An innovative SCs mobilizing regimen uses plerixafor to obtain adequate SCs yield from the blood of "poor responders" ("poor mobilizers") [5, 30, 43]. Our data also confirmed the efficacy of this mobilization protocol using plerixafor (combined with rHuG-CSF); the $CD34^+$ count was higher in PB, and cell yield was superior in the harvest [23–25, 30]. A successful SC transplant can be expected when the yield of $CD34^+$ cells is $2–4 \times 10^6$/kg/bm (or more likely $\geq 5 \times 10^6$/kg/bm) [5, 30]. Finally, our preclinical SCs cryoinvestigation has confirmed that the ratio of more primitive SCs ($CD34^+/CD90^+$ subset) in PB could also be a useful mobilization predictive factor to determine optimized timing for cell harvesting and predictor of the quality of harvest [24–26].

However, the use of an effective SC transplant requires both high-quality collection/harvesting methods and CP systems to obtain an adequate cell yield, as well as quantitative/qualitative cell recovery. In practice, CP of BM-derived SCs incorporates the following steps: 1) marrow aspirate processing (pre-freezing depletion of red blood cells and plasma); 2) cell exposure to a freezing medium with CA (equilibration); 3) freezing the mixture of cells in medium; 4) cryostorage at -130 ± 10 °C (mechanical freezer or nitrogen steam) or at -196 °C (liquid nitrogen); 5) thawing in a water bath at temperature 37 ± 3 °C.

The PB-derived SCs CP should be modified, or in other words, adapted to conditions that depend on superior mononuclear cell number, the presence of proteins (albumin) in the plasma, and the lack of lipid or bone particles in the harvest. Following the thawing procedure, SCs are immediately applied (reinfused) across a central venous catheter to the patient. Recipients tolerate well this reinfusion, lacking DMSO-related adverse effects. The incidence of potential reinfusion-associated side effects (typically nausea and vomiting) is regularly a function of the DMSO concentration (quantity) in thawed cell suspension [5, 4–9, 16, 22, 23]. In this context, there are reports that the use of lower DMSO concentration (5%) rather than higher (10%) results in a superior $CD34^+$ recovery (inferior apoptotic and necrotic $CD34^+$ cell incidence) with an elevated engraftment potential of these cells [31]. At last, there are data that the concentrations of DMSO from 2.2% to 3.5% are also adequately qualified for a satisfactory cell recovery following SCs transplant [29].

Finally, let us summarize our activities in cryo-practice using primarily controlled-rate freezing systems (with fusion-heat compensation) vs. uncontrolled-rate method ("dump-freezing") in experimental or (pre)clinical settings. Our experimental and (pre)clinical results are comparable with data from the literature, as well as above cited studies [5, 17–20, 30].

In an experimental setting, we have found that the recovery/clonogenicity of less primitive SCs populations (pluripotent and committed progenitors: CFU-Sd12 and CFU-GM) was higher in the presence of 5% vs. 10% of DMSO. On the contrary, it has been verified that the recovery/viability of very primitive SCs (marrow-repopulating ability cells) was superior when 10% of DMSO was applied. These results mean a different cryobiological "request" of marrow-repopulating ability cells vs. more mature progenitors. We have demonstrated in these experimental studies that deviations in cell recovery, clonogenicity, and viability are significantly related to the cell-specific CP strategy used (freezing technique with appropriate DMSO concentration) [5, 17].

Briefly, SC transplants were used for the treatment of our patients with acute lymphoblastic leukemia and non-lymphoblastic leukemia, chronic myeloid leukemia, multiple myeloma (MM), Hodgkin lymphoma and non-Hodgkin lymphoma, as well as patients with severe aplastic anemia, and multiple sclerosis [5, 23–25, 30]. Mobilization of SCs was accomplished with rHuG-CSF (by standard dose, 12–16 μg/kg/bm) following chemotherapy, for instance, by salvage (platinum-based) regimen (lymphoma patients), as well as applying a poly-chemotherapy pre-treatment (cyclophosphamide, adriamycin and dexamethasone) or using high dose cyclophosphamide (MM patients). For "poor mobilizers", the second mobilization using rHuG-CSF (16 μg/kg/bm) with plerixafor (24 to 48 mg around 6–11 hrs prior to cell collection) was completed ($CD34^+$ cell yield $\geq 4 \times 10^6$/kg/bm in the harvest) [30]. The SCs harvesting (by Cobe®-Spectra or Spectra-Optia®; Terumo-BCT, USA) was initiated only at the "cut-off" value of circulating $CD34^+$ cells, at 20×10^6/L or more [5, 30]. Harvested cells were frozen by our original controlled-rate freezing procedure using an optimized DMSO concentration (final DMSO concentration of 10%) and stored at -130 ± 10 ºC (mechanical freezer) or at -196 ºC (liquid nitrogen) and thawed directly prior to the clinical application (using water bath at 37 ± 3 °C) [5, 30]. Hematopoietic reconstitution was rapid – the average time for neutrophil recovery was on the 12th day (range 6–26 days), and average platelet recovery was on the 12th day (range 5–44 days) [5, 15, 23–25, 30]. The overall SC transplant efficacy was dependent on the type, stage, and chemosensitivity of the disease, presence of co-morbidities, general health status, and age of the patient, as well as the degree of human leukocyte antigens-HLA matching [5, 14–16, 30].

Lastly, our preclinical results demonstrated that platelet recovery was superior when the strictly equalized six-step controlled-rate freezing (CR = 1 °C/min), with compensation of the released fusion heat (CR = 2 °C/min) during the "phase transition" period, in combination with lower DMSO concentration (6% in autologous plasma), was used. Only minor intergroup differences (between protocols) for parameters of cell recovery, integrity, and functionality were observed [19, 20].

Conclusion

The increased use of myeloablative treatments (combined with SCs rescue), as well as intensified application of cell-mediated therapies, has resulted in superior requirements for both the SCs and practical operating procedures to improve cell yield and recovery, as well as minimize cellular damages during harvesting and/or CP.

CP is a well-working system that protects cellular organelles, isolated cells, cell clusters, or tissues during freezing (by ultra-low cooling), long-term cryostorage, and rapid thawing procedures, and it is beneficial in cases where cells are vulnerable and unstable during durable preservation in liquid-state. The major CP requirement is to reduce cell thermal damage ("cryoinjury-score") and its consequences. Existing CP techniques applicable in cryo-practice should be re-evaluated and improved. Further basic research and (pre)clinical cryo-investigations are recommended or required to define well operating "cryo-biosystem" (specific for each cell type) in order to obtain an optimized post-thaw cell recovery and viability.

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Clinical and radiological characteristics of patients with spontaneous and post-traumatic subarachnoid hemorrhage: a retrospective observational study

Kliničke i radiološke karakteristike bolesnika sa spontanim i posttraumatskim subarahnoidalnim krvarenjem: retrospektivna opservaciona studija

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Abstract

Background/Aim. Several serious complications can accompany both spontaneous and post-traumatic subarachnoid hemorrhage (SAH) such as the development of intracranial hypertension, hydrocephalus, rebleeding, cerebral hypoxia, cerebral vasospasm, impaired pituitary function, electrolyte imbalance, and electrocardiographic (ECG) abnormalities. Although there is a declining trend in mortality, the treatment of SAH and its complications represents a challenge even in imposing neurosurgical centers. The aim of the study was to compare some clinical characteristics and complications between spontaneous and post-traumatic SAH. **Methods.** The retrospective study included 138 patients treated at the Department of Neurosurgery from January 2018 to January 2023. There were 71 patients with spontaneous and 67 patients with post-traumatic SAH. **Results.** A predominance of spontaneous SAH in female and post-traumatic SAH in male patients ($p < 0.001$) was found. There was a statistically significant difference in the frequency of hydrocephalus between groups of spontaneous and post-traumatic SAH patients ($p = 0.013$). Cerebral vasospasm was significantly more prevalent in patients with spontaneous SAH ($p < 0.001$). A statistically significant association was also obtained between the thickness of the coagulum in these two groups ($p < 0.001$). Patients with spontaneous SAH were significantly more likely to have a negative T wave in ECG findings ($p < 0.001$). Furthermore, there was no statistically significant difference regarding electrolyte imbalance in these two groups of patients with SAH. **Conclusion.** There were statistically significant differences between gender distribution, the frequency of abnormal ECG findings in the form of a negative T wave, greater coagulum thickness, vasospasm occurrence, and a higher rate of hydrocephalus in patients with spontaneous SAH compared to patients with post-traumatic SAH.

Key words:
brain injuries; electrocardiography; hydrocephalus; intracranial aneurysm; sex factors; subarachnoid hemorrhage; vasospasm intracranial.

Apstrakt

Uvod/Cilj. Kod bolesnika sa spontanim i posttraumatskim subarahnoidalnim krvarenjem (SAK), moguća je pojava teških komplikacija kao što su razvoj intrakranijalne hipertenzije, hidrocefalus, ponovno krvarenje, cerebralna hipoksija, cerebralni vazospazam, poremećena funkcija hipofize, poremećaj ravnoteže elektrolita i elektrokardiografske (EKG) abnormalnosti. Iako je trend mortaliteta u opadanju, lečenje bolesnika sa SAK i njenim komplikacijama predstavlja izazov čak i u velikim neurohirurškim centrima. Cilj rada bio je da se uporede učestalost nekih kliničkih karakteristika i komplikacija između bolesnika sa spontanim i posttraumatskim SAK. **Metode.** Retrospektivnom studijom obuhvaćeno je 138 bolesnika lečenih u Odeljenju za neurohirurgiju u periodu od januara 2018. do januara 2023. godine. U analizu je bio uključen 71 bolesnik sa spontanim i 67 bolesnika sa posttraumatskim SAK. **Rezultati.** Utvrđena je prevaga spontanog SAK kod žena, a posttraumatskog SAK kod muškaraca ($p < 0,001$). Utvrđena je statistički značajna razlika u učestalosti hidrocefalusa između bolesnika sa spontanim i posttraumatskim SAK ($p = 0,013$). Cerebralni vazospazam bio je značajno češći kod bolesnika sa spontanim SAK ($p < 0,001$). Utvrđena je statistički značajna povezanost u pogledu debljine koaguluma između te dve grupe ($p < 0,001$). Bolesnici

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sa spontanim SAK su značajno češće imali negativan T talas u EKG nalazu ($p < 0,001$). Takođe, nije bilo statistički značajne razlike u pogledu poremećaja ravnoteže elktrolita između te dve grupe bolesnika sa SAK. **Zaključak.** Rezultati našeg istraživanja ukazuju na postojanje statistički značajne razlike vezane za pol bolesnika, učestalost abnormalnog EKG nalaza u vidu negativnog T talasa, veće debljine koaguluma, pojave vazospazma i veće stope hidrocefalusa kod bolesnika sa spontanim SAK, u odnosu na bolesnike sa posttraumatskim SAK.

Ključne reči:
mozak, povrede; elektrokardiografija; hidrocefalus; aneurizma, intrakranijalna; pol, faktor; krvarenje, subarahnoidno; vazospazam, intrakranijalni.

Introduction

Nontraumatic subarachnoid hemorrhage (SAH) represents a type of hemorrhagic stroke that most often arises spontaneously due to aneurysm rupture, occurs through the penetration of blood into the subarachnoid space (Figure 1), and is thought to be responsible for about 3% of all strokes, with an estimated annual incidence of approximately 600,000 cases worldwide [1]. Spontaneous SAH affects a disproportionately large number of people under the age of 65 compared to ischemic stroke and represents a major burden on the healthcare system and society [2]. The development of delayed cerebral ischemia due to vasospasm (Figure 2) significantly worsens the prognosis of patients [3]. It is considered that most intracranial aneurysms are not congenital but develop gradually during life. Furthermore, it is estimated that an average of 2% to 3% of adults without risk factors have brain aneurysms, but this number increases proportionally with age [4]. The most common place where saccular aneurysms occur is the branches of the intracranial arteries of the base of the brain (Figure 3), especially in the area of the circle of Willis [4, 5]. Although most intracranial aneurysms will never rupture, the risk for hemorrhage increases with increasing aneurysm size, although paradoxically, most ruptured aneurysms are less than 1 cm in diameter [4].

In 10% of patients with spontaneous SAH, the cause cannot be detected, while a smaller (5%) number of cases arises due to other vascular pathology (arteriovenous malformations, arteriovenous fistula, reversible cerebral vasoconstriction syndrome) [6]. On the other hand, traumatic brain injuries (TBI) are the most common cause of SAH (Figure 4). Furthermore, post-traumatic SAH occurs in approximately 33–60% of cases of severe and moderate TBI,

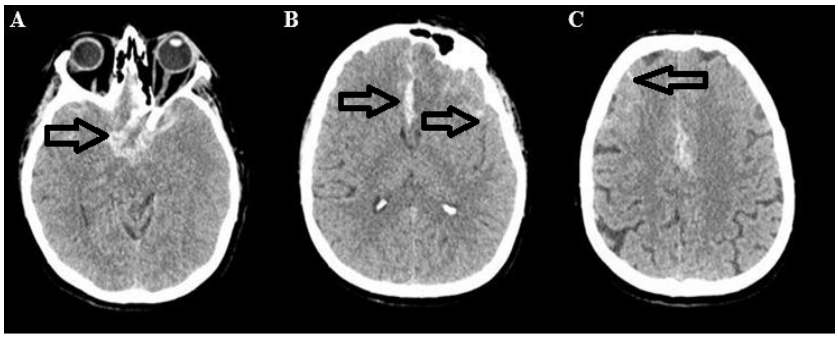

Fig. 1 – Non-contrast axial computed tomography indicates spontaneous subarachnoid hemorrhage (marked by arrows) extending into the basal and suprasellar cisterns (A), into the proximal Sylvian fissure and the anterior part of the interhemispheric fissure (B), reaching the right frontal convexity (C).

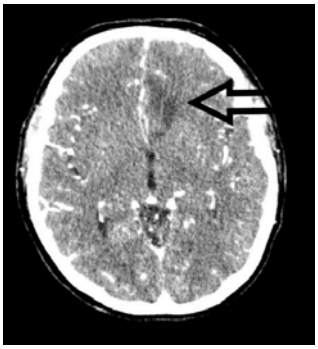

Fig. 2 – Computed tomography angiography in the axial plane demonstrates a subacute ischemic zone on the frontal parasagittal left (indicated by arrow) as a consequence of cerebral vasospasm.

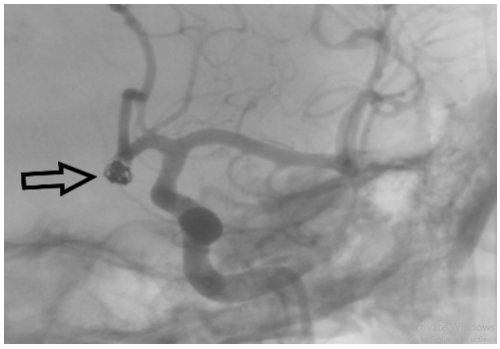

Fig. 3 – Digital subtraction angiogram shows an embolized bleeding aneurysm at the A1/A2 junction of the left anterior cerebral artery (indicated by arrow).

such as brain contusion and subdural hematoma [7]. The leading mechanisms of injury are injuries in traffic accidents, violence, and falls, while the patients are mostly males between 15 and 44 years of age. Brain trauma associated with post-traumatic SAH is a prognostic factor of poor outcomes in patients because it is associated with numerous complications [7, 8].

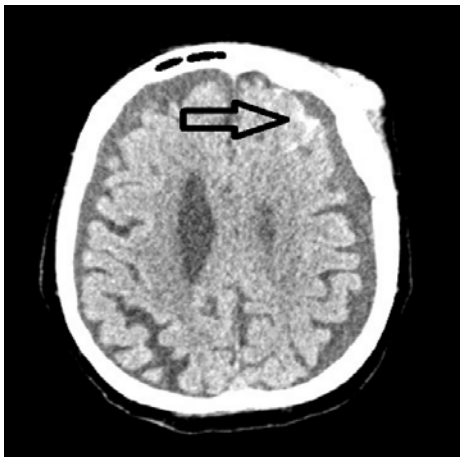

Fig. 4 – Non-contrast axial computed tomography demonstrates post-traumatic subarachnoid hemorrhage with cerebral contusion in the region of the left superior frontal gyrus (marked by arrow). A subcutaneous hematoma can be seen frontally on the left side.

Approximately 11% of patients with SAH die before receiving medical care, in addition to approximately 40% of patients who die within four weeks of admission to the hospital [9]. The mortality rate is estimated to be about 50% in the population, with a declining trend [10]. Moreover, several serious complications can accompany both spontaneous and post-traumatic SAH, such as the development of intracranial hypertension, hydrocephalus, re-bleeding (recurrence of SAH), cerebral hypoxia, cerebral vasospasm, impaired pituitary function, electrolyte imbalance, and electrocardiographic (ECG) abnormalities. Although there is a declining trend in mortality, the treatment of SAH and its complications represents a challenge even for experienced neurosurgical centers [7, 8, 10].

Methods

This retrospective study included 138 patients treated at the Department of Neurosurgery, University Clinical Center Niš, Serbia from January 2018 to January 2023.

The data processed in this research was collected from the available medical records. In all patients included, computed tomography (CT) with CT angiography (CTA) of the brain was performed initially upon admission, as well as control CT during hospitalization.

Based on the etiology of SAH, patients were divided into the following groups: Group I – patients with spontaneous SAH and Group II – patients with post-traumatic SAH.

According to the protocol of our Neurosurgery Department, the control CT scan was performed three days after the initial one or even earlier in cases of clinical deterioration of the patient. Furthermore, CTA was not performed in patients with chronic renal failure, allergy to iodinated contrast media, decompensated heart failure, and type 2 diabetes mellitus with metformin as regular therapy. Digital subtraction angiography (DSA) of the brain was performed in all of the patients with spontaneous SAH in order to exclude or confirm the presence of aneurysms, arteriovenous malformations, or other malformations of the brain blood vessels as a potential source of bleeding. In patients with spontaneous SAH and a positive CTA finding, DSA was urgently performed. Moreover, if the presence of a brain aneurysm was confirmed, subsequent endovascular embolization and stent graft placement or microsurgical clipping of the aneurysm was performed.

In patients with post-traumatic SAH, DSA was performed only in cases where there was a doubt about whether spontaneous SAH preceded head trauma and caused additional post-traumatic SAH, more precisely in six patients.

Follow-up brain CT reports were used to determine the presence of hydrocephalus and re-hemorrhage after SAH. The radiological characteristics of SAH were graded by using the modified Fisher scale (mFS), while independently of the mFS grade, the thickness of the coagulum was also measured, where a coagulum with a thickness of more than 1 mm was considered thick. Neurological status and clinical presentation of the patients were evaluated in order to suspect the presence of vasospasm after SAH. Consequently, CTA and DSA were performed to confirm vasospasm and were reviewed by radiologists and the neurosurgical council. The criteria for excluding patients from the research were the following: patients with incomplete documentation, patients with an initial brain CT performed at a local hospital that was not available for analysis, and patients in whom an associated brain injury was the dominant cause of the patient's clinical deterioration, such as traumatic epidural hematoma, subdural hematoma, and massive brain contusions. Besides, patients under 18 years of age were not included in our analysis.

Patients with aneurysmal SAH were treated after the neurosurgical and radiological intervention by maintaining the mean arterial pressure close to the upper limit of 100 mmHg, oral administration of nimodipine (60 mg every 4 hrs) or intravenously (2 mg/h), as well as with hemodynamic optimization.

A venous blood sample was taken from each patient upon admission. Routine biochemical parameters, such as sodium and potassium values, were determined immediately using standard biochemical methods on an AU680 Clinical Chemistry Analyzer (Beckman Coulter, Brea, CA, USA). The electrolyte reference values were for sodium 135–148 mmol/L and potassium 3.5–5.5 mmol/L.

Each patient underwent a standard ECG examination on admission with six precordial leads, which was interpreted by a cardiologist. The cardiologist assessed dynamic ST-

segment abnormalities, as well as the presence of negative T waves and abnormal U waves.

Statistical analysis

Data entry and tabulation were performed using the MS Office 2016 Excel program. Statistical calculations were performed with the program SPSS (IBM SPSS Statistics – version 23). Standard and basic statistical methods were used for qualitative and quantitative assessment of the results. The normality of the distribution of individual values was assessed by the Kolmogorov-Smirnov test. The Chi-squared (χ^2) test was used to test whether the distributions of categorical variables differ from each other. The statistical hypothesis was tested at the level of significance for the risk of $\alpha = 0.05$, i.e., the difference between the samples was considered significant if $p < 0.05$. The Mann-Whitney *U* test was used when the assumptions of the *t*-test were not met, and independence within the samples and mutual independence was assumed.

Results

In the whole group of patients, there were 78 male and 60 female patients. In the group of patients with spontaneous SAH, there were 46 (64.79%) females and 25 (35.22%) males, while in the group of patients with post-traumatic SAH, there were 14 (20.90%) female and 53 (79.10%) male patients. Our results indicated a statistically significant difference in the frequency of spontaneous and post-traumatic SAH according to gender (χ^2 statistic was 27.0247 and $p < 0.001$). The mean age of patients in the whole group was 61.53 ± 14.82 years. In the group of patients with spontaneous SAH, the mean age was 59.94 ± 11.48 years, while in the group of patients with post-traumatic SAH, it was 59.42 ± 20.45 years. There was no statistically significant age difference between patients with spontaneous and post-traumatic SAH (the *Z*-score was -1.79925 and $p = 0.072$) (Table 1).

The incidence of hydrocephalus in both groups of patients with SAH is shown in Table 2. There was a statistically significant difference in the frequency of

Table 1

Demographic characteristics of the patients with spontaneous and post-traumatic subarachnoid hemorrhage (SAH)

Variable	Spontaneous SAH	Post-traumatic SAH	*p*
Gender			
male	25 (35.22)	53 (79.10)	< 0.00001*
female	46 (64.79)	14 (20.90)	
Age (years)	59.94 ± 11.48	59.42 ± 20.45	0.072†

All values are expressed as numbers (percentages) or mean ± standard deviation.
***Chi-squared test; †Mann-Whitney *U*-test.**

Table 2

Clinical and radiological characteristics in patients with spontaneous and post-traumatic subarachnoid hemorrhage (SAH)

Variable	Spontaneous SAH n = 71	Post-traumatic SAH n = 67	*p**
Hydrocephalus			
with	19 (26.76)	6 (8.95)	0.013
without	52 (73.24)	61 (91.05)	
Recurrence of SAH			
yes	7 (9.86)	2 (2.99)	0.197
no	64 (90.14)	65 (97.01)	
Vasospasm			
with	17 (23.94)	1 (1.49)	0.00009
without	54 (76.06)	66 (98.51)	
Coagulum thickness			
thick	48 (67.59)	24 (35.82)	0.0002
thin	23 (32.39)	43 (64.18)	
mFS			
I	18 (25.35)	23 (34.32)	0.110
II	32 (45.07)	19 (28.36)	
III	14 (19.71)	21 (31.34)	
IV	7 (9.86)	4 (5.97)	

mFS – modified Fisher scale. All values are expressed as numbers (percentages).
***Chi-squared test.**

hydrocephalus between these two groups (χ^2 statistic with Yates correction was 6.2157 and $p = 0.013$). Regarding the incidence of re-bleeding in both groups of patients with SAH, it was found that there was no statistically significant difference in the frequency of re-bleeding between these two groups (χ^2 statistic with Yates correction was 1.6632 and $p = 0.197$). A statistically significant association was also determined between the thickness of the coagulum and spontaneous and post-traumatic SAH (χ^2 statistic was 13.9564 and $p = 0.0002$), where a thick coagulum was considered a coagulum with a thickness of 1 mm or more (Table 2).

The incidence of vasospasm was also considered in Table 2. Cerebral vasospasm was significantly more prevalent in patients with spontaneous SAH ($p = 0.00009$). On the other hand, there was no statistically significant association between the grades of the mFS and spontaneous and post-traumatic SAH (χ^2 statistic was 6.0308 and $p = 0.110$) (Table 2).

Initial mean values of serum sodium, the incidence of sodium level disturbances (hyper- or hyponatremia), and mean values of serum potassium, hypo- or hyperkalemia in both groups of examined SAH patients are shown in Table 3. There was no statistically significant difference regarding electrolyte imbalance in these two groups of patients with SAH. Elevation of the ST segment was observed in 5 (7.04%) patients with spontaneous and 1 (1.49%) with post-traumatic SAH, while ST depression was present in 7 (9.85%) patients with spontaneous and 1 (1.49 %) with post-traumatic SAH. No statistically significant difference was observed between the presence of abnormal U wave, elevation, and depression of the ST segment in two groups of patients with SAH ($p = 0.689$). Patients with spontaneous SAH were significantly more likely to have a negative T wave (χ^2 statistic was 12.9772 and $p = 0.0003$) (Table 3).

Discussion

According to the relevant scientific literature, the most common age of patients with ruptured aneurysms is between 40 and 65 years, which coincides with the results from our study [11, 12]. The mean age of patients with spontaneous SAH in our research was 59.94 ± 11.48 years, and in the group with post-traumatic SAH, it was 59.42 ± 20.45 years. The frequency of females in patients with spontaneous SAH was 1.8 times higher than in males, which is in accordance with the results of other studies where women were found to have about 1.7 times greater risk for spontaneous SAH than men, but this difference was evident only in patients older than 50 years [13]. A possible explanation for the higher incidence of spontaneous SAH in females after the age of 50 could be a decrease in the protective hormones estrogen and progesterone after reaching menopause [14–16]. Frontera et al. [15] found in their study of 580 patients with spontaneous SAH that 68% of patients were female, which corresponds to the results of our study. In contrast to the spontaneous SAH, the results of our study indicate that the frequency of men was significantly higher in post-traumatic SAH, which could be explained by the fact that men are more often involved in traffic accidents and more often suffer from serious injuries at work [11–16].

SAH is known to be associated with ECG abnormalities [17]. The reported prevalence of ECG changes in patients with SAH ranges from 27% to 100% [17]. Abnormal U wave is one of the most common abnormalities that occur in patients with SAH, with a frequency between 50–60%, and after that,

Table 3

Electrolyte and electrocardiographic abnormalities in patients with spontaneous and post-traumatic subarachnoid hemorrhage (SAH)

Variable	Spontaneous SAH	Post-traumatic SAH	*p*
Sodium values			
serum natrium disorders	135.79 ± 6.84	136.42 ± 3.24	0.072†
hypernatremia	2 (2.81)	1 (1.49)	0.917*
hyponatremia	28 (39.43)	6 (8.95)	
Potassium values			
serum potassium disorder	3.40 ± 0.71	4.03 ± 0.56	0.960†
hyperkalemia	2 (2.82)	1 (1.49)	0.865*
hypokalemia	15 (21.12)	6 (8.95)	
ST segment changes			
ST elevation	5 (7.04)	1 (1.49)	
ST depression	7 (9.85)	1 (1.49)	0.689*
Abnormal U wave			
T wave shapes	36 (50.70)	12 (17.91)	
negative T wave	26 (36.62)	7 (10.48)	0.0003*
normal T wave	45 (63.38)	60 (89.52)	

All values are expressed as numbers (percentages) or mean ± standard deviation.
***Chi-squared test; †Mann-Whitney *U*-test.**

changes in ST segment, T wave, and QT interval are found in about 50% of patients [17, 18]. Our results indicate that patients with spontaneous SAH were significantly more likely to have a negative T wave. The higher occurrence of negative T waves in patients with spontaneous SAH compared to the patients with post-traumatic SAH may be associated with electrolyte disturbances, especially low potassium levels, which was also observed in our study in patients after the spontaneous SAH. Electrolyte disturbances, particularly low serum potassium, are thought to cause ECG abnormalities [19, 20]. Some authors emphasize that the time when the ECG is performed in patients with SAH is of critical importance, bearing in mind that during the first 72 hrs, the most pronounced changes on the ECG could be seen in these patients [21, 22].

Moreover, SAH is also associated with electrolyte imbalance and homeostasis disturbances, such as hyponatremia occurring in 10–34% of patients with SAH [23]. Presumably, it is caused by hypothalamic dysfunction, most often due to sodium loss influenced by an increase in the concentration of brain natriuretic peptides [24, 25].

In our study, there was no statistically significant difference regarding electrolyte imbalance between post-traumatic and spontaneous SAH groups. The frequency of hyponatremia in our study coincides with some previously published research [24, 26, 27]. Several conducted studies have suggested that cerebral salt-wasting syndrome and inappropriate antidiuretic hormone syndrome are the most common causes of hyponatremia after SAH [28, 29]. Elevated brain natriuretic peptide and atrial natriuretic peptide levels after SAH are thought to lead to natriuresis causing hyponatremia [30]. Furthermore, a vasodilator peptide, adrenomedullin, is secreted into the cerebrospinal fluid from the choroid plexus and may have a natriuretic effect on the kidneys [26].

In addition to hyponatremia, in our study, there were 15 (21.12%) patients with hypokalemia in spontaneous and 6 (8.95%) patients in the post-traumatic SAH group. There was no statistically significant difference in the frequency of hypokalemia between the groups of patients with spontaneous and post-traumatic SAH. Hypokalemia is thought to occur due to the release of catecholamines after the onset of SAH. High levels of circulating catecholamines lead to excessive activation of Na/K-ATPase by stimulating beta-adrenergic receptors. This results in the displacement of the potassium ions into the intracellular space [31, 32].

Cerebral vasospasm usually occurs on the third day after SAH reaches a peak frequency between the sixth and eighth day and lasts for two to three weeks. Cerebral vasospasm can lead to decreased cerebral blood flow and impaired oxygen supply to the brain, which can cause cerebral ischemia and infarction in a number of patients [4, 33]. The incidence of cerebral vasospasm remains unknown; it is ascribed to great difficulties in reporting incidence data, both due to difficulties in diagnosing and defining the cerebral vasospasm, and ranges from 1.5% to 91%, which corresponds to the results we obtained [34, 35]. In addition, the factor that affects the occurrence of vasospasm the most is the amount and localization of SAH on brain CT in the first four days after bleeding, regardless of the way the ruptured aneurysm was previously treated [36]. The occurrence of vasospasm is significantly correlated with the amount of blood in the subarachnoid space. According to the results we obtained, cerebral vasospasm was significantly more prevalent in patients with spontaneous SAH. Vasospasm occurs most frequently and most intensively in the vicinity of a bleeding aneurysm, primarily due to the amount of blood in the proximity of the ruptured aneurysm. In patients with SAH of the non-aneurysmal etiology, the occurrence of vasospasm is significantly less frequent, which may explain the low occurrence of vasospasm in patients after traumatic SAH in our study [37].

The Fischer scale was established in 1980, assuming it was sufficient to predict the risk of developing cerebral vasospasm. This assumption was confirmed in a small sample (41 patients) in 1983 [38]. According to one study, which had a significantly higher number of patients, this correlation existed but was not statistically significant [39]. On the other hand, the mFS scale has wide application and enormous clinical significance as a grading system that correlates with vasospasm based on the amount and localization of SAH [40]. However, certain limitations were also observed with mFS in terms of different interpretations and scoring by clinical doctors, and it is considered by some authors that the criteria of this scale must be clearer [40, 41].

How the blood affects the appearance of vasospasm has not been completely elucidated. Theories that try to explain the mechanism of vasospasm are based on the release of vasoactive substances during the breakdown of blood. The amount and extent of SAH predict symptomatic vasospasm and delayed cerebral ischemia as a consequence of hemolysis, leading to inflammation, endothelial injury, and the release of oxygen-free radicals, which lead to vasoconstriction and promote it [42]. Contemporary research is focused on discovering new drugs that would be more effective in the treatment of vasospasm. Endothelin receptor antagonists inhibit the action of the vasoconstrictor endothelin-1, and it has been shown that endothelins play an important role in the development of cerebral vasospasm [43, 44]. On the other hand, the nonglucocorticoid 21-aminosteroid tirilazad inhibits lipid peroxidation and has a neuroprotective effect with antioxidant effects [45]. The neuroprotective effect of erythropoietin in maintaining vascular autoregulation was also investigated, which could have implications in the treatment of vasospasm after SAH [46].

Acute hydrocephalus occurs in *circa* 20% of patients with SAH [47, 48]. Hydrocephalus resolves spontaneously within 24 hrs in 30% of patients but may worsen and quickly lead to a fatal outcome [49]. The results of our research indicate that the incidence of hydrocephalus in the group with spontaneous SAH was 19 (26.76%) of 71 patients, while in the group with post-traumatic SAH, it was 6 (8.95%) of 67 patients. There was a statistically significant difference in the frequency of hydrocephalus between these two groups ($p = 0.013$). We obtained a statistically significant association between the thickness of the coagulum and spontaneous

and post-traumatic SAH ($p < 0.001$), where a thick coagulum was considered a coagulum with a thickness of 1 mm or more. In addition, thicker coagulum is more often observed after aneurysmal SAH, and in patients with a higher mFS score as a consequence of a larger amount of blood and basal localization, they lead to obstruction of the cerebrospinal fluid pathways and basal cisterns [50–52]. The results of our study are consistent with the results of some previous studies, indicating that acute hydrocephalus occurs in 15% to 58.4%, and chronic hydrocephalus develops in 4.3% to 37% after aneurysmal spontaneous SAH [50–53]. Acute hydrocephalus is most often caused by blood clots in the aqueduct of Sylvius, openings of the fourth ventricle, as well as in the subarachnoid basal cisterns, which obstruct the flow of cerebrospinal fluid [50, 54]. Chronic hydrocephalus can occur later and is caused by the formation of adhesions between the pia and arachnoid mater, which also obstructs the flow of cerebrospinal fluid [50, 51].

Should the patient survive the initial SAH, the most dangerous early complication is re-bleeding from a previously ruptured aneurysm. The frequency of this complication ranges from 8% to 23% in the first 72 hrs after the initial SAH [55]. Studies have shown that about 50% to 90% of re-bleeding episodes arise within the first 6 hrs after the primary rupture [56, 57]. The consequences of re-bleeding are serious, with a reported mortality rate of 20 to 60 [58, 59]. There was no statistically significant difference in the frequency of re-hemorrhage between the groups of spontaneous and post-traumatic SAH in our research ($p = 0.197$). In our study, the frequency of re-hemorrhage in both groups of patients was in accordance with the data from the relevant scientific literature [60–62]. The mechanism of re-bleeding is complex and influenced by many factors. Moreover, several risk factors for re-bleeding have been identified. Beck et al. [60] pointed out that large and multiple aneurysms were associated with re-hemorrhage after SAH. Large aneurysms have a more fragile wall, which explains the increased risk for re-hemorrhage. Ohkuma et al. [57] indicated that elevated systolic blood pressure (> 160 mmHg) on admission was associated with re-bleeding. Poor clinical status (e.g., high Hunt and Hess score) on admission is associated with an increased risk of re-bleeding [59, 62].

Limitations of the study

A limitation of our study is that we did not include risk factors and comorbidities that may have been associated with re-bleeding, so we do not have an adequate explanation for the results presented here.

Conclusion

There were statistically significant differences between gender distribution, the frequency of abnormal ECG findings in the form of a negative T wave, greater coagulum thickness, vasospasm occurrence, and a higher rate of hydrocephalus in patients with spontaneous SAH compared to patients with post-traumatic SAH. Additional and more comprehensive prospective studies with larger groups of patients are needed to confirm the strength of the evidence presented here.

Conflict of interest

The authors declare no conflict of interest.

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Age-independent association between high-sensitivity C-reactive protein and blood pressure in middle-aged adults

Povezanost između visoko osetljivog C-reaktivnog proteina i krvnog pritiska nezavisna od životnog doba kod sredovečnih osoba

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Abstract

Background/Aim. There is growing evidence suggesting that high-sensitivity C-reactive protein (hs-CRP) is a reliable biomarker in patients with hypertension. While the relationship between hypertension and age is well established, the connection between hs-CRP and age remains unclear. The aim of the study was to determine a relationship between hs-CRP and age, body mass index (BMI), and blood pressure in middle-aged people. **Methods.** This cross-sectional survey was conducted in Shanghai, China, and it included data from 1,677 healthy male participants aged 18 to 50 years and 1,127 healthy female participants aged 19 to 49 years recruited during routine health examinations. The hs-CRP, BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were recorded. **Results.** The participants were first separated into four age quartile groups, in which an increase in BMI, SBP, and DBP was observed but not in hs-CRP. Afterward, the participants were divided into four hs-CRP quartile groups, in which an increase in BMI, SBP, and DBP was noted, but not in age. Finally, using Pearson correlation, positive correlations were found between hs-CRP, BMI, SBP, and DBP, but no correlation was discovered between age and hs-CRP. **Conclusion.** The authors showed that age is likely a confounding factor that correlates with SBP, DBP, and BMI, but it does not directly correlate with hs-CRP.

Key words:
age factors; biomarkers; blood pressure; body mass index; c-reactive protein.

Apstrakt

Uvod/Cilj. Sve više dokaza ukazuje na to da je visoko osetljivi C-reaktivni protein (*high-sensitivity C-reactive protein* – hs-CRP) pouzdan biomarker kod bolesnika sa hipertenzijom. Dok je veza između hipertenzije i životnog doba dobro utvrđena, veza između hs-CRP i životnog doba još uvek nije dovoljno jasna. Cilj rada bio je da se utvrdi povezanost između hs-CRP i životnog doba, indeksa telesne mase (ITM) i krvnog pritiska kod osoba srednjih godina. **Metode.** Ova studija preseka sprovedena je u Šangaju, u Kini i uključila je podatke o 1 677 zdravih muških ispitanika, od 18 do 50 godina, i 1 127 zdravih ženskih ispitanika, od 19 do 49 godina, koji su prikupljeni tokom redovnih zdravstvenih pregleda. Beleženi su hs-CRP, ITM, sistolni krvni pritisak (SKP), i dijastolni krvni pritisak (DKP). **Rezultati.** Ispitanici su prvo bili podeljeni prema životnom dobu u četiri grupe, kvartilno, u kojima je utvrđen porast BMI, SKP i DKP, ali ne i hs-CRP. Zatim su ispitanici podeljeni kvartilno u četiri grupe, prema hs-CRP u kojima je utvrđen porast vrednosti BMI, SKP i DKP, ali ne i starosti. Na kraju, korišćenjem Pearson-ove korelacije, nađena je pozitivna korelacija između hs-CRP, ITM, SKP i DKP, ali ne i korelacija između životnog doba i hs-CRP. **Zaključak.** Životno doba je verovatno pridruženi faktor, jer je u korelaciji sa SKP, DKP i ITM, ali nije u direktnoj korelaciji sa hs-CRP.

Ključne reči:
životno doba, faktor; biomarkeri; krvni pritisak; telesna masa, indeks; c-reaktivni protein.

Introduction

C-reactive protein (CRP), a low-weight protein produced by the liver, is considered a classic acute-phase protein that increases in response to stress, inflammation, and various illnesses [1]. CRP detection is a standard test performed in clinical practice. However, standard CRP detection has limited sensitivity with a lower detection limit of 3–8 mg/L, making it an unreliable prediction biomarker [2]. To address this, a more sensitive assay for CRP, known as high-sensitivity CRP (hs-CRP), has been developed and proved to be a useful biomarker in various conditions such as Parkin

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son's disease [3], diabetes [4], postoperative complications [5], sepsis [6], and chronic obstructive pulmonary disease (COPD) [7]. In the realm of hypertension or cardiovascular disease, the hs-CRP has been widely studied and is recognized as one of the key risk factors in assessing cardiovascular risk [8, 9].

Most of the diseases involved in the hs-CRP studies are age-related. Age has typically been considered a confounding factor that is accounted for in hs-CRP studies. However, the relationship between age and hs-CRP has not been thoroughly explored, which results in varying findings from different studies. Some studies have found a positive correlation between hs-CRP and patient age. For instance, Demirbas et al. [10] reported a positive correlation between hs-CRP and age in psoriasis patients. Milan-Mattos et al. [11] reported that the natural aging process increased IL-6 and hs-CRP levels. In patients with carotid intima-media thickness, Kim et al. [12] reported a significant positive correlation between hs-CRP and age. In young children with cardiovascular risk, Rondo et al. [13] reported that age is positively correlated with hs-CRP levels. In infants and young adults with diabetes, Coulon et al. [14] reported a significant correlation between hs-CRP and age or duration of diabetes.

However, other studies did not find any relationship between hs-CRP and age. For instance, there was no relationship between serum hs-CRP and age or weight both in smokers and nonsmokers in COPD patients [15]. In patients with atrial fibrillation, Hermida et al. [16] reported no obvious multiplicative interaction between hs-CRP and age, gender, or race. In an investigation of 213 systemic lupus erythematosus patients and 134 controls, Barnes et al. [17] did not find a relationship between hs-CRP and age, gender, race, etc. Song et al. [3] reported that age is not correlated with hs-CRP in patients with *de novo* Parkinson's disease. Liu et al. [18] reported that age is not correlated with hs-CRP in patients with depression. Feldman and Sbong [19] reported no relation between hs-CRP and age in patients with inflammatory or infectious disorders. Allam et al. [20] found no obvious correlation between serum hs-CRP and age in patients with bronchial asthma.

Moreover, in addition to being a biomarker of a variety of diseases, hs-CRP has been shown to be not only a marker of various illnesses but also a risk factor in healthy individuals for non-alcoholic fatty liver [21], abdominal obesity [22], hypoadiponectinemia [23], etc. Despite this, most studies on hs-CRP and hypertension have focused on patients with the abnormalities, leaving the relationship between hs-CRP level and blood pressure (BP) in healthy individuals largely unstudied.

In this investigation, the relationship between hs-CRP and age, body mass index (BMI), and BP was evaluated by recruiting healthy adults aged 18 to 50 years in Shanghai, China. The recruitment process was conducted during routine physical examinations and involved a medical history inquiry, hematological and clinical chemistry, and electrocardiograph (ECG) by certificate doctors. The levels of hs-CRP, BMI, systolic BP (SBP), and diastolic BP (DBP) were measured in healthy adults and analyzed for any correlations.

Methods

Study population

In total, 1,677 males and 1,127 females aged 18 to 50 years, with a median age of 29 and 28 years, respectively, were recruited from Yangpu Hospital in Shanghai, China, during a routine health examination conducted between April and May 2019. Participants were asked to complete a questionnaire covering their personal information, career, life habits, and medical history. All participants were subjected to physical examination, medical history inquiry, hematology, clinical chemistry, electrocardiograph, urinalysis, etc. After being reviewed by certificated medical physicians, participants who showed no abnormalities in any of the parameters except for BP were included in the study. The investigation complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Yangpu Hospital, Tongji University School of Medicine (No LL-2017-SCI-002). Oral or written informed consent forms were obtained from all the participants in this investigation.

Laboratory measurements

After overnight fasting, body weights and heights were measured in the morning, and body weight mass calculation was conducted using the equation "BMI = body weight (kg)/height (m^2)". After at least 10 min relaxation in the medicine ward of the hospital, the participants were manually measured for SBP and DBP from the right arm by a physician using a mercurial sphygmomanometer (Yuyue Co., Ltd, Jiangsu, China) with a stethoscope. For hs-CRP detection, venous blood (approximately 2 mL) was collected from the participants and processed into serum *via* centrifugation; the samples were kept at 2–8 ˚C until analysis. Then, hs-CRP was detected by using Dimension RXL Analyzer (Siemens Healthcare, Germany) with the method of particle-enhanced turbidimetric immunoassay (PETIA) as described elsewhere [24]. The C-reactive Extended Range Flex reagent was also brought from Siemens (Germany). The detection range was 0.5 to 250.0 mg/L. Calibration and quality control samples were included in each run of the analyses.

Statistical analysis

Data were statistically analyzed using SPSS V20.0 for Windows (SPSS Inc., Chicago, IL, USA). Values below the limit of detection (LOD) were considered negative and given an arbitrary value of LOD/$\sqrt{2}$ [25]. Based on age or hs-CRP levels, participants were divided into four quartile groups: Q1 (1st quartile), Q2 (2nd quartile), Q3 (3rd quartile), and Q4 (4th quartile). The differences in BMI, SBP, DBP, and hs-CRP or age between the groups were examined using one-way ANOVA followed by T-test or *post-hoc* test. Then, a Pearson correlation analysis was applied for the relationship between age, hs-CRP, gender, BMI, SBP, and DBP. Cut-off values for statistical significance were set at $p < 0.05$.

Results

Participants

The results of the measurements are presented in Table 1, revealing that the average age of the participants was 30.9 years, with no significant difference between males (30.9 years) and females (31.0 years, $p > 0.05$). The average hs-CRP level was 1.87 mg/L, with no significant difference between males (1.89 mg/L) and females (1.85 mg/L, $p > 0.05$). The average BMI was 23.4 kg/m^2, with a slightly higher average in males (24.0 kg/m^2) than in females (22.6 kg/m^2, $p < 0.01$). The average SBP was 119.3 mmHg, with a higher average in males (123.0 mmHg) than in females (113.7 mmHg, $p < 0.01$). Similarly, the average DBP was 81.5 mmHg, with a higher average in males (83.1 mmHg) than in females (79.1 mmHg, $p < 0.01$). All participants were considered healthy with no known underlying diseases.

Changes among the age-quartile groups

Initially, the participants were divided into four age-quartile groups: 18–23 years (1st quartile), 24–28 years (2nd quartile), 29–36 years (3rd quartile), 37–50 years (4th quartile). Differences in hs-CRP, BMI, SBP, and DBP were examined between the age-quartile groups. No statistical significance was noted for the hs-CRP between the groups, while data of BMI, SBP, and DBP increased with age from the 1st to the 4th quartile (Table 2).

Changes among the hs-CRP-quartile groups

Similarly, the participants were then divided into four hs-CRP-quartile groups: 0.36–0.95 mg/L (1st quartile), 0.96–1.31 mg/L (2nd quartile), 1.32–1.99 mg/L (3rd quartile), 2.00–3.00 mg/L (4th quartile). Differences in age, BMI, SBP, and DBP were examined between the hs-CRP-quartile groups. No statistical significance was noted for the age between groups, while data of BMI, SBP, and DBP increased as the hs-CRP escalated from the 1st to the 4th quartile (Table 3).

Pearson correlation analysis

Pearson correlation analysis was utilized to assess the interdependence between age, hs-CRP, BMI, SBP, and DBP. Table 4 revealed that age positively correlated with BMI, SBP, and DBP but not with hs-CRP. Similarly, hs-CRP positively correlated with BMI, SBP, and DBP but not with age. Positive correlations were observed between BMI, SBP, and DBP.

Table 1

Summary results of hs-CRP, BMI, SBP, and DBP in the participants

Gender	Number	Age years	hs-CRP mg/L	BMI kg/m^2	SBP mmHg	DBP mmHg
Male	1,677	30.9 ± 7.8	1.89 ± 1.78	24.0 ± 3.7*	123.0 ± 13.4*	83.1 ± 9.7*
Female	1,127	31.0 ± 8. 6	1.85 ± 1.92	22.6 ± 3.5	113.7 ± 13.1	79.1 ± 8.6
Total	2,804	30.9 ± 8.1	1.87 ± 1.84	23.4 ± 3.7	119.3 ± 14.0	81.5 ± 9.5

hs-CRP – high-sensitivity C-reactive protein; BMI – body mass index; SBP – systolic blood pressure; BDP – diastolic blood pressure.
Data are presented as mean ± standard deviation. Student *t*-test was used for comparison between males and females. *$p < 0.01$.

Table 2

Comparisons of hs-CRP, BMI, SBP, and DBP between age-quartile cohorts

Quartile cohort	Age years	Number	hs-CRP mg/L	BMI* kg/m^2	SBP* mmHg	DBP* mmHg
1st	18–23	594	1.88 ± 1.82	22.6 ± 4.0	115.5 ± 12.7	78.4 ± 8.2
2nd	24–28	737	1.83 ± 1.64	23.0 ± 3.8	116.2 ± 13.2	79.7 ± 8.8
3rd	29–36	743	1.94 ± 1.98	23.9 ± 3.6	120.3 ± 14.0	82.1 ± 9.5
4th	37–50	730	1.85 ± 1.89	24.0 ± 3.0	124.4 ± 14.1	85.1 ± 9.9

Data are presented as mean ± standard deviation. A one-way Analysis of Variance (ANOVA) was used for parameter comparison among quartiles. *$p < 0.01$.
For abbreviations, see Table 1.

Table 3

Comparisons of age, BMI, SBP, and DBP between hs-CRP-quartile cohorts

Quartile cohort	hs-CRP mg/L	Number	Age years	BMI* kg/m^2	SBP* mmHg	DBP* mmHg
1st	0.36–0.95	690	30.7 ± 8.3	21.8 ± 3.0	116.3 ± 13.5	79.8 ± 9.0
2nd	0.96–1.31	705	30.7 ± 8.1	22.4 ± 3.1	117.7 ± 13.6	80.2 ± 8.9
3rd	1.32–1.99	701	31.7 ± 8.2	23.9 ± 3.3	120.2 ± 14.0	82.0 ± 9.7
4th	2.00–3.00	708	30.6 ± 7.9	25.5 ± 4.0	122.9 ± 14.1	83.9 ± 9.6

Data are presented as mean ± standard deviation. A one-way Analysis of Variance (ANOVA) was used for parameter comparison among quartiles. *$p < 0.01$.
For abbreviations, see Table 1.

Table 4

Pearson correlation coefficient

Parameters	Age	hs-CRP	BMI	SBP	DBP
Age	1	0.019	0.198*	0.259*	0.270*
hs-CRP		1	0.387*	0.188*	0.167*
BMI			1	0.446*	0.351*
SBP				1	0.736*
DBP					1

Correlation between age, hs-CRP, BMI, SBP, and DBP were analyzed by using Pearson correlation analysis. *$p < 0.01$.
For abbreviations, see Table 1.

Discussion

The aim of this study was to examine the role of hs-CRP as a biomarker of hypertension in young, healthy adults and to assess the impact of age on hs-CRP levels. Results showed that hs-CRP levels were positively correlated with SBP, DBP, and BMI in healthy participants with an average age of 30.9 years (ranging from 18 to 50 years), which was in line with previous studies on hypertension [26] and other cardiovascular disorders [27]. By including young, healthy adults, we sought to minimize the bias between age and underlying disease. Analysis of the data using ANOVA and Pearson correlation analysis revealed that changes in hs-CRP were not related to age, and there was no correlation or trend observed between age and hs-CRP levels.

The hs-CRP level of 1.87 mg/L obtained in this investigation is in line with other studies in Chinese young adults. As reported by Wang et al. [28], the average hs-CRP was determined as 1.89 mg/L in 14,046 healthy adults aged 35 to 64 years. The mean SBP of 119.3 mmHg and the mean DBP of 81.5 mmHg were considered normal values, which is consistent with the results in the nationwide survey conducted in China from October 2012 to December 2015; the mean SBP and DBP in 88,540 adults aged 25–34 years were calculated as 118.9 mmHg and 73.2 mmHg, respectively [29]. The mean BMI in this investigation was 23.4 kg/m^2, which is consistent with the mean BMI (20.8–25.1 kg/m^2) obtained from approximately 100,000 residents in China mainland [30] and the mean BMI of 24.1 kg/m^2 in 2,893 Chinese subjects in Hong Kong [31].

Inflammation is widely recognized as the key contributor to the development of atherosclerosis, which in turn can lead to hypertension. Hs-CRP is a significant marker of inflammation and has been strongly linked to cardiovascular disease. Not only does hs-CRP serve as an inflammatory biomarker, but it also has a direct impact on the pathogenesis of hypertension. That is because hs-CRP can promote vasoconstriction, leukocyte adherence, platelet activation, oxidation, thrombosis, and upregulation of angiotensin type-1 receptor expression. These actions all contribute to the development of hypertension [32].

Although inflammation is observed in many age-dependent diseases, there is no direct correlation between inflammation and age. In healthy children under 12 years old (mean age 5.2 years), the inflammatory cytokines of IL-1Ra, IP-10, and TNF-α decreased with age [33]. In participants aged 24 to 90 years, Lin et al. [34] demonstrated that the rates of change of T cells ($CD4^+$ and $CD8^+$), B cells and NK cells were relatively stable throughout life. In a review including more than 50 studies, researchers did not find evidence of age-related changes in any of the Th1 (IL-2, IFN-γ), Th2 (IL-4, IL-6, and IL-10), or proinflammatory cytokines (IL-1β, IL-8, TNF-α) [35].

Several other risk factors for arterial hypertension are equally important, such as genetics, kidney function, endocrine status, family history, overweight/obesity, poor diet, tobacco use, alcohol consumption, chronic conditions, and gender. The subjects in this investigation with genetic predisposition were from Shanghai, China. The authors separated the parameters of overweight/obesity and gender from the assessment of the relationship between hs-CRP and hypertension. All subjects were considered healthy and free of any abnormalities in the parameters of kidney function, family history, poor diet, tobacco use, and alcohol consumption after being thoroughly reviewed by certified medical physicians. Endocrine status and chronic conditions were not included in the routine physical examination, and their role in both hs-CRP and hypertension has not been addressed in this investigation.

One limitation of this investigation is that the demographic of the participants was not evenly distributed between males and females. The data showed that males had a higher BMI, SBP, and DBP than females. Despite this imbalance, the analysis found no significant differences in hs-CRP between the genders, indicating that this imbalance did not impact the data interpretation. Additionally, participants were considered healthy based on a variety of examinations but not on their BP values. As a result, participants with a BMI ≥ 30 kg/m^2 (159 individuals), SBP ≥ 140 mmHg (204 individuals), and DBP ≥ 90 mmHg (501 individuals) were still included in the investigation, even though they may have underlying health issues.

These individuals were considered part of the targeted population for the investigation.

Conclusion

In the present investigation, by evaluating the levels of hs-CRP, BMI, SBP, and DBP in a sample of young, healthy adults in Shanghai, China, we found that age does not directly correlate to hs-CRP. Instead, hs-CRP may serve as a risk factor that correlates to BP.

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Conflict of Interest

The authors declare no conflict of interest.

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Comparative assessment of the depth of invasion of early-stage oral cavity carcinomas based on intraoral ultrasound and computerized tomography findings

Komparativna procena dubine invazije tumora usne duplje u ranom stadijumu na osnovu nalaza intraoralnog ultrazvuka i kompjuterizovane tomografije

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Abstract

Background/Aim. The depth of invasion of oral cavity carcinoma (OCC) and the nodal involvement define the treatment selection, outcome, and prognosis of the disease. In determining the stage of OCC, the most widely applied methods are computerized tomography (CT) and magnetic resonance imaging (MRI), whose limitations can be overcome to some extent by using intraoral ultrasound (IOUS). The aim of the study was to evaluate the imaging presentation of early-stage OCC, determine the depth of invasion (DOI) and the greatest diameter (GD) of the tumor using the IOUS and CT methods, and compare them with histopathological (HP) findings. **Methods.** The study was designed as a prospective one, with a time limitation of three months. Eleven patients with clinical early-stage OCC underwent a native CT examination of the head and neck as well as a contrast-enhanced phase, and then IOUS of the lesion was performed. Using both methods, DOI and GD values were measured, and the values were correlated with HP findings. The analysis of the obtained data was performed using the statistical package SPSS 22 and Pearson correlation coefficient. **Results.** A significant correlation (p = 0.001) was established between the DOI values measured by IOUS and CT examination with the measurements obtained by HP processing. On the other hand, by comparing the GD measured on IOUS and CT examination, no correlation was established with the HP report. **Conclusion**. Measurements of DOI obtained by IOUS significantly correlated with those in the HP report, while overcoming the limitations of the CT method in the evaluation of small-sized tumors and tumors that cannot be shown due to artifacts.

Key words:
histological techniques; mouth neoplasms; neoplasm invasiveness; tomography, x-ray computed; ultrasonography.

Apstrakt

Uvod/Cilj. Dubina invazije karcinoma usne duplje i zahvaćenost regionalnih limfnih čvorova definišu terapijski modalitet, ishod lečenja i prognozu bolesti. U određivanju stadijuma karcinoma usne duplje, najšire primenjivane metode su kompjuterizovana tomografija (KT) i magnetna rezonanca (MR), čija ograničenja se donekle mogu prevazići upotrebom intraoralnog ultrazvuka (IOUZ). Cilj rada bio je da se proceni vizuelni prikaz karcinoma usne duplje ranog stadijuma, odredi dubina invazije (DI) i najveći dijametar tumora (NDT) metodama IOUZ i KT, a zatim da se uporede sa histopatološkim (HP) nalazom. **Metode.** Studija je dizajnirana kao prospektivna sa vremenskim ograničenjem od tri meseca. Kod 11 bolesnika sa ranim kliničkim stadijumom karcinoma usne duplje, urađen je nativni i postkontrastni pregled glave i vrata pomoću KT, a potom pregled lezije metodom IOUZ. Koristeći obe metode merene su vrednosti DI i NDT, koje su potom korelisane sa HP nalazom. Analiza dobijenih podataka izvršena je upotrebom statističkog paketa SPSS 22 i Pearson-ovog koeficijenta korelacije. **Rezultati.** Ustanovljena je značajna korelacija (p = 0,001) između vrednosti DI izmerenih pomoću metoda IOUZ i KT, sa merama dobijenim HP obradom. Sa druge strane, upoređivanjem vrednosti za NDT izmerenih na IOUZ i KT pregledu, nije ustanovljena korelacija sa HP izveštajem. **Zaključak.** Mere DI dobijene metodom IOUZ značajno su korelisale sa onim u HP izveštaju, uz prevazilaženje ograničenja KT metode u evaluaciji tumora malih dimenzija i tumora koji se zbog artefakata ne mogu prikazati.

Ključne reči:
histološke tehnike; usta, neoplazme; neoplazme, invazivnost; tomografija, kompjuterizovana, rendgenska; ultrasonografija.

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Introduction

Malignancies of the oral cavity are the most common malignancies of the head and neck [1]. Tongue is the origin of tumors in half of the cases from developed countries [2]. Smoking, alcohol, and, to a lesser extent, human papillomavirus (HPV) infection are the risk factors for oral cavity carcinoma (OCC) [2]. The incidence of different localizations of OCC, in addition to the socio-epidemiological status, also depends on the habits of the population [1, 2]. In Eastern European countries, the frequency of carcinoma of the tongue is higher, while due to the habit of chewing tobacco, squamous cell carcinoma (SCC) in the lower gingivobuccal sulcus is the most common sublocation of OCC in India [3].

The 2010 staging manual of OCC incorporates the greatest diameter (GD) of the tumor as a relevant factor for determining the stage of the primary tumor and as a prognostic factor for disease-specific survival (DSS), but also for progression-free survival (PFS) [4]. The updated 8th edition of the American Joint Committee of Cancer (AJCC) 2017 introduces depth of invasion (DOI) as the independent prognostic factor of OCC [5]. Recently, a retrospective study indicated that DOI is a prognostic factor only for the DSS in patients with early stage OCC, along with cofactors such as lympho-vascular invasion and histopathological (HP) tumor grade [6].

The most common imaging methods for tumor staging and evaluating DOI are computerized tomography (CT), magnetic resonance imaging (MRI), and, lately, intraoral ultrasound (IOUS) [3]. Known limitations of CT and MRI for OCC examination are artifacts originating from metal dental restorations. On the other hand, a limiting factor of IOUS is the eventual inaccessibility of the lesion and the impossibility of its complete coverage by the probe [3]. These methods should be complementary to achieve the best diagnostic results. To our knowledge, IOUS examinations of the OCC have not been performed in Serbia so far.

Therefore, this study aimed to evaluate the imaging presentation of early-stage OCC, to determine the DOI and GD by IOUS and CT methods, and to compare it with HP findings.

Methods

The study was designed as a prospective one and conducted with the approval of the Ethics Committee of the Faculty of Dental Medicine, University of Belgrade, Serbia (No. 36/33 from December 14, 2022).

The inclusion criteria for this research were the following: clinical T1 or T2 tumor stage, clinical and radiological N0 stage, clinical and radiological M0 stage, and accessibility of the lesion by hockey stick IOUS probe.

The exclusion criteria for this study were the following: incomplete postoperative HP report (DOI not specified), hypersensitivity to iodinated contrast media (contrast CT was missing), and surgery that was not performed.

Out of 52 patients referred to the Center for Radiological Diagnostics with an oral cavity lesion, 11 met all the inclusion criteria for the study.

Patients underwent a non-contrast and contrast-enhanced CT examination of the head and neck (ranging from the base of the skull to the upper aperture of the thorax) performed on a 64-slice machine (Philips Ingenuity Core 64, The Netherlands) in order to assess the local tumor extent and detect lympho-nodal disease. Contrast CT examination was performed in the arterial-venous phase using the split bolus technique: 60 mL + 10 s pause + 40 mL with a flow rate of 2 mL/s. Before the contrast injection, during the break, and at the end of the contrast scan, patients received saline in the following amount: 14 mL + 30 mL + 40 ml. Sections were made at 0.8 mm thickness and reconstructed at 0.625 mm in the bone and soft tissue window (IntelliSpace Portal 10, Philips, The Netherlands).

In order to better display and separate the tissues and organs in the vestibulum of the oral cavity, CT scanning was performed using the inflated cheeks technique. We instructed our patients to perform the maneuver of throwing the head back and raising the tip of the tongue to the palate for a more optimal view of the OCC of the ventral side of the tongue. Metal artifact reduction CT algorithm (OMAR, Philips, The Netherlands) was applied as well.

The IOUS was performed with the unit SonoScapeS50 PRO (Guangdong, China), using the hockey stick multifrequency probe (1012, F-5.5MHz–10MHz). A glove with a probe immersed in the gel was placed directly on the surface of the lesion. The normally present saliva served as an interface between the surface of the lesion and the surface of the glove. Ultrasound sections were made in all directions of the lesion, and the characteristic sections were forwarded to the Picture Archiving and Communication System (PACS) station.

Patient examinations (CT and IOUS) were evaluated at the PACS station independently by two radiologists. All measurements were repeated three times, and the obtained separate values of DOI and GD were expressed as the mean value.

Statistical analyses were performed using SPSS 22.0 (SPSS Statistics for Windows, SPSS, Inc., Chicago, IL, USA). Pearson's coefficient was used to show the correlation between DOI and GD values measured by IOUS and CT and corresponding HP measures [7]. The values of $p < 0.05$ were set as significant. Intraclass Correlation Coefficient (ICC) test was used to determine inter-observer reliability for measurements taken on ultrasound and CT scans. We performed a *post hoc* analysis based on the results of the study, and the achieved power was 75.8%. Power for 11 respondents was calculated for the difference between two dependent means, $\alpha = 0.05$, and the effect size was calculated based on the difference between the two dependent means and standard deviation of difference ($dz = 0.76$, $rh = 0.947$). That was performed in the G*power program (version 3.1.9.2.) [8].

Results

After the surgery, all patients were diagnosed with SCC, and the HP T stage was reported as well. Seven (63.64%) patients were male, and 4 (36.36%) were female. The average age of the patients was 57.64 years (from 45 to 67 years). Nine (81.8%) patients had tongue carcinoma, and 2 (18.2%) had buccal mucosa carcinoma.

The most common site of OCC origin was the margin of the tongue (Table 1).

In 2 out of 11 patients, the lesion could not be detected by CT, but only by IOUS, due to the presence of numerous artifacts originating from metal dental restorations (Figures 1, 2, and 3 illustrate the DOI measurements assessed by IOUS and CT).

ICC interobserver tests showed a high correlation between the measured values of DOI and GD (in the range from

Table 1
Sublocations of early stage oral cavity carcinoma.

Localization	n (%)
Tongue	
lateral margin	5 (45.5)
ventral side	3 (27.3)
dorsal side	1 (9.1)
Buccal mucosa	2 (18.2)
Total	11 (100.0)

n – number. All values are expressed as numbers (percentages).

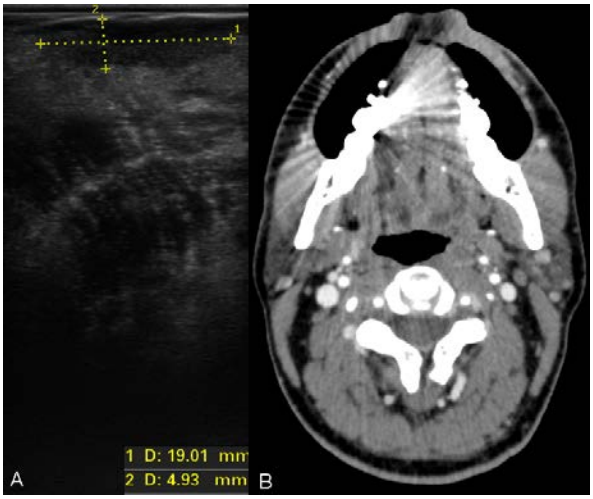

Fig. 1 – Oral cavity carcinoma (OCC) localized on the lateral edge of the tongue with measured DOI and GD: A) IOUS shows a hypoechoic and not sharply demarcated lesion towards the deeper tissues of the tongue; B) A transversal CT scan showing that it was not possible to detect OCC due to the presence of artifacts originating from metal dental restorations. IOUS – intraoral ultrasound; DOI – depth of invasion; CT – computed tomography; GD – greatest dimension.

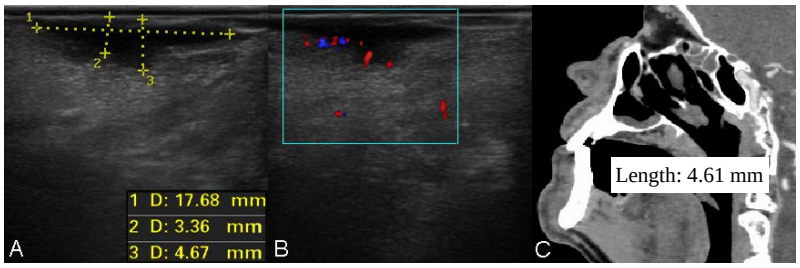

Fig. 2 – Oral cavity carcinoma (OCC) localized on the ventral side of the tongue with measured DOI and GD: A) A hypoechoic, plaque lesion with an unsharp border towards the deeper tissues of the tongue, shown by the IOUS; B) Visualization of OCC vascularization using IOUS – note the dominantly fringe distribution of Color Doppler signal (blue and red color); C) Contrast-enhanced CT in sagittal reformation with the tip of the tongue on the hard palate showing OCC with measured DOI, localized on the ventral side of the tongue. There is a well-opacified plaque lesion on the ventral side of the tongue. For abbreviations, see Figure 1.

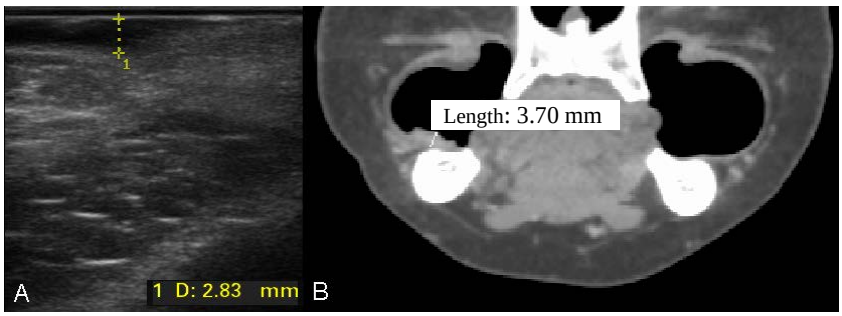

Fig. 3 – A) IOUS of oral cavity carcinoma (OCC) localized on the buccal mucosa with measured DOI. Note the hypoechoic, plaque lesion with an unsharp border towards the deeper buccal tissues. B) Contrast-enhanced CT in coronal reformation and in distension technique (inflated cheeks) presenting the DOI measuring of well-opacified OCC, localized on the buccal mucosa. For abbreviations, see Figure 1.

0.959 to 1). Statistical analysis of data established a strong correlation between DOI values measured by IOUS and CT with the measurements obtained by HP processing. On the other hand, by comparing GD values assessed by IOUS and CT with HP reports, no correlation was established (Table 2).

Table 2

Correlation of DOI and GD values measured by IOUS and CT methods with the HP findings

Parameter	PCC	*p*-value
DOI		
IOUS-HP	0.947	0.001
CT-HP	0.868	0.05
GD		
IOUS-HP	0.277	0.595
CT-HP	-0.612	0.388

DOI – depth of tumor invasion; GD – greatest diameter of tumor; CT – computed tomography; PCC – Pearson's correlation coefficient; HP – histopathology; IOUS – intraoral ultrasonograph.

Discussion

During the last decade, IOUS has been used in the evaluation of various benign and malignant oral cavity lesions [9]. Malignant tumors of the tongue, walls, and floor of the oral cavity are, in 90% of cases, SCCs and appear as hypoechoic lesions on IOUS examination [9, 10]. Most publications refer to SCC of the tongue, which also constituted most of our study (91.8%). A systematic review from 2019 analyzed 19 published studies of different designs and levels of evidence, with 3 to 109 enrolled patients with tongue cancer [11]. The authors stated the significant role of IOUS in determining the dimensions and margins of the tongue SCC; however, due to large variations in the analyzed publications, they suggest that the reliability of IOUS be proven through prospective and standardized studies. Other publications reported a high correlation of 0.95 between tumor thickness (TT) and DOI measured by intraoperative IOUS and HP reports [5, 12]. Whether preoperatively or during the surgery, the IOUS exam showed high sensitivity in depicting the dimensions and deep margins of tongue SCC, which is very important for adequate resection of early-stage carcinomas [10, 11]. Our prospective study showed a high correlation (0.947) between DOI measurements obtained by IOUS and those in HP reports, almost identical to the cited study [12].

The 8th edition of the staging manual of the AJCC introduces DOI as an independent prognostic factor for oral cavity malignancies [5]. Depth of invasion reflects the proximity of lympho-vascular structures and thus the likelihood of lymph node involvement [11]. In published literature, for several reasons, the term DOI is often replaced with TT. Upon the definition, TT is the sum of the largest exophytic and endophytic part of the tumor, and DOI is the greatest depth of tissue infiltration by the tumor, measured perpendicular to the line of the surrounding preserved mucosa [13]. A retrospective study by Weimar et al. [14] analyzed T1–T3 stages of OCC and the correlation of TT determined by CT and MRI examination with the HP report. As DOI was not referred to in the majority of HP reports and the overall survival rate was the same whether TT or DOI was measured, the authors concluded that TT may be a surrogate for DOI [14–16].

Despite emphasizing DOI as an important prognostic factor in the 8th AJCC tumor, node, metastazis-TNM classification, GD still stands as an indispensable factor in determining the stage of the primary tumor [5]. Our study also analyzed GD, which, although in the prognostic sense, is considered less important relative to DOI and certainly remains a significant factor in planning the operative procedure itself. Additionally, GD is a measure regularly reported in radiology and HP reports; it may also indicate the reliability of the applied imaging modalities in assessing the overall extent of the lesion [13, 16]. Our results showed the absence of correlation between IOUS and CT measurement of GD with those in the HP report, which illustrates the lower reliability of these techniques in measuring GD. That could be addressed towards the limitation in obtaining GD of irregularly shaped tumors located on movable organs (tongue) or irregular-shaped oral cavity surfaces. Additionally, it can be partly attributed to the errors of subjective assessment of GD. The discrepancy between imaging and HP measurements is also influenced by limiting factors in the pathologist's work [16].

Namely, the processing of tissue in formaldehyde leads to its shrinkage (least for the tongue 91%), and we should not forget the volume variations of the delivered resected tissue, as well as the differences in the direction of cutting the specimen [16].

We excluded OCC where the DOI and GD were not mentioned in the HP report, which is one of the reasons for the small number of patients analyzed in relation to the total number of examined patients (11 out of 52) and, therefore, the main limitation of our study. Our study showed a higher degree of correlation between applied imaging methods and HP in the DOI value compared to a previously published study, but it should be noted that the time interval from diagnostic processing to surgery in all our patients was up to four weeks. Weimar et al. [14] reported a higher (83%) radiological-HP correlation of TT measurements in patients in whom four weeks or less elapsed from CT/MRI examination to surgery, which is close to our CT/HP correlation results. However, the correlation between IOUS and HP measurements of DOI in our study was 95%, which is obviously higher than the CT/MRI correlation [14, 16]. The mentioned difference can be explained by the proportion of carcinomas on the base of the tongue in the mentioned studies, which were not included in our research.

A meta-analysis of early-stage OCCs showed a correlation of 0.82 between IOUS and HP measurements of TT, which is lower compared to our results (0.947) [17]. A recent study from 2020 reported a higher degree of correlation between IOUS and HP measurements of TT/DOI, identical to our results (0.95) [12]. Nevertheless, IOUS is a new, operator-dependent diagnostic method with a long learning curve, limited to probe-accessible parts of the oral cavity [18].

A retrospective study from 2021 stated that TT is not a significant prognostic factor for early-stage OCC and that DOI is not an independent and sufficient prognostic factor. Comparing the early OCC staged both with the 7th and 8th editions of the AJCC manual, the authors concluded that according to the 8th edition, upstaging occurred in 23% of cases, which decreased DSS but did not affect PFS. According to their results, different sublocations of OCC should be considered separately due to their specific architecture, lymphovascular network, possibility of perineural spread, and the type of infiltrative growth pattern should be taken into consideration as well [6]. In our study, 2 (18.2%) patients had SCC of the lower gingivobuccal sulcus. These buccal SCCs are the most common OCCs in South Asia due to the habit of chewing tobacco and have a greater infiltrative-destructive potential compared to tongue carcinomas [8]. OCCs in the lower gingivobuccal sulcus were accessible to IOUS examination, while for CT examination, it was necessary to apply the inflated cheek technique [19]. For adequate CT imaging of OCCs located on the ventral side of the tongue, we modified the proposed maneuvers [3]. However, in two patients with lateral edge tongue carcinoma, artifacts from dental fillings obscured CT tumor visualization. The 8th edition of the AJCC manual points out that OCC is usually evaluated with CT or MRI but also claims that lesions with a DOI of 4 mm or less may remain unrecognized using these imaging methods [5]. Respecting these facts, the systematic review by Marcello Scotti et al. [20] stressed the IOUS as a reliable imaging technique in the evaluation of initial oral cavity lesions.

The limitations of our study are the small sample size and the single-center experience. Strict inclusion criteria that we applied have to be considered, which referred to the initial (clinical T1/T2) tumor lesions, their accessibility to the IOUS examination, as well as to the fact that the values of DOI and GD were stated in the HP report. Although numerous studies have shown a high degree of correlation of IOUS with HP staging of OCC at accessible sites, the retrospective nature of any systematic review does not preclude the need for future prospective studies on the reliability of IOUS compared with other imaging modalities [20]. Our prospective study supports this view, aiming to present the results of the IOUS application in the evaluation of OCC in Serbia, where, as far as we know, no results have been published yet.

Conclusion

Measurements of DOI obtained by IOUS significantly correlated with those in the HP report, suggesting that IOUS is a reliable tool in the evaluation of small tumors and tumors that cannot be seen on CT examination due to metal dental artifacts. This research emphasizes the role of IOUS in the multimodal diagnostic approach of OCC.

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Correlation between clinical severity and quality of life in moderate to severe psoriasis patients: real-world evidence

Korelacija između težine kliničke slike i kvaliteta života kod bolesnika sa umerenom do teškom psorijazom: dokazi iz stvarnog sveta

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Abstract

Background/Aim. Psoriasis is a chronic multisystem, inflammatory, and immune-mediated dermatological disease of a relapsing nature. Not only does it affect objective parameters such as skin and joints, with different intensity involvement and with changes and the degree of changes, but it also significantly affects the health-related quality of life (QoL). The aim of the study was to determine the clinical severity and QoL of patients with moderate to severe psoriasis and examine the association between those parameters before and after the treatment. **Methods.** This cross-sectional study included 183 patients diagnosed with moderate to severe psoriasis. The severity of the clinical picture was determined by calculating the Psoriasis Area and Severity Index (PASI) by a dermatologist, while the QoL was assessed using the Dermatology Life Quality Index (DLQI) questionnaire and psoriasis-related stress by the Psoriasis Life Stress Inventory (PLSI). Disease severity and QoL were measured at the baseline visit and after the 16th week of therapy. **Results.** The average PASI score at the beginning of therapy was 23.1 ± 6.5, while after 16 weeks, this value was 4.36 ± 4.86. The DLQI score was 20.8 ± 5.0 at the start of therapy and 6.20 ± 6.16 after 16 weeks, while the PLSI score was 35.37 ± 8.84 initially and 12.75 ± 12.82 after 16 weeks of therapy. A strong correlation was found between PASI and PLSI scores ($r = 0.702$, $p < 0.001$) in the 16th week of therapy, while the correlation between DLQI and PASI scores was moderate ($r = 0.683$, $p < 0.001$). No significant differences between PASI and DLQI scores were found ($r = 0.080$, $p = 0.284$) nor between PASI and PLSI scores ($r = 0.109$, $p = 0.140$) at baseline. **Conclusion.** Patients with severe psoriasis experience a significant reduction in their QoL, accompanied by a high level of psychosocial stress. Observed improvements in QoL have shown a moderate correlation, while lower levels of psychosocial stress have strongly correlated with the severity of the clinical presentation, which may indicate a complex interaction between psychological factors and physical health in patients with psoriasis.

Key words:
psoriasis; quality of life; severity of illness index; surveys and questionnaires; stress, psychological.

Apstrakt

Uvod/Cilj. Psorijaza je hronično multisistemsko, inflamatorno i imunski posredovano dermatološko oboljenje recidivirajuće prirode. Ovo oboljenje ne samo da utiče na objektivne parametre kao što su zahvaćenost kože i zglobova sa različitim intezitetom zahvaćenosti i stepenom intenziteta promena, već utiče i na kvalitet života (KŽ) povezanim sa zdravljem. Cilj rada bio je da se utvrde težina kliničke slike i KŽ kod obolelih sa umerenom do teškom psorijazom, kao i povezanost ispitivanih parametara pre i posle lečenja. **Metode**. Studijom preseka obuhvaćeno je 183 bolesnika sa dijagnozom umerene do teške psorijaze. Težina kliničke slike određivana je izračunavanjem indeksa *Psoriasis Area and Severity Index* (PASI) od strane dermatologa, dok je pomoću upitnika *Dermatology Life Quality Index* (DLQI) procenjivan KŽ, a pomoću *Psoriasis Life Stress Inventory* (PLSI), meren je nivo stresa povezanog sa psorijazom. Težina bolesti i KŽ mereni su na početku primene terapije kao i posle 16. nedelje primene terapije. **Rezultati**. Prosečna vrednost PASI indeksa na početku terapije bila je 23,1 ± 6,5, dok je nakon 16. nedelje ta vrednost iznosila 4,36 ± 4,86. Vrednost DLQI skora na početku terapije iznosila je 20,8 ± 5,0, a nakon 16. nedelje 6,20 ± 6,16, dok je skor PLSI iznosio 35,37 ± 8,84 na početku, a 12,75 ± 12,82 posle 16. nedelje primene terapije. Utvrđeno

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je postojanje jake korelacije između PASI i PLSI skora (r = 0,702, $p < 0,001$) posle 16. nedelje, dok je korelacija između DLQI i PASI skora bila umerena (r = 0,683, $p < 0,001$). Nije nađena značajna razlika između rezultata PASI i DLQI (r = 0,080, $p = 0,284$), niti između PASI i PLSI skorova (r = 0,109, $p = 0,140$) na početku terapije. **Zaključak**. Bolesnici sa teškim formama psorijaze doživljavaju ozbiljan pad KŽ, uz visok nivo psihosocijalnog stresa. Uočeno poboljšanje KŽ pokazalo je umerenu korelaciju, dok su niži nivoi psihosocijalnog stresa snažno korelisali sa težinom kliničke slike, što može ukazivati na kompleksnu interakciju između psiholoških faktora i fizičkog zdravlja bolesnika sa psorijazom.

Ključne reči:
psorijaza; kvalitet života; bolest, indeks težine; ankete i upitnici; stres, psihički.

Introduction

Psoriasis is a chronic, multisystem, inflammatory, and immune-mediated dermatosis whose prevalence and incidence at the global level have increased in the last three decades [1]. Psoriasis has a detrimental impact on the quality of life (QoL), diminishes patients' self-esteem, causes psychosocial stress, and enhances social stigmatization. Impairments in the domain of QoL in patients with psoriasis are more severe compared to other dermatological diseases and are comparable to cardiovascular diseases, joint inflammation, and depression [2].

Guidelines for treatment and classification differ globally, and treatment depends on the patient's age and comorbidities. Most often, the severity of psoriasis is determined by the Psoriasis Area and Severity Index (PASI) score and the body surface area (BSA). A PASI and BSA score below 10 typically implies a mild presentation, whereas scores surpassing 10 encompass a spectrum ranging from moderate to severe psoriasis. The Delphi consensus from the International Psoriasis Council proposed a new classification system so that, due to the observation of the disease only through these parameters, the severity of the disease would not be underestimated. Under the framework of the revised classification, comprehensive evaluation factors encompass the extent of affected regions, the medical history of the condition, and the patient's overall QoL. That evolved perspective reflects an awareness that gauging disease severity involves more than just physiological markers; it recognizes the broader implications for the patient's holistic well-being. Consequently, the contemporary classification not only accommodates a more nuanced assessment process but also emphasizes the necessity of acknowledging the multifaceted impact of the disease on the individual's overall health and QoL [3].

The recommended instrument for determining the severity of psoriasis in many guidelines is the PASI score. Infiltration, desquamation, erythema, affected parts of the body, and assessment of the involvement of the body with lesions are considered in the estimate of this value. The maximum score can be 72, while PASI $\geq$ 10 indicates that it is a severe form of psoriasis. If the decrease in PASI values observed at the baseline visit and the end of the induction phase (usually after 16 weeks) is greater than 75%, the therapeutic response is considered adequate [4].

In addition to the PASI score as an objective assessment measure, the Dermatological Life Quality Index (DLQI) is used to determine the self-assessment of QoL of patients as a subjective measure, and they are used in clinical research and daily practice. The use of this instrument is increasing when assessing the overall clinical picture of patients with dermatological diseases due to a growing understanding of the impact of the disease on everyday life and the patient's well-being. Although specific QoL assessment questionnaires exist for psoriasis, the DLQI is still the most applicable in psoriasis testing. The use of this questionnaire has many advantages, such as validity, reliability, and a short time to fill it in. Besides the fact that it has been used in numerous studies in psoriasis, it is also used in other dermatological diseases, so we can easily compare QoL across all dermatoses [5].

The research underscores the necessity of adopting a comprehensive approach for psoriasis patients, as their QoL is hampered across various domains, prominently marked by elevated stress levels. Assessing psoriasis-related stress is pivotal due to its direct influence on QoL and symptom exacerbation. The Psoriasis Life Stress Inventory (PLSI) is of paramount significance in this context, as it stands as a disease-specific tool that accurately addresses stress stemming from skin alterations offering distinct advantages over generic questionnaires [6].

Although both instruments are often used together and do not measure the same parameters, the correlation between PASI and DLQI has often been investigated. Some studies show a moderate correlation between PASI and DLQI values, as anticipated, suggesting enhanced QoL with decreased disease severity [7–9]. Likewise, the amount of perceived stress measured by the PLSI questionnaire reflects a similar trend [6]. Conversely, some authors argue that there is no correlation between QoL and stress with objective measures. This perspective arises from the fact that psoriasis is a condition that systemically affects all aspects of life, regardless of clinical severity [10].

The aim of this research was to examine the association between the disease severity of psoriasis and patients' QoL before and after the treatment. As far as we are aware, no study has been published in our country that compares PASI with DLQI and PLSI results.

Methods

A cross-sectional study was conducted on 183 subjects diagnosed with moderate to severe psoriasis under real-world conditions to evaluate the correlation between the patient-

reported outcomes (DLQI and PLSI) and PASI score. The Ethics Committee of the University Clinical Center Kragujevac, Serbia approved this study (No. 01.21–375, from October 29, 2021), and it was conducted according to the Declaration of Helsinki ethical principles [11]. The patients had to have a signed voluntary consent form, a confirmed diagnosis of psoriasis, a PASI value of 10 or higher, and be treated with conventional systemic, phototherapy, and/or biological therapy for 16 weeks to participate in the study. All patient outcomes were tracked at the baseline visit and at the 16th week of treatment. G*Power 3.0.10 was used to estimate the sample size and study power. The minimum sample size for using a two-sided significance test with an alpha significance level of 0.05 and a study strength of at least 90% was 180, resulting in a study power of 91.8%. A sufficient number of respondents was obtained after 13 months of successive data collection from November 2021 to December 2022.

DLQI is a self-administered questionnaire used to assess QoL in dermatological patients. It is divided into domains that include symptoms and feelings, daily activities, leisure, school or work, personal relationships, and treatment effects. Answers are on a four-point scale that ranges from 0 to 3. The results are observed on a linear scale, with higher scores indicating more detrimental effects of the dermatological disease on QoL. Answers can be also obtained and summed across domains using the same method [12].

PLSI is used to measure the psychosocial stress that psoriasis sufferers experience in trying to cope with everyday life events. Chronic stress is classified into two categories: stress due to the appearance of skin changes and social stigmatization and stress due to the physical aspects of the disease as well as the administration of treatment. Patients answer whether any of the above-mentioned situations happened in the last month, and if so, how much the patient thinks that the given situation caused him stress. The total score is obtained by summing the answers from 15 questions (0–45). Test values greater than 10 indicate that given patients are exposed to psychosocial stress due to psoriasis [13].

PASI is a widely used instrument for determining the severity and distribution of lesions in patients with psoriasis. On the body, head, trunk, and lower and upper extremities individually, erythema, desquamation, and infiltration are graded from 0 to 4, and skin involvement by lesions is graded from 0 to 6. Higher scores imply more changes and involvement, with a maximum value of 72 [14].

Questions about socio-demographic information and the disease itself were included in the general questionnaire.

Depending on the data, all patient information is displayed and analyzed using the appropriate statistical methods. The mean and standard deviation were used to represent continuous variables, and the Chi-squared test was used to determine the frequency of categorical variables. The degree of linear relationship between PASI and DLQI was examined by correlation analysis. Statistical analysis was performed *via* SPSS for Windows, version 23.0 of the Statistical Package for the Social Sciences (IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics of the study population are shown in Table 1. In this study, 59% of the patients were males, whereas 41% were females. The average age of the patients was 47.5 ± 14.9 years.

Correlation analysis examined the relationship between the severity of the disease and QoL of patients with psoriasis and was performed for two time points of therapy application – baseline visit and 16th week of therapy. As shown in Figure 1, a moderately positive correlation was established in the 16th week ($r = 0.683$, $p < 0.001$) between the observed features. Patients with a higher PASI score, which indicates a more severe psoriasis form, also had a higher DLQI score value, i.e., a worse QoL. No statistically significant difference between these characteristics was found at the baseline visit ($r = 0.080$, $p = 0.284$).

When comparing PASI and PLSI values in the 16th week of therapy, a statistically significant strong positive correlation ($r = 0.702$, $p < 0.001$) between these characteristics was established. As in the case of the correlation between PASI and DLQI scores, in this case, as well, no statistically significant correlation was found at the baseline visit between PASI and PLSI ($r = 0.109$, $p = 0.140$) (Figure 2).

Table 1

Characteristics of the study population (n = 183)

Characteristics	Baseline	After 16 weeks
Age, years	47.5 ± 14.9	
Gender, n (%)		
male	108 (59)	
female	75 (41)	
PASI	23.14 ± 6.50	4.36 ± 4.86
DLQI	20.57 ± 5.83	6.20 ± 6.16
PLSI	35.37 ± 8.84	12.75 ± 12.82
Psoriasis onset age, years	34.5 ± 13.7	
Psoriasis duration, years	12.9 ± 10.3	

PASI – Psoriasis Area and Severity Index; DLQI – Dermatology Life Quality Index; PLSI – Psoriasis Life Stress Inventory.
All values are expressed as mean ± standard deviation or as numbers (percentages).

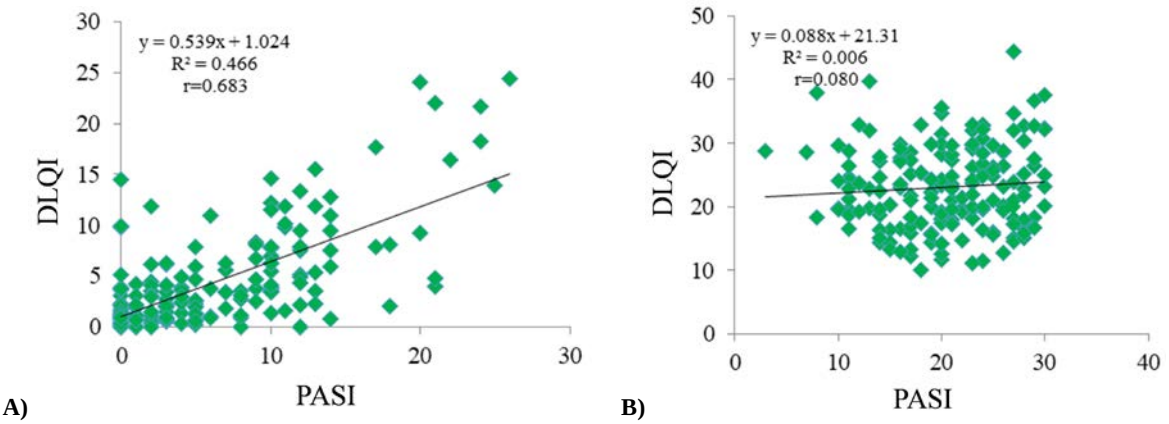

Fig. 1 – Correlation of PASI and DLQI score at baseline visit (A) and after 16 weeks of therapy (B). For abbreviations, see Table 1.

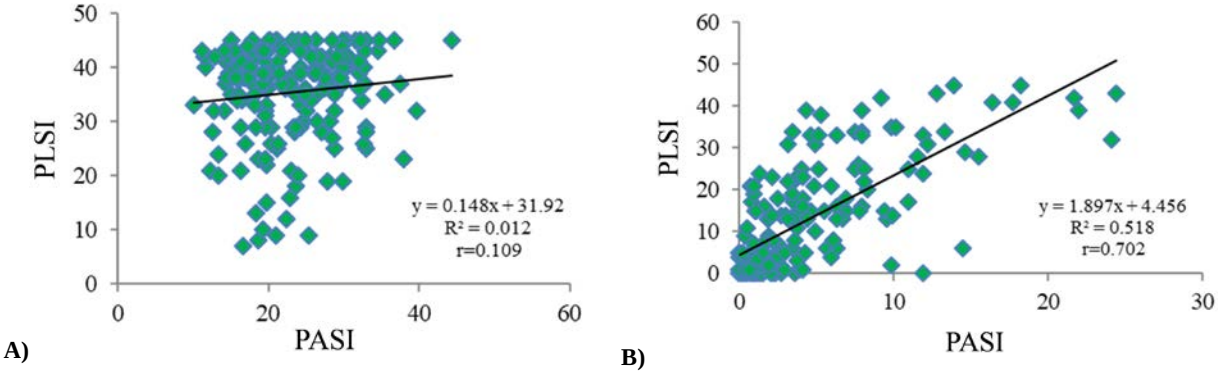

Fig. 2 – Correlation of PASI and PLSI score at baseline visit (A) and after 16 weeks of therapy (B). For abbreviations, see Table 1.

After the induction phase, in the 16th week of treatment, patient-reported outcomes were the following: 6.20 ± 6.16 for DLQI score, 12.75 ± 12.82 for PLSI, and 4.36 ± 4.86 for PASI (Table 1).

Discussion

This research aimed to analyze the relationship between psoriasis severity and patient's QoL before and after treatment, using patient-reported outcomes. Regardless of gender or age, patients with psoriasis frequently experience stigmatization, social maladjustment, and feelings of humiliation due to physical appearance, which contribute to everyday stress and impaired QoL [15]. Estimating health-related QoL is complicated because diagnostic assessments are often not precise enough to effectively convey the degree of psoriasis influence on an individual's life.

A study evaluating stress in psoriasis patients found that 66.26% experienced high stress related to the disease, with only 4.57% reporting low perceived stress, irrespective of disease severity [16]. Our data are in accordance with that research because the high mean values of experienced psychosocial stress were determined in presented patients with psoriasis. There were also great impairments found in the QoL of the examined population, which is in line with studies of a similar design, where the QoL was examined in patients with more severe forms of the disease [17, 18]. When reviewing studies with a design akin to ours, we identify parallel trends in study population characteristics. The mean age is 42.6 ± 2.9 years, and the average psoriasis duration closely mirrors our findings, standing at 12.5 ± 10.5 years, compared to our 12.9 ± 10.3 years [19].

In terms of correlation, studies demonstrate that PASI and DLQI have a moderate to high positive correlation after the induction phase of treatment in moderate to severe psoriasis. The authors emphasize that a decrease in the PASI score by 75% or more from the baseline visit plays a crucial role in this correlation. They also note that, as this percentage increases, the strength of the correlation grows [8]. Herédi et al. [20] report a highly positive correlation between PASI and DLQI (r = 0.81). Such data were confirmed in our study, where, after the 16th week, a strong positive correlation was shown. Considering that our research included patients with more severe forms of the disease and significantly impaired QoL, we assume that the therapy's high effectiveness led to substantial clearance and, consequently, notable QoL improvement. Similar data are presented by Lacour et al. [21] in a study that dealt with an estimation of the correlation between patient-reported and physician-reported outcomes in their work; the correlation between PASI and DLQI scores after

six months was assessed as highly correlated (r = 0.70). Numerous investigations reveal a moderate positive correlation between these attributes, yet it is worth noting that these studies were not restricted based on PASI values [7, 22]. Contrary to these data, individual studies show no statistically significant correlation (r = 0.172) between QoL and physical assessment methods [9]. For instance, Silva et al. [23] attributed the lack of correlation between PASI and DLQI at baseline and after the 16th week of treatment to factors such as the patient's low socioeconomic status, which can impact QoL regardless of disease presence and the chronic nature of the condition, suggesting a higher level of disease acceptance.

The individual's psychological well-being is linked to therapeutic success. Skin diseases, such as psoriasis, exacerbate or become a source of depression and stress, perpetuating a vicious circle; thus, further study and a deeper understanding of the skin-psychology connection are required [24]. In a study from Serbia where QoL was assessed, no statistically significant difference was found between PASI and PLSI scores (r = 0.119). However, it should be taken into account that their study did not consider this correlation at the beginning and end of the induction phase and that milder forms of psoriasis were included in the study [10]. This data can be elucidated by the inclusion criteria of our study, where only patients with a PASI value exceeding 10 were enrolled. Patients with challenging-to-treat psoriasis or those with lesions in conspicuous areas were excluded if their PASI score was below 10. For such patients, daily stress linked to treatment inefficacy, lesion location, and impaired QoL is expected [19], thus resulting in a weaker correlation. We posit that a substantial association emerges primarily when the condition is more severe and treatment proves effective.

Conclusion

In summary, our study sheds light on the intricate interplay between psoriasis severity and the well-being of individuals grappling with moderate to severe forms of the condition. The discernible reduction in QoL and the palpable psychosocial stress experienced by these patients underscore the multifaceted impact of psoriasis beyond its physical manifestations. We observed that amelioration in patients' QoL correlated moderately with objective reductions in psoriasis severity, while the mitigation of psychosocial stress demonstrated a strong relation to disease severity. The utilization of comprehensive indices, including patient-reported assessments, emerges as pivotal not only for clinical research but also for informing routine practice. Our findings advocate for a holistic approach to psoriasis management that encompasses both physical and psychological aspects, recognizing the need for a more nuanced evaluation of the disease's impact. As we move forward, understanding the intricate dynamics between these parameters will be essential in enhancing the quality of care provided to individuals navigating the challenges posed by psoriasis.

Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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The reliability of dental panoramic tomographs in determining the upper and lower third molar root morphology

Pouzdanost ortopantomograma u proceni morfologije korenova gornjih i donjih umnjaka

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Abstract

Background/Aim. The shortcomings of the orthopantomography (OPG) method and radiographic misinterpretations may lead to poor treatment planning and complications during or after the third molar extraction. The aim of this study was to determine the validity and reliability of OPG findings concerning post-extraction wisdom tooth root morphology, as well as whether the degree of clinical expertise affects assessment accuracy. **Methods.** The cross-sectional study included 200 patients who were referred for third molar extraction. Preoperative OPGs were evaluated by the examiners, who were classified by their level of experience into three groups: students, residents, and professors. True root morphologies were recorded after the extraction, and the accuracy of the assessment was evaluated using various statistical tests. **Results.** The majority of assessments were accurate for the lower and upper third molars with a single root. The professor group was the most accurate when compared to the assessments made by the students and residents (p = 0.0015). Weighted Cohen's kappa (κ_w) values for intra-respondent accuracy gradually increased from the student to professor group (0.06, 0.28, 0.34, respectively). The highest discrepancy in inter-respondent accuracy was determined between the student and professor groups (poor; κ_w = 0.25584). **Conclusion.** In this study, the results that confirm the reliability of the OPG scan for the detection of accurate third molars root morphology have not been achieved. The level of clinical experience affects diagnostic accuracy, but complex clinical cases should be evaluated using different methods.

Key words:
evaluation study; molar, third; radiography, panoramic; tooth root.

Apstrakt

Uvod/Cilj. Nedostaci ortopantomografske (OPT) metode, kao i neadekvatne interpretacije radiografskih snimaka mogu imati za posledicu pogrešno planiranje i komplikacije koje nastaju tokom ili nakon ekstrakcije umnjaka. Cilj rada bio je da se utvrdi validnost i pouzdanost OPT nalaza u proceni morfologije korenova umnjaka nakon ekstrakcije, kao i to da li stepen kliničkog iskustva utiče na preciznost procene. **Metode.** Studija preseka sprovedena je na 200 pacijenata kojima je bila indikovana ekstrakcija umnjaka. Preoperativna OPT procena sprovedena je od strane ispitivača koji su na osnovu nivoa iskustva bili svrstani u tri grupe: studente, specijalizante i profesore. Nakon ekstrakcije umnjaka beležena je morfologija njihovih korena, a preciznost procene izvršena je primenom različitih statističkih testova. **Rezultati.** Najviši procenat uspešnih procena utvrđen je za jednokorene gornje i donje umnjake. Postojala je statistički značajna razlika u pogledu uspešnosti procene profesora u odnosu na procenu studenata i specijalizanata (p = 0,0015). Vrednosti *weighted* Cohen's *kappa* (κ_w) su se postepeno uvećavale idući od grupe studenata ka grupi profesora (0,06, 0,28, 0,34, redom). Najveće razmimoilaženje odgovora postojalo je kada su se poredile grupa studenti i grupa profesori (slabo, κ_w = 0,25584). **Zaključak.** U ovoj studiji nisu pokazani rezultati koji potvrđuju pouzdanost OPT snimka za procenu tačne morfologije korena trećih molara. Nivo kliničkog iskustva utiče na uspešnost procene, ali kompleksnije kliničke slučajeve trebalo bi procenjivati različitim dijagnostičkim metodama.

Ključne reči:
procena, istraživanja; umnjaci; ortopantomografija; zub, koren.

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Introduction

The surgical removal of wisdom teeth is one of the most common oral surgery procedures [1–3]. Indications are numerous, and without disputing the importance of complications that may occur during or after the surgery, it should be emphasized that a well-planned intervention reduces the chances of their occurrence. In addition, well-designed preoperative planning may shorten surgery time and reduce postoperative trauma. Clinical examination supported by radiographic evaluation of tooth angulation, impaction type, and relation to the adjacent anatomical structures is the key factor for proper extraction planning [4–7]. However, the interpretation of X-rays should always be taken into consideration. There are wide variations in root numbers and shapes [8–10]. Those diversities reflect a frequent discrepancy between radiographic presentation and true root morphology (Figure 1 a–j).

Orthopantomography (OPG) is the primary radiographic method usually used in everyday surgical practice [11–16]. It is a two-dimensional image of the lower third of the face, along with teeth and temporomandibular joints. This extraoral tomographic technique provides a clear view of the upper and lower alveolar processes as structures that lie within the focal trough. Structures outside the focal plane are blurred or invisible [17]. Those shortcomings and the insufficiencies for fine anatomical/pathological details should not be neglected during the X-ray analysis [18]. Additionally, image distortion, magnification, and the superimposition of different structures may mislead the clinician [19].

Radiographic misinterpretation might not only be the result of OPG deficiencies but it may also be related to the examiner's experience. Although knowledge is required, clinical practice and training are recommended for proper radiographic judgment [15, 20]. Superimposition, fused or

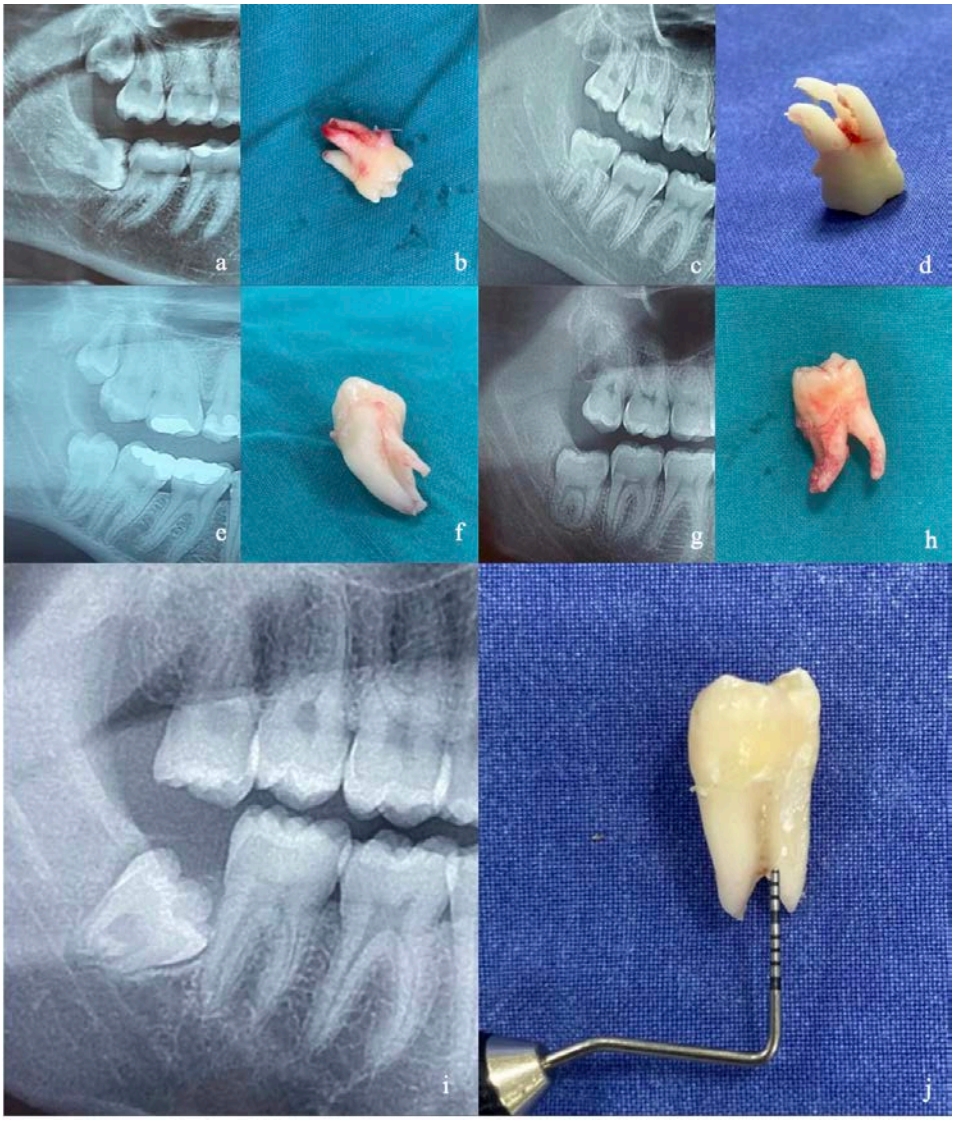

Fig. 1 – The discrepancy between radiographic presentation and true root morphology. Preoperative orthopantomography of lower third molar (a, e) and upper third molar (c, g, i). True morphology of lower third molar (b, f) and upper third molar (d, h, j). Demonstration of incomplete fusion > 3 mm which was considered as separate roots (j).

accessory roots, and dilacerations are burdening factors even for well-trained clinicians and often require additional radiographic methods.

Therefore, the primary aim of this study was to determine the validity and reliability of OPG findings concerning post-extraction wisdom tooth root morphology. Additionally, we wanted to determine whether the degree of clinical expertise affects assessment accuracy.

Methods

The cross-sectional study was carried out at the University of Belgrade, Faculty of Dental Medicine, Clinic for Oral Surgery, Serbia, from October 2021 to October 2022, in concordance with the Helsinki Declaration and with the approval of the local Ethics Committee.

Inclusion and exclusion criteria (participants/examiners)

A total of 265 adult patients were assessed for the study, and 200 met the following inclusion criteria: patients with an indication for the extraction of impacted, semi-impacted, or erupted wisdom teeth; preoperative OPG performed at least three months before the tooth extraction; adult patients over 18 years of age, in good physical and mental condition [American Society of Anesthesiologists (ASA) I classification].

The exclusion criteria were the following: unfinished root formation or the presence of the associated root/tooth pathology; patients with poor oral hygiene; pregnant or breastfeeding women; smokers and drug addicts.

Patients who met the inclusion criteria were informed about the procedure, required radiographic analysis, and utilization of their OPG images for the research. Written consent for participation was obtained from all included patients.

The examiners involved in the study were classified into three respondent groups: student group (20 fourth-year dentistry students that have passed the Radiology exam), resident group (10 residents from the Oral Surgery Department), and professor group (2 full-time professors from the Oral Surgery Department).

The examiners were randomly given an even number of OPGs for the evaluation (10 OPGs per student, 20 OPGs per resident, and 100 OPGs per professor). The randomization process was performed using a table of random numbers for three groups of respondents using an online program [21].

Outcomes

The primary outcome variables were the assessed OPG root number (aOPGrn) and the true post-extraction root number (TRN). The gender and age of the patient and the tooth scheduled for extraction were recorded in the study chart. Each examiner performed a radiographic evaluation separately, and the assumed OPG findings regarding the wisdom tooth root number were recorded. On the day of surgery, several weeks later, after the tooth extraction, the actual number of roots was again recorded by a separate investigator blinded for the examiners' assessments. Fused roots were counted as a single root, and in the case of incomplete fusion, when the roots were more than 3 mm long after the furcation, they were counted individually.

Statistical methods

Data were analyzed using a commercially available software program (SPSS 22.0, IBM Corp., Armonk, NY, USA). Data were summarized by intervention group (*per* protocol analysis). Parameters presented by continuous variables were described using measures of central tendency (mean, median) and dispersion (standard deviation, minimum, maximum). For categorical variables, the frequency and percent in each category were presented and analyzed with a Chi-squared test (χ^2). Inter-rater (Cohen's weighted kappa – κ_w) statistics were done to evaluate the total agreement between the two methods (medcalc ver. 20.104). Sensitivity and specificity tests were utilized to determine the predictive validity of radiographic interpretation.

The sample size and power of the study were calculated in the G*power program (ver. 3.1.9.4. Germany). There is a 95% chance of correctly rejecting the null hypothesis of no difference between expected and observed proportions with 148 participants ($\alpha = 0.01$, dz = 0.37, Df = 2). The *post-hoc* achieved power was 98.8% for 200 participants [difference between two frequencies (Goodness-of-fit tests: Contingency tables), $\alpha = 0.05$, Df = 2]. The level of significance was set at 0.05.

Results

The study included 200 patients, 95 (47.5%) of whom were men and 105 (52.5%) were women. The patients' ages ranged from 17 to 28 years (20.99 ± 2.51 on average). For the male patients, the average was 21.41 ± 2.50 years and 20.60 ± 2.48 years for females.

Out of the 200 extracted third molars, 64 had a single root, 109 had two roots, 22 had three, and just 5 had four roots (Table 1).

Table 1

Wisdom tooth root number distribution

Third molars	One root	Two roots	Three roots	Four roots	Total
Upper	49	33	15	3	100
Lower	15	76	7	2	100
Total	64 (32)	109 (54.5)	22 (11)	5 (2.5)	200 (100)

All values are expressed as numbers (percentages).

When comparing the upper and lower wisdom teeth, the frequency of the correct assessments varied. The incidence of improper OPG interpretation increased with the root number (Table 2).

Sensitivity and specificity tests describing the accuracy of the correct assessment for each respondent group are presented in Table 3. A statistically significant difference in correct assessment of OPG relating to the TRN was found in the student group for two- and three-rooted third molars (p = 0.001, p = 0.000, respectively), as well as for the two-rooted molars in the resident group (p = 0.009).

Among the 200 teeth, 65 (32.5%) were evaluated radiographically correctly by all three respondents. In 43.5% of cases, the estimation was discordant (two respondents had the same correct assessment, but the third was incorrect). All three respondent groups failed to evaluate correctly 48 (24%) teeth.

The compliance between aOPGrn and wisdom tooth TRN for all three respondent groups is presented in Table 4. The professors' respondent group had the highest percentage (63%) of correct OPG assessments, while the students' group had the lowest (46.5%) performance. There were statistically significant differences between the groups' correct answers (p = 0.0015).

The respondents' reliability in making a correct OPG assessment was measured through intra-respondent accuracy (Table 5). There was a gradual increase in κ_w values from the first to the third group (0.06, 0.28, and 0.34, respectively). The students' reliability was accessed as poor (κ_w < 0.20), while the residents' and professors' reliability were accessed as fair (κ_w between 0.21 and 0.40). Inter-respondents' concordance in OPG assessments demonstrated the highest discrepancy between the student and professor groups (poor; κ_w = 0.25584).

Discussion

The OPG is not a reliable radiographic method for third molar root assessment [1]. Due to the lack of a third dimension on OPG, there are many mismatches between radiographic assessments and the true wisdom tooth root morphology. However, the level of clinical expertise and OPG interpretation experience affects assessment accuracy.

Table 2

The frequency of the correctly assessed orthopantomography root number

Third molars	One root	Two roots	Three roots	Four roots
Upper	43.5	35.3	42.2	22.2
Lower	51.1	40.4	19	0

All values are expressed as percentages.

Table 3

Sensitivity and specificity tests

Root number	Student group		Resident group		Professor group	
	sensitivity	specificity	sensitivity	specificity	sensitivity	specificity
One	45.3	52.9	56.3	37.5	68.8	39.7
Two	57.8*	67.0*	68.8‡	49.5‡	63.3	37.4
Three	4.5†	48.3†	40.9	37.1	54.5	36.0
Four	0.0	52.3	20.0	38.5	20	35.9

Statistically significant differences: *p = 0.001; †p = 0.000; ‡p = 0.009.
All values are expressed as percentages.

Table 4

The compliance between aOPGrn and TRN for the three respondent groups

Parameter	Student group	Resident group	Professor group	Total
True	93 (46.5)	121 (60.5)	126 (63.0)	340 (56.7)
False	107 (53.5)	79 (39.5)	74 (37.0)	260 (43.3)
Total	200 (100)	200 (100)	200 (100)	600 (100)

aOPGrn – assessed orthopantomography root number; TRN – true post-extraction root number.
All values are expressed as numbers (percentages).

Table 5

Intra-respondents' accuracy in orthopantomography assessment and inter-respondent concordance

Parameter	Intra-respondents accuracy			Inter-respondents compliance		
	student group	resident group	professor group	students/residents	residents/professors	students/professors
κ_w values	0.06	0.28	0.34	0.41	0.41	0.25

κ_w – weighted kappa. κw and strength of agreements: < 0.20 – poor; 0.21–0.40 – fair; 0.41–0.60 – moderate; 0.61–0.80 – good; 0.81–1.00 – very good.

In permanent dentition, the upper and lower third molars are the teeth with the widest range of morphological variations [12]. In our study sample, similar to the studies of Bell et al. [1], Zhang et al. [9], and Tomaszewska et al. [10], the majority (49%) of upper ones had a single root, compared to the lower ones that were mostly (76%) two-rooted. With the root numbers increasing, there was a higher chance of OPG misinterpretation, which came to attention, especially in multirooted lower third molars. They usually had mesial and distal roots and were easily recognized as two-rooted teeth. However, in the cases when they had three or four roots, they were correctly recognized in only 19% and 0%, respectively. Upper third molars, on the other hand, were typically identified as single or three-rooted teeth.

The present study discovered differences in sensitivity among participating respondent groups. Sensitivity was significantly higher for the assessment of two-rooted teeth within the resident and professor groups. That could be due to the prevalence of third molar root morphology and the ease of precise identification on OPG images. On the contrary, the student group demonstrated significantly lower sensitivity when assessing three-rooted and four-rooted teeth. We assumed that the main reason for that was inexperience and that the complex root morphology required a refined manner of OPG observation and interpretation. Moreover, experienced observers probably rely on previous observations, making it easier to predict the true root morphology.

In everyday practice, dentists mostly interpret X-rays by themselves, and it is assumed that the precision of OPG assessment directly depends on years of clinical experience and expertise [15]. When observing different respondent categories, we found that the professor group had the highest (63%) percentage of correct answers, followed by the residents (60.5%) and the students (46.5%). One of the reasons might come from the fact that those who extracted a lot of wisdom teeth experienced accessory root fractures, different failures, and complications during the extractions and developed practical skills and experience for improved radiographic evaluation. With time, they adopt an explicit vision and become sensitive to details that are overlooked by the less experienced doctors. They incorporate acquired knowledge and expect the worst from every wisdom tooth extraction. For that kind of clinician, it is of essential value to determine, for instance, if the upper wisdom tooth is three-rooted or if the lower one has two mesial roots. Unexperienced students are not aware of those anatomical varieties and have not developed skills for radiographic detail recognition. Because of that, in this study, they were evaluated as having poor diagnostic accuracy ($\kappa_w = 0.06$).

Inter-respondent compliance in the assessment among the groups was consistent. Although the different κ_w values supported the idea of clinical experience importance, the professors/residents group ($\kappa_w = 0.41$) and the professors/students group ($\kappa_w = 0.25$) had a fair assessment match. In other words, the greater the experience, the fewer the discrepancies in the OPG interpretations among observer groups. Those findings are supported by the study of Richter et al. [15], who stated a strong relationship between the number of images read and diagnostic accuracy.

All the patients included in the study had preoperative OPG, although not all images were taken at the same radiology center. That could imply that the quality of the OPGs was not the same for all the patients. Additionally, frequent eccentric tooth positions, root dilacerations, fusions, and number variations were the contributing factors to low diagnostic reliability [1]. Nevertheless, the overall number of false assessments was similar to those described in the literature. Even for the experts, the error rates may vary from 19% to 41% [15, 20]. After all, it is not easy to perform a correct OPG evaluation, and misinterpretations may occur even with trained eyes. That is one of the reasons why clinicians must always be aware and cautious. Whenever in doubt, whenever the root anatomy is not easily recognized and may differ from the one presented on the OPG, the clinician should consider additional radiographic methods for precise assessment. In conditions where the wisdom tooth is deeply impacted close to the alveolar inferior bundle, close to the maxillary sinus, or when other pathologies are present, indications for the cone beam computer tomography (CBCT) radiographic method should be considered [16]. CBCT is the best and most accurate method for wisdom tooth root assessment. However, the radiation exposure is much higher than OPG, so the indication for CBCT has to be reserved for the designated conditions [5]. The surgeons should aspire to minimize the occurrence of complications during or after third molar extraction by approaching every case individually and making decisions based on a thorough clinical and radiographic examination. They should be able to recognize complex morphology cases requiring detailed radiographic analysis, which exceeds the capabilities of OPG images.

Conclusion

In the majority of cases, the reliability of the OPG method for the evaluation of wisdom tooth root number and morphology is insufficient. The level of clinical experience and expertise affects diagnostic accuracy, but complex clinical cases should be evaluated by different, more accurate methods, i.e., CBCT images.

Conflicts of interest

The authors declare no conflict of interest.

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LETTER TO THE EDITOR
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Using body mass index for the estimation of the nutritional status of school children – are international standards good enough?

Korišćenje indeksa telesne mase za procenu uhranjenosti školske dece – da li su međunarodni standardi dovoljno dobri?

To the Editor:

Most scientific studies agree that the simplest method for large population studies of the nutritional status of children is to directly measure body mass and body height and calculate body mass index (BMI), out of which nutritional status is estimated using either local (national) norms or international criteria. The most commonly used international norms are those from the International Obesity Task Force (IOTF) [1], the Centers for Disease Control and Prevention (CDC) [2] criteria, and the World Health Organization (WHO) [3] standards. As we do not have national norms in Serbia, there is a dilemma over which of the given norms would be more appropriate to use in our population. Therefore, our objective was to evaluate the differences between the three international norms and find out whether there is a systematic error that can affect the assessment of the nutritional status of school children.

This epidemiological cross-sectional study was approved by the Ethics Committee of the Faculty of Medicine in Belgrade (No. 2650/IV-11, from April 10, 2018). The measurements were carried out from January to June 2018 at the Institute of Medical Physiology "Richard Burjan" of the Faculty of Medicine, University of Belgrade, and the Sports Medicine "Malićević" in Belgrade, as well as within fieldwork in 17 primary schools in each city municipality of Belgrade, Serbia. After receiving information about the research details, written informed consent was given for each subject by one of the parents/guardians for the data to be used in this study. In summary, 7,880 children aged 9–15 years were included in this large study (6.16% of the total of 127,811 children in the Belgrade region).

BMI was calculated out of body mass and body height and expressed in kilograms *per* square meter (kg/m^2). The assessment of the nutritional level from the BMI value was carried out according to the criteria of IOTF [1], WHO [3], and CDC [2].

In our study, we have found the following: according to the CDC [2] definition, the BMI value defines the lowest cases of malnutrition; the WHO [3] criteria from the BMI value defines the fewest children with normal nutritional status; the

Table 1

The prevalence of classes of nutritional status according to different definitions in children aged 9–15 years

Nutritional status	IOTF [1]	CDC [2]	WHO [3]
Girls			
malnutrition	297 (7.7)	121 (3.1)	374 (9.7)
normal	2,473 (64.2)	2,647 (68.7)	2,206 (57.2)
pre-obesity	835 (21.7)	671 (17.4)	733 (19.0)
obesity	249 (6.5)	415 (10.8)	541 (14.0)
total	3,854 (100.0)	3,854 (100.0)	3,854 (100.0)
Boys			
malnutrition	244 (6.1)	149 (3.7)	342 (8.5)
normal	2,357 (58.5)	2,334 (58.0)	1,870 (46.4)
pre-obesity	1,035 (25.7)	801 (19.9)	832 (20.7)
obesity	390 (9.7)	742 (18.4)	982 (24.4)
total	4,026 (100.0)	4,026 (100.0)	4,026 (100.0)
Total children			
malnutrition	541 (6.9)	270 (3.4)	716 (9.1)
normal	4,830 (61.3)	4,981 (63.2)	4,076 (51.7)
pre-obesity	1,870 (23.7)	1,472 (18.7)	1,565 (19.9)
obesity	639 (8.1)	1,157 (14.7)	1,523 (19.3)
total	7,880 (100.0)	7,880 (100.0)	7,880 (100.0)

IOTF – International Obesity Task Force; CDC – Centers for Disease Control and Prevention; WHO – World Health Organization. All values are expressed as numbers (percentages).

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Table 2

Head-to-head comparison of international standards for defining nutritional status from body mass index values of children

Parameter	WHO [3]				
	malnutrition	normal	pre-obesity	obesity	total
CDC [2]					
malnutrition	100.0	0.0	0.0	0.0	100.0
normal	9.0	81.8	9.2	0.0	100.0
pre-obesity	0.0	0.0	75.1	24.9	100.0
obesity	0.0	0.0	0.0	100.0	100.0
	χ^2 = 12.833 (n = 7,880; *df* = 9), $p < 0.0001$; κ = 0.733; V = 0.737				
IOTF [1]					
malnutrition	100.0	0.0	0.0	0.0	100.0
normal	3.6	84.4	12.0	0.0	100.0
pre-obesity	0.0	0.0	52.7	47.3	100.0
obesity	0.0	0.0	0.0	100.0	100.0
	χ^2 = 13.229 (n = 7,880; *df* = 9), $p < 0.0001$; κ = 0.661; V = 0.748				
	CDC [2]				
	malnutrition	normal	pre-obesity	obesity	total
IOTF [1]					
malnutrition	49.9	50.1	0.0	0.0	100.0
normal	0.0	97.4	2.6	0.0	100.0
pre-obesity	0.0	0.3	72.0	27.7	100.0
obesity	0.0	0.0	0.0	100.0	100.0
	χ^2 = 14.048 (n = 7,880; *df* = 9), $p < 0.0001$; κ = 0.789; V = 0.771				

χ^2 – chi-squared; κ – coefficient of agreement between definitions of nutritional status; V – Cramer's V coefficient of degree of association; *df* – degree of freedom; n – total number of participants. For the abbreviations of other terms, see Table 1. All values are expressed as percentages.

application of the IOTF [1] criteria defines the highest prevalence of pre-obesity and the fewest children with obesity (Table 1).

Statistically significant differences were found between the results obtained using all three definitions ($p < 0.0001$). On the other hand, that also confirmed a very good agreement between different definitions of nutritional status, the largest being between the definitions of CDC and IOTF (κ = 0.789) and the smallest between WHO and IOTF (κ = 0.661); the degree of association was high (Cramer's V coefficient 0.737–0.771) (Table 2).

Although there were high levels of agreement and association, we have found significant differences in our study between the three standards: nearly a quarter of the subjects classified as obese by the WHO definition were classified into the pre-obese category by the CDC criteria; nearly half of the subjects classified as obese according to the WHO criteria were classified into the pre-obesity category by the IOTF definition; half of the subjects classified as normal by the CDC were classified into the malnutrition category according to the IOTF criteria, while nearly 28% of the subjects classified as obese by the CDC were classified into the pre-obese category by the IOTF definition.

That fully agrees with the findings of numerous international [4–6] and national studies conducted around the world: the United Kingdom [7], Portugal [8], India [9], Chile [10], Canada [11], Ireland [12], Argentina [13], and France [14].

As there are obvious and significant differences between international standards, developing and using national norms is recommended, which is our main conclusion.

Conflict of interest

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

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CASE REPORTS

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Myocarditis as the first manifestation of eosinophilic granulomatosis with polyangiitis

Miokarditis kao prva manifestacija eozinofilne granulomatoze sa poliangiitisom

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Abstract

Introduction. Myocarditis is not a rare diagnosis, but its etiology often remains unknown as it requires extensive diagnostic work. Eosinophilic granulomatosis with polyangiitis (EGPA) or Churg-Strauss syndrome is a very rare systemic disease that is not easy to diagnose. Myocarditis in EGPA is uncommon and usually occurs in the late stages of the disease. **Case report.** A 22-year-old man was admitted with acute coronary syndrome. Using coronary angiography, the presence of stenoses on the epicardial coronary arteries was ruled out, and a working diagnosis of myocardial infarction with non-obstructive coronary arteries (MINOCA) was established. Then, we found inflammatory syndrome, eosinophilia, and a lot of systemic symptoms and signs. The diagnostic work included extensive laboratory tests, which ruled out infectious agents. Then, immunological tests, a computed tomography scan of the chest, cardiac magnetic resonance imaging (MRI) and a biopsy of the bone marrow, nasal mucosa, and skin were performed. We managed to establish the diagnosis of myopericarditis by cardiac MRI. The cause of myocarditis – EGPA, was found only after the histopathological finding of the skin biopsy, which enabled adequate immunosuppressive therapy. **Conclusion.** The accurate diagnosis was crucial for the correct, causal treatment of the patient, especially because he needed life-long immunosuppressive therapy. In order for such complex patients to receive adequate treatment, a multidisciplinary approach and perseverance in the diagnostic evaluation of the etiology of myocarditis are necessary.

Key words:
diagnosis; histological techniques; myocarditis; eosinophilia; churg-strauss syndrome.

Apstrakt

Uvod. Miokarditis nije retka dijagnoza, ali njegova etiologija često ostaje nepoznata, jer zahteva obiman dijagnostički rad. Eozinofilna granulomatoza sa poliangiitisom (EGPA) ili Churg-Strauss-ov sindrom je vrlo retka sistemska bolest, čiju dijagnozu nije lako postaviti. Miokarditis u EGPA nije čest i obično se javlja u kasnim stadijumima bolesti. **Prikaz bolesnika.** Muškarac, starosti 22 godine, primljen je pod kliničkom slikom akutnog koronarnog sindroma. Koronarnom angiografijom isključeno je prisustvo stenoza na epikardnim koronarnim arterijama i postavljena je radna dijagnoza infarkta miokarda bez opstrukcije koronarnih arterija (*myocardial infarction with non-obstructive coronary arteries* – MINOCA). Potom su utvrđeni inflamatorni sindrom, eozinofilija i mnogobrojni simptomi i znaci sistemske bolesti. Dijagnostički rad uključio je obimna laboratorijska ispitivanja, kojima su isključeni infektivni agensi kao uzročnici. Zatim su urađena imunološka ispitivanja, kompjuterizovana tomografija grudnog koša, magnetna rezonanca (MR) srca i biopsija koštane srži, nosne sluznice i kože. Postavljena je dijagnoza mioperikarditisa, koja je potvrđena pomoću MR srca. Uzrok miokarditis – EGPA, je utvrđen tek nakon patohistološkog nalaza biopsije kože, što je omogućilo adekvatnu imunosupresivnu terapiju. **Zaključak.** Precizna dijagnoza bila je presudna za ispravno – kauzalno lečenje bolesnika, posebno zbog toga što mu je potrebna doživotna imunosupresivna terapija. Kako bi ovako kompleksni bolesnici dobili adekvatnu terapiju, neophodan je multidisciplinarni pristup i istrajnost u dijagnostici etiologije miokarditisa.

Ključne reči:
dijagnoza; histološke tehnike; miokarditis; eozinofilija; angiitis, granulomatozni.

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Introduction

Myocarditis is a common diagnosis, but its etiology often remains unknown. Eosinophilic granulomatosis with polyangiitis (EGPA) or Churg-Strauss syndrome is a very rare disease, a sporadic vasculitis described in 1951 [1]. It involves small and medium arteries and veins and is defined as eosinophil-rich and granulomatous inflammation involving the respiratory tract, combined with necrotizing vasculitis of small and medium vessels associated with asthma and eosinophilia [2]. The diagnosis is based on the presence of four or more criteria according to the American College of Rheumatology (ACR): asthma, eosinophilia > 10% in peripheral blood, paranasal sinusitis, transient pulmonary infiltrates, histological evidence of vasculitis with extravascular eosinophils and mononeuritis multiplex or polyneuropathy. When four or more criteria are present, the sensitivity for the diagnosis is 85%, and the specificity is 99.7% [3].

Myocarditis in EGPA is not common and usually occurs in the late stages of the disease [4].

We presented a young man with unusual myocarditis caused by a very rare systemic disease. The diagnosis required extensive multidisciplinary work, enabling adequate treatment and a good outcome.

Case report

A 22-year-old man was presented to the local hospital with a four-day history of intermittent chest pain in the form of tightness. On the day of admission, the pain lasted for one hour continuously. The electrocardiographic (ECG) finding showed QS in V1–V3 and negative T wave in V4–V6. The level of troponin (Tn) was 4,273.8 ng/L, 215 times above the upper limit (< 19.8 ng/L). It was interpreted as an acute coronary syndrome (ACS), and coronary angiography was performed. However, no narrowing of the coronary arteries was found. The working diagnosis of myocardial infarction with non-obstructive coronary arteries (MINOCA) was established. Complete laboratory tests were performed after the admission to the hospital, and their results showed some abnormalities: sedimentation rate 115 [normal values (NV) < 20], C-reactive protein (CRP) 89 mg/L [reference range (RR) 0.0–5.0), leukocytes (Le) 20.7 × 10^9/L (RR 4.0–9.0), eosinophils (Eo) 49.3% (RR 0.0–6.0), aspartate aminotransferase 51 U/L (RR 10–31), lactate dehydrogenase 1,020 U/L (RR 220–450), brain natriuretic peptide (BNP) 555.1 pg/mL (NV < 100). The rest of the evaluated parameters were within normal limits. Echocardiography showed no regional wall motion abnormalities, a left ventricle (LV) ejection fraction (EF) of 47%, and pericardial effusion 4–8 mm behind the posterior wall. A working diagnosis of perimyocarditis of unknown cause was made. Due to the pronounced inflammatory syndrome and high eosinophilia, an infectious disease specialist was consulted. The infectiologist prescribed the antiparasitic drug albendazole and asked for testing for trichinella and cysticercosis. A detailed history revealed that the patient runs a dog kennel, and his father is a hunter, so they often eat game meat. He had been treating asthma for a year and a half prior to admission.

In the following days, the patient became febrile in the late afternoon, reaching a temperature of 38.2 °C, followed by the appearance of vesicles on the skin of both hands and the left foot. The dermatologist characterizes the skin changes as dyshidrotic papules and hemorrhagic vesicles and prescribes an ointment containing an antibiotic, antifungal and corticosteroid, and antihistamine tablets. After the appearance of skin changes, he gives information about intermittent temperatures, weakness, and muscle pain for the past month. As there was no improvement (high value of Tn, associated with weakness and feverishness), and for further diagnostic work (cause of myocarditis, other diseases), the patient was referred from the local hospital to the University Clinical Center, more precisely, the Cardiology Clinic.

On admission, the patient was hemodynamically stable (blood pressure 130/90 mmHg, heart rate 95/min, and oxygen saturation 96%). The ECG was the same as described nine days earlier (Figure 1).

Changes according to the type of vasculitis were present on the palms and soles. On a heart ultrasound, we found the following: LV contractility at the lower normal limit (EF 50%), LV dimensions were normal (53/33 mm), walls were hypertrophic (septum and posterior wall 12 mm), the left atrium was enlarged (volume 78 mL), ratio of velocity E and A waves of mitral in-flow (E/A) was 2.07, and minimal separation of the pericardial sheets was found. In the laboratory tests, TnI was 1.113 ng/mL (upper limit

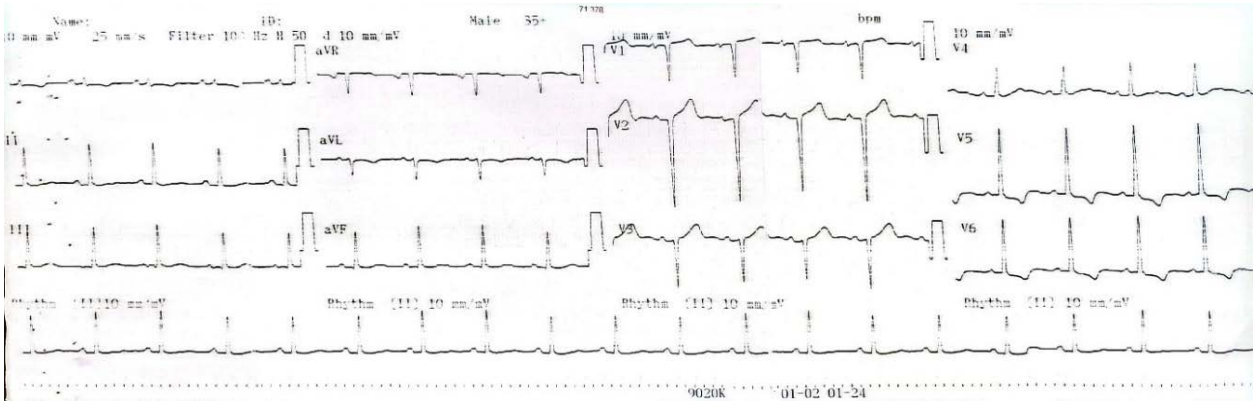

Fig. 1 – Electrocardiographic finding on admission: QS in V1–V3 and negative T wave in D1, aVL, V4–V6.

0.04 ng/mL) – 28 times above the upper limit, BNP 634.6 pg/mL, CRP 65.2 mg/L, Le 23.3 × 10^9/L (RR 4.0–9.0), Eo 53.4%. A multidisciplinary team consisting of an infectious disease specialist, a hematologist, an immunologist, and a pulmonologist was consulted. A chest computed tomography scan revealed mediastinal and hilar lymphadenomegaly (Figure 2).

A cardiac magnetic resonance imaging (MRI) was performed, and the diagnosis of myopericarditis was confirmed (Figures 3 and 4). Ten minutes after the

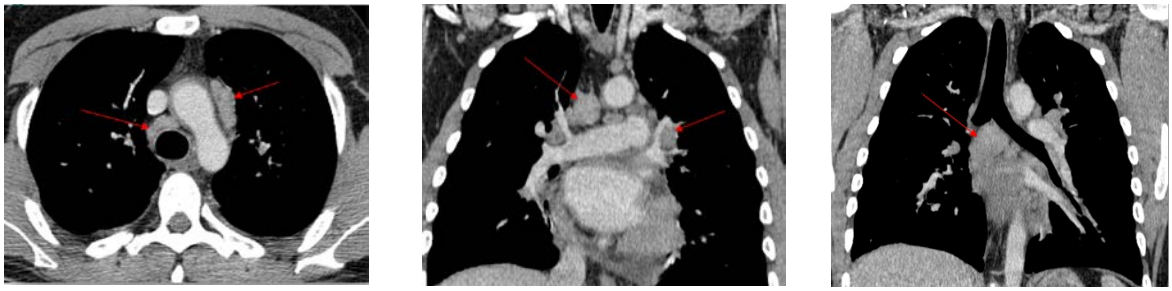

Fig. 2 – Computed tomography scan of the chest: enlarged lymph nodes in mediastinum (red arrows).

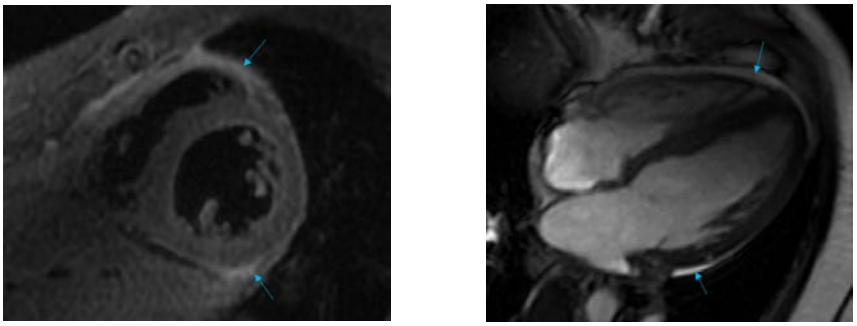

Fig. 3 - Cardiac magnetic resonance imaging, T2-weighted black blood triple inversion recovery sequence, short-axis view: signal of the myocardium is normal, no signs of edema. Pericardial effusion - hypersignal area (arrows, left picture). Cine bright blood sequence, four-chamber view; pericardial effusion – hypersignal area (arrows, right picture).

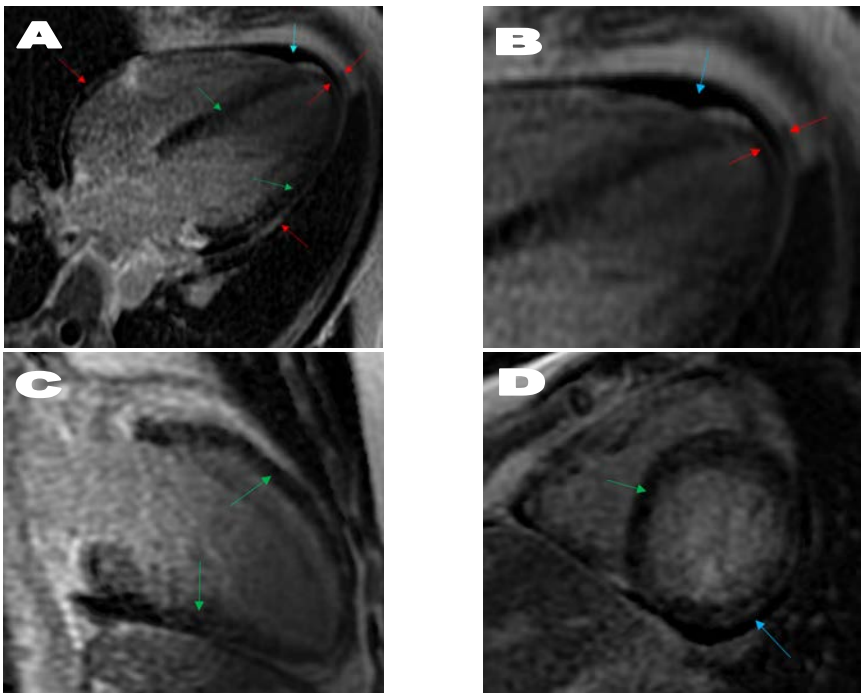

Fig. 4 – Cardiac magnetic resonance imaging, phase-sensitive inversion recovery (PSIR), four-chamber view (A, B), two-chamber view (C), short-axis view (D); to perform this sequence we choose inversion time to achieve appropriate nulling of the normal myocardium to accentuate late gadolinium enhance (LGE). Normal myocardium must be dark. In this case, that was not possible and we can conclude that LGE is diffuse heterogeneous transmural (green arrows). Pericardial effusion is hyposignal on PSIR (blue arrows). LGE of thickened pericardium is hypersignal (red arrows).

application of contrast, diffuse heterogeneous late gadolinium enhancement (LGE) of myocardium was found, which is the less common staining pattern in eosinophilic myocarditis. More common is subendocardial LGE, but cardiac MRI was performed more than three weeks after the symptoms began. A small pericardial effusion and signs of focal pericarditis can also be seen.

We have ruled out infection with parasites, protozoa, bacteria, viruses, and fungi with microbiological and immunological tests. Only Aspergillus immunoglobulin (Ig) M and IgG antibodies were positive. Additional immunological analyzes were performed: IgG 22.8 g/L (RR 7–16), IgE 818 IU/mL (NV < 100), IgA and IgM normal, complement (C) 3 2.09 g/L (RR 0.9–1.8), C4 0.476 g/L (RR 0.100–0.400); antinuclear antibodies (ANA), anti double stranded (ds) DNA antibodies, Sjögren's syndrome antibodies [anti-Ro (SS-A) and anti-La (SS-B)] and anti-Smith (Sm) antibodies within normal limits; cytoplasmic antineutrophil cytoplasmic antibody (ANCA) and perinuclear ANCA negative.

In addition, the patient gave information that he had frequent infections of the upper respiratory tract and that he had rhinosinusitis four months ago. At that time, an endocranium MRI was performed, confirming the existence of chronic pansinusitis.

The first working diagnosis was ACS; the second was MINOCA, then perimyocarditis and heart failure, and then parasitosis or some other infectious agent causing hypereosinophilia with myocardial involvement. The hematologist suspected T lymphoma of the mediastinum and asked for a biopsy of the mediastinal lymph glands. The pulmonologist suspected Churg-Strauss syndrome and indicated further investigation in that direction, but only after ruling out malignant diseases. Therefore, we referred the patient to the Institute for Pulmonary Diseases, where a biopsy of the bone marrow and nasal mucosa was performed. No pathological substrate was found, except for increased Eo with hypolobulation of the nucleus and cell enlargement in bone marrow histopathological (HP) findings. An electromioneurography was performed, which does not meet the electrodiagnostic criteria for polyneuropathy. We have noticed changes – papules on his right hand, and hence, an excisional skin biopsy was performed from that site (Figures 5 and 6). The HP finding indicated EGPA, formerly known as Churg-Strauss syndrome: necrotic vasculitis with eosinophilic granulomas present in the dermis (Figure 7).

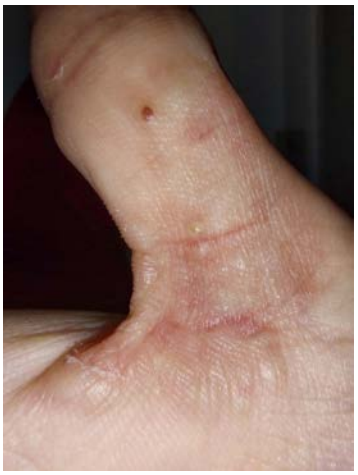

Fig. 5 – Papules on the right thumb.

Fig. 6 – The thumb after biopsy.

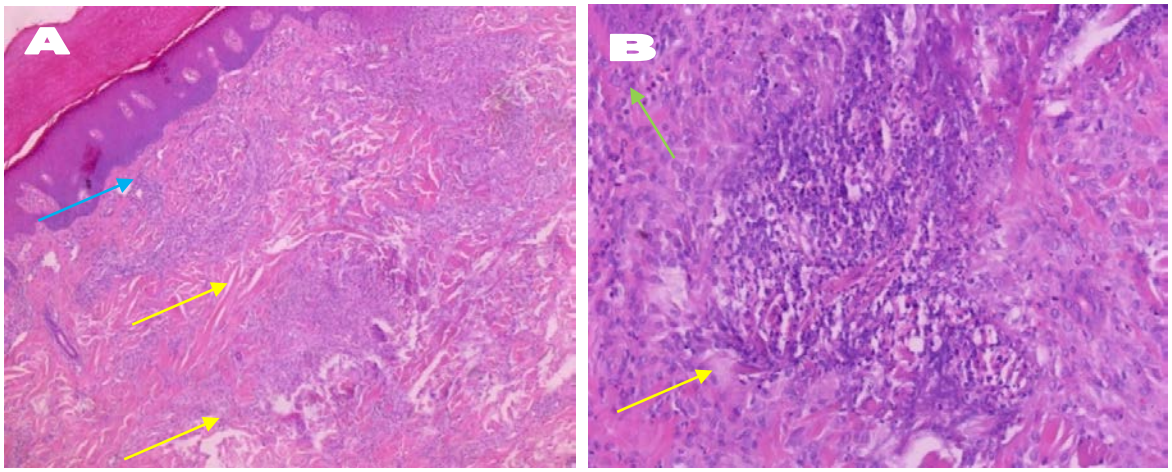

Fig. 7 – Histopathological finding of the dermis, hematoxylin-eosin staining: A) Necrotic vasculitis with eosinophilic granulomas (yellow arrows) in the dermis: Inside the dermis small blood vessels are affected by vasculitis (blue arrow) and granulomatous formations around necrotic foci (blue arrow) with lymphocytic and numerous eosinophilic infiltrate (green arrow) (×50); B) Eosinophilic granuloma (yellow arrows), lymphocytic and eosinophilic infiltrate (green arrow) (×200).

The patient's diagnosis of EPGA was confirmed by HP examination, and treatment with prednisone 1 mg/kg of body weight was started. Very quickly, there was an improvement. The disappearance of skin changes was noticed, new ones did not appear, CRP decreased to the RR, Le and Eo fell, and BNP decreased. The prednisone dose was gradually reduced until it was at the maintenance dose of 20 mg a day, which he receives to this day, six months after the onset of the disease. The patient feels good, and the disease is under control. EF was slightly improved (55%).

Discussion

Considering that our patient had pronounced eosinophilia and inflammatory syndrome dominated within laboratory tests, we considered the differential diagnosis of eosinophilia with myocardial involvement. We started from the most common and most likely causes, considering the patient's age, lifestyle, and habits. The cause of eosinophilia can be hypersensitivity to antibiotics, neurological drugs, vaccines, tuberculostatic agents, nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, diuretics, digoxin, and others. However, the patient was not taking any medication, except for an asthma spray, nor had he recently received any vaccines. He had not been vaccinated against severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) infection. A common cause of eosinophilia could also be various infections: viral (for instance, human immunodeficiency virus – HIV), parasitic (*Toxocara canis*, Ascaris), protozoal (*Toxoplasma gondii*). However, we have ruled out all infectious agents. Following were autoimmune diseases (EGPA, giant cellular arteritis, sarcoidosis, allergic bronchopulmonary aspergillosis, polyarteritis nodosa), among which we found the cause of myocarditis. Nevertheless, it was necessary to exclude malignant diseases (lymphomas – Hodgkin, T and B cell, acute leukemias), primary hypereosinophilic syndrome, and chronic eosinophilic leukemia, which was the reason for performing a bone marrow biopsy. We did not consider primary immunodeficiency diseases (Hyper IgE syndrome, Omenn syndrome) because we previously came to the diagnosis of EGPA. In some cases, eosinophilia with myocardial involvement remains idiopathic/undefined [5].

Our patient had asthma, eosinophilia, and paranasal sinusitis. There were no transient pulmonary infiltrates or neurological criteria, so we had to look for HP confirmation of the disease, according to the recommendations of ACR [3]. We got it after a skin biopsy, which is a simpler and less invasive method compared to an endomyocardial biopsy (EMB), especially because there is no high-volume center with highly experienced staff for EMB in our country.

Myocardial contractility was satisfactory, and high BNP dominantly resulted from diastolic heart failure. Namely, myocardial involvement and damage in EGPA occur due to direct tissue eosinophilic infiltration and the release of cytotoxic proteins, which are directly involved in remodeling, fibrogenesis, cardiotoxicity, and fibrous degeneration [6]. Eo also have direct procoagulant activity, leading to a prothrombotic microenvironment. When Eo are > 20% in the peripheral blood, they infiltrate the myocardium [6]. They can lead to restrictive or dilated cardiomyopathy, which is precisely one of the reasons for the poor long-term prognosis of these patients [4, 6–8]. In our patient, Eo were over 50% in the peripheral blood.

According to the criteria of the European Respiratory Society (ERS) task force, which consist of asthma, eosinophilia, and at least two of the additional features of EGPA [9, 10], we could reach a diagnosis even without biopsy and HP findings, based on the presence of asthma, eosinophilia, paranasal sinusitis, and purpura. Compared with the criteria of ACR, new features in the ERS document are cardiomyopathy, glomerulonephritis, alveolar hemorrhage, palpable purpura, and ANCA-positivity [9, 10]. However, additional diagnostics were necessary to rule out malignancy in our case.

ACR and the European Alliance of Associations for Rheumatology published new criteria and their weights for patients with a diagnosis of vasculitis of small or medium vessels [11]. According to these criteria, Eo $\geq 1 \times 10^9$/L are scored with 5 points, obstructive airway disease and nasal polyps are scored with 3 points each, extravascular eosinophilic-predominant inflammation on biopsy – 2 points, and mononeuritis multiplex or motor neuropathy, not due to radiculopathy – 1 point. On the other hand, the presence of ANCA positive result and hematuria reduced the score by 3 points and 1 point, respectively. If the score is ≥ 6, vasculitis could be classified as EGPA with 85% sensitivity and 99% specificity, and these criteria are validated for use in research [11]. In our case, the score was 10, so the diagnosis of EGPA could be established.

The diagnosis was made, and the therapy started 51 days from the first day of hospitalization in the local hospital and 17 months from the onset of asthma. However, asthma and chronic sinus disease are only the prodromal phases of the disease. It is realistically possible to make a diagnosis in the second – eosinophilic phase, when eosinophilia occurs in the peripheral blood, followed by tissue infiltration of Eo with or without the formation of granuloma (upper and lower respiratory, gastrointestinal, renal tract, myocardium) [4]. The third stage is vascular, when systemic necrotizing vasculitis of small blood vessels occurs, especially in peripheral nerves, kidneys, and skin [4]. From the beginning of the disease to the diagnosis, it takes 8 to 49.7 months on average [12, 13]. Eosinophilic myocarditis of any etiology requires immediate treatment with glucocorticoids to prevent myocardial fibrosis. On the other hand, although this therapy leads to rapid clinical improvement, it will delay the establishment of the underlying diagnosis for an extended period [14]. Furthermore, glucocorticoid therapy is contraindicated until infection and malignancy have been ruled out [15], which we have done.

Our patient was ANCA-negative, which is consistent with literature data that cardiac and neuropathic manifestations are more common in ANCA-negative patients [16]. The two main clinical subgroups of patients with EGPA are ANCA positive, in which small vessel vasculitis predomi-

nates, and ANCA negative, in which organ damage is predominantly caused by eosinophilic tissue infiltration [7]. Cardiac involvement is present in 27–47% of patients, depending on whether it is observed only clinically or in patients undergoing systemic heart MRI, where changes are found in 47% of patients [4, 6]. The most common manifestations of myocardial involvement are heart failure, cardiomyopathy, myocarditis, pericarditis, acute myocardial infarction, and coronary vasculitis. Myocardial involvement is an independent predictor of mortality and morbidity in EGPA [6, 17]. Myocardial involvement is associated with many Eo in the blood and the absence of ANCA [4]. It is estimated that the mortality of patients with myocarditis caused by EGPA in the first few months from the onset of the disease is about 50% [18, 19].

How rare myocarditis is associated with EGPA is shown by the fact that a search of PubMed found only 116 results, which include these two keywords, most of which are reports of individual cases.

In patients with EGPA and myocardial involvement, monitoring Tn and creatine kinase-myocardial band (CK-MB), ECG, and heart ultrasound are necessary. In recent years, MRI of the heart has gained increasing importance [20]. In our case, cardiac MRI showed an unusual LGE distribution of eosinophilic myocarditis. We have one of two Lake Louise Criteria, myocardial enhancement without edema [21]. That could be an unusual form of eosinophilic myopericarditis in resolution. Coronary angiography excludes coronary artery disease. An endomyocardial biopsy can confirm the diagnosis in a center with great experience. Additional diagnostics aim to identify other causes of hypereosinophilic syndrome, which may involve the myocardium [20].

The treatment of these patients includes glucocorticoid therapy in the acute phase of the disease and as maintenance therapy. Initially, 1 mg/kg of weight of prednisone is given. The maintenance dose is the minimum effective dose. In severe forms, pulse glucocorticoid therapy for 1–3 days is indicated. Remission is achieved in > 85% of patients. If there is no response or a relapse occurs, immunosuppressants (cyclophosphamide, azathioprine, methotrexate) are added [12]. Biological therapy (rituximab), which targets IL-5, is also used [10].

Conclusion

We presented a patient with an unusual clinical presentation of myocarditis associated with numerous systemic manifestations, with dominant eosinophilia. The positive HP findings enabled us to diagnose EGPA and start adequate glucocorticoid treatment. This therapy could not have been prescribed earlier until we ruled out infection and malignancy. If it had been given, it would have masked a finding crucial for the correct diagnosis of EGPA, which requires lifelong treatment. This case showed the need for multidisciplinary work and perseverance in the diagnostic evaluation of the etiology of myocarditis for the treatment to be adequate.

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CASE REPORT

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Unusual case of Parkes-Weber syndrome in a patient with spontaneous subarachnoid hemorrhage

Neobičan slučaj Parkes-Weber-ovog sindroma kod bolesnika sa spontanom subarahnoidnom hemoragijom

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Abstract

Introduction. Parkes-Weber syndrome (PWS) is a complex and rare genetic disease of combined vascular malformations that primarily occur in the extremities and can involve the pelvic blood vessels. In extremely rare cases, the disease is manifested by endocranial and spinal involvement. The treatment of such patients represents a challenge for neurosurgical centers and requires a multidisciplinary approach. **Case report.** We present the case of a 46-year-old male patient admitted to the emergency department due to spontaneous subarachnoid hemorrhage (SAH), moderate flaccid paraparesis, and urinary incontinence. The patient was previously diagnosed with PWS, while the genetic evaluation proved the *RASA1* gene mutation. Furthermore, he experienced a spontaneous SAH and was hospitalized 26 years ago, while six years ago, he underwent a right nephrectomy due to multiple hilar aneurysms of the right renal artery and its branches. Digital subtraction angiography of the endocranium was performed, which detected no aneurysmal dilatations or arteriovenous malformations (AVM). The magnetic resonance imaging recorded spinal intradural AVM in the vertebral levels between T12 and L3, which completely filled the dural sac. After the conservative treatment, there was a significant improvement in the patient's neurological and clinical condition. **Conclusion**. To the best of our knowledge, this is the only case report of a patient with PWS who had a spinal intradural AVM and spontaneous SAH without high-output heart failure and with a history of a previous nephrectomy.

Key words:
arteriovenous malformations; congenital abnormalities; diagnosis; genes; magnetic resonance imaging; mutation; rasa1 protein, human; subarachnoid hemorrhage.

Apstrakt

Uvod. Parkes Weber-ov sindrom (PWS) je kompleksna i retka genetska bolest, koja se manifestuje kombinovanim vaskularnim malformacijama, prvenstveno na krvnim sudovima ekstremiteta, mada mogu biti zahvaćeni i krvni sudovi karlice. Izuzetno retko bolest može imati endokranijalnu i spinalnu lokalizaciju. Lečenje takvih bolesnika predstavlja izazov neurohirurškim centrima i zahteva multidisciplinarni pristup. **Prikaz bolesnika.** Prikazujemo 46-godišnjeg bolesnika, koji je primljen u Urgentni centar zbog spontane subarahnoidne hemoragije (SAH), flakcidne parapareze i urinarne inkontinencije. Bolesniku je prethodno postavljena dijagnoza PWS, a genetskom analizom dokazana je mutacija *RASA1* gena. Takođe, bolesnik je imao SAH i bio hospitalizovan pre 26 godina, dok je pre šest godina, zbog višestrukih hilarnih aneurizmi desne renalne arterije i njenih grana, načinjena desnostrana nefrektomija. Urađena je digitalna subtrakciona angiografija endokranijuma, pri čemu nisu otkrivene aneurizmatske dilatacije i arteriovenske malformacije (AVM). Metodom magnetne rezonance nađena je intraduralna AVM na nivou između pršljenova T12 i L3, koja je u potpunosti ispunila duralnu vreću. Nakon konzervativnog lečenja došlo je do značajnog poboljšanja subjektivnog i kliničkog stanja bolesnika. **Zaključak.** Prema nama dostupnim podacima u referentnoj naučnoj literaturi, ovo je jedini prikaz bolesnika sa PWS sa spinalnom intraduralnom AVM i spontanim SAH bez srčane insuficijencije i sa istorijom prethodne nefrektomije.

Ključne reči:
arteriovenske malformacije; anomalije; dijagnoza; geni; magnetska rezonanca, snimanje; mutacija; rasa1 protein, humani; krvarenje, subarahnoidno.

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Introduction

Parkes-Weber syndrome (PWS) is a complex and rare genetic disease of combined vascular malformations that primarily occur in the extremities and can involve the pelvic blood vessels [1]. Research has shown that the mutation of the *RASA1* gene is responsible for abnormal connections, vascular malformations, and changes in the size of the affected blood vessels. The most common clinical manifestations are "port wine stains" on the skin, venous varicosities, unilateral limb overgrowth, high-flow arteriovenous malformation (AVM), and high-output heart failure [2]. In extremely rare cases, the disease is manifested by endocranial and spinal involvement [3], while the treatment of such patients represents a challenge for neurosurgical centers and requires a multidisciplinary approach. To the best of our knowledge, this is a first reported case of a patient with PWS and spinal AVM who underwent a prior nephrectomy and presented with a perimesencephalic spontaneous subarachnoid hemorrhage (SAH) without intracranial vascular malformation.

Case report

We present a case of a 46-year-old male patient admitted to the emergency department due to severe pain in the lumbar spine with sciatic propagation, as well as sudden headache and neck stiffness. The patient described the headache as the worst in his life.

Furthermore, we obtained relevant medical data from the medical records and heteroanamnesis, evidencing that a patient was previously diagnosed with PWS syndrome, while the genetic evaluation proved the *RASA1* gene mutation. Moreover, the patient's family history of PWS and other vascular malformation syndromes was negative. The patient had previously been under periodical multidisciplinary assessment for the past 30 years. Henceforth, he experienced a spontaneous SAH and was hospitalized 26 years ago, while six years ago, he underwent a right nephrectomy due to multiple hilar aneurysms of the right renal artery and its branches.

During the initial assessment, the patient was drowsy, arterial pressure values were 200/100 mmHg and was rated with a Glasgow Coma Scale score of 14. Neurological examination showed no cranial nerve gross neurological deficits; the neck was stiff, while moderate flaccid paraparesis and urinary incontinence were recorded. An urgent computed tomography (CT) scan of the brain was performed, which revealed spontaneous SAH and intraventricular hemorrhage (IVH) predominantly in the fourth and third cerebral ventricles with a minimal amount of blood in both occipital horns (Figure 1). The Hunt and Hess score was estimated as grade I, the modified World Federation of Neurosurgical Societies grading scale score was evaluated as grade II, while the modified Fisher scale grade was grade IV.

Moreover, a digital subtraction angiography (DSA) of the endocranium was performed, during which no aneurysmal dilatations and AVMs were detected. Magnetic resonance (MR) imaging of the lumbar and thoracic spine was performed, where dilated tortuous intradural blood vessels were present from T12 to L3 vertebral levels, which completely filled the dural sac, measuring 20 × 35 × 90 mm (Figure 2). Above the described AVM, a dilated blood vessel with a diameter of 13 mm was observed up to the recorded level of the C7 vertebral body. That was followed by spinal DSA, which detected the aforementioned AVM feeding from the basin of the tenth intercostal artery on the right side with the formation of numerous small aneurysms and high-flow venous drainage towards the intradural venous plexus. Two dural arteriovenous fistulas were observed at the L2 vertebral level as well (Figures 3 and 4).

On the other hand, an ultrasound of the inguinal region was performed, which recorded an AVM measuring 3 × 1.5 cm in the right inguinal region. Additionally, multiple liver hemangiomas were observed on the abdominal ultrasound examination.

During the hospital treatment, the patient was in an algid stage of the disease, with occasional opisthotonus posture; the pain was controlled with opioid and nonsteroidal analgesics, as well as corticosteroids. Since he exhibited clinical signs of hydrocephalus, a control CT scan of the brain was performed,

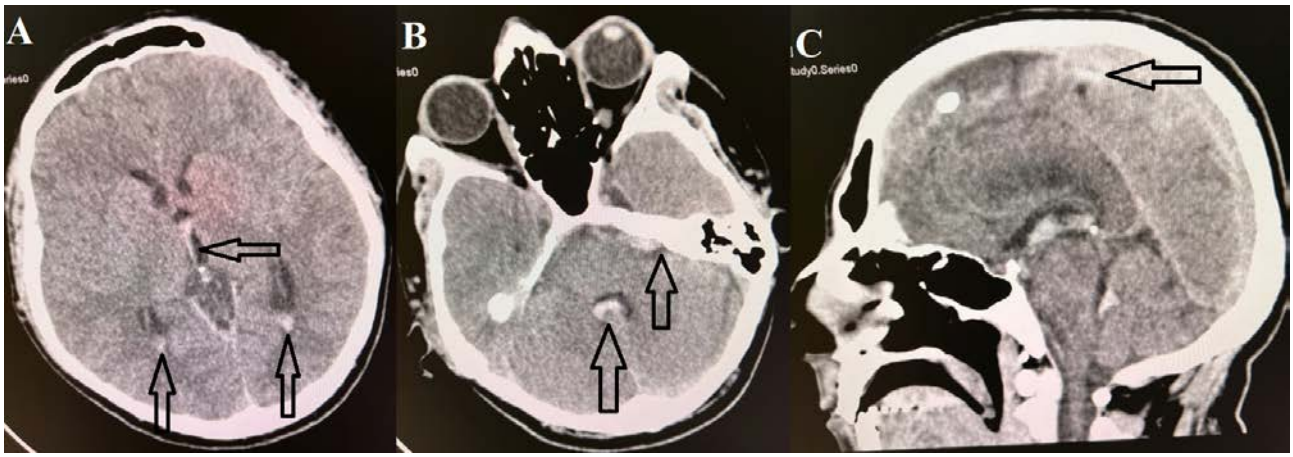

Fig. 1 – Non-contrast axial (A, B) and sagittal (C) tomograms showing subarachnoid hemorrhage recorded in the occipital horns of: A) the lateral and third ventricle, B) fourth ventricle and prepontine cisterns, and C) in the parietal sulci (arrows).

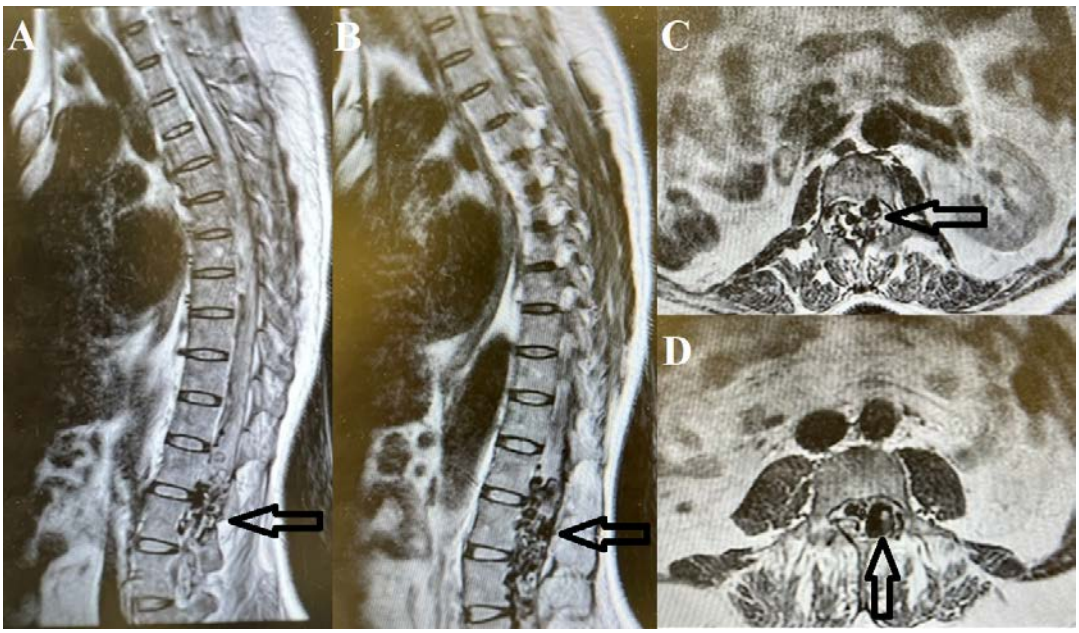

Fig. 2 – Sagittal (A, B) and axial (C, D) magnetic resonance tomograms – at the vertebral levels between T12 and L3, there is a malformation in the spinal canal consisting of numerous tubular and dominantly intradural convoluted flow voids, corresponding to dilated and tortuous blood vessels, measuring 20 × 35 × 90 mm and corresponding to the nidus of arteriovenous malformation (arrows).

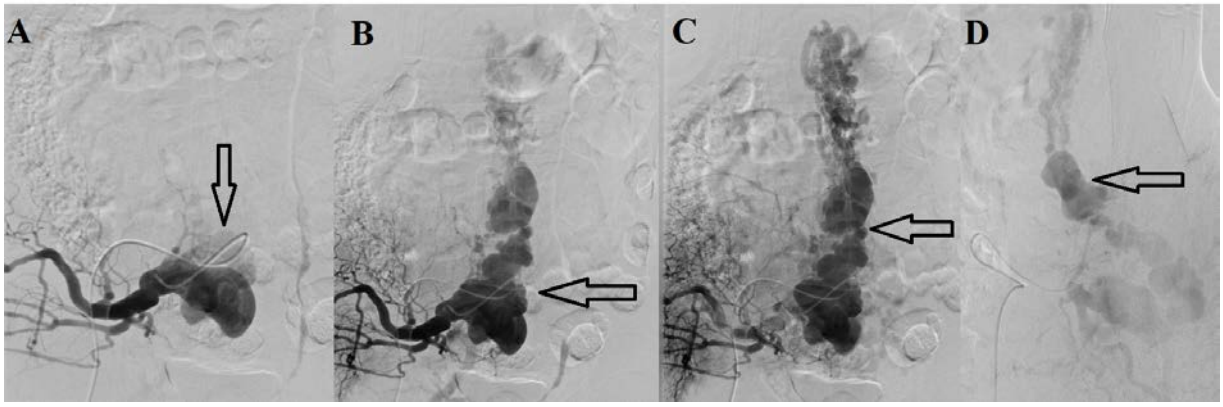

Fig. 3 – Digital subtraction angiography through arterial (A), parenchymal (B, C), and venous (D) phases shows a wide, high-flow arteriovenous fistula (arrows) with rapid venous drainage from the basin of the right internal iliac artery through markedly ectatic and tortuous efferent blood vessels to the spinal venous plexus.

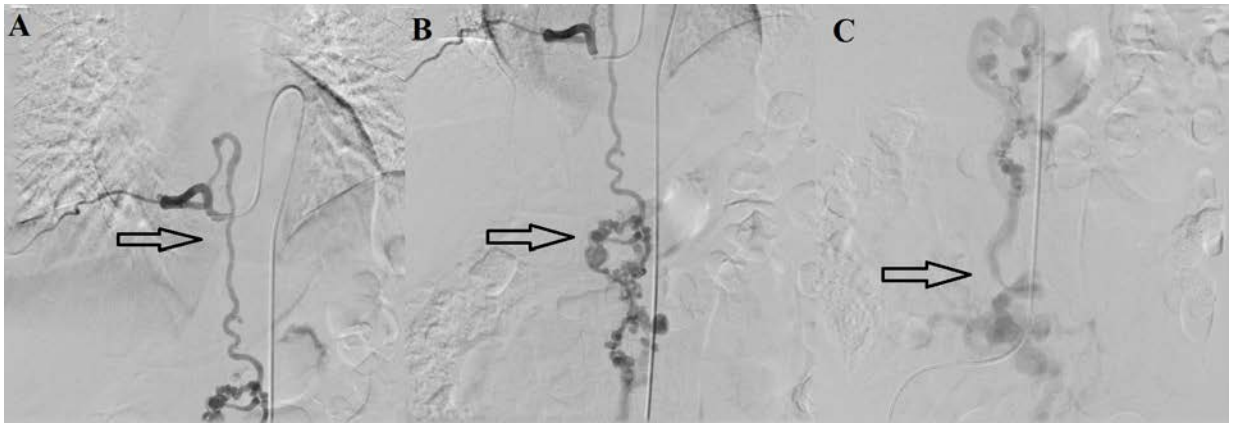

Fig. 4 – Digital subtraction angiograms through arterial (A), parenchymal (B), and venous (C) phases show an extensive arteriovenous malformation (arrows) with a dominant feeder from the basin of the right X intercostal artery, with the formation of numerous smaller aneurysmal dilatations on the afferent branches and with rapid venous drainage through ectatic and extremely tortuous venous plexus.

which indicated the existence of hydrocephalus with periventricular lucency and IVH (Figure 5).

The patient and his family were proposed surgical treatment of hydrocephalus with ventriculoperitoneal shunt or external ventricular drainage, as well as subsequent endovascular treatment of the AVM. However, the patient and his relatives refused further treatment.

Therefore, his hydrocephalus was treated conservatively for seven days with acetazolamide (250 mg a day), furosemide (20 mg twice a day), dexamethasone (8 mg three times a day) with a gradual dose reduction, mannitol (125 mL four times a day) for four days with rehydration and other symptomatic therapy, as well as daily checkup of electrolytes, urea, and creatinine. After the conservative treatment, there was a significant improvement in the patient's subjective and clinical condition, and he was discharged with a recommendation to return for a follow-up CT scan of the brain and further treatment of the AVM. Follow-up examinations were performed after one year and after 18 months, while the patient had paraplegia and urinary incontinence in the clinical presentation and still refused surgical treatment and control radiological diagnostics.

Discussion

PWS is most usually clinically manifested by unilateral limb overgrowth, high-output heart failure, distal arterial ischemia, and venous ulceration, while aneurysms, intracranial and spinal AVM, and malignancies occur less frequently [1, 2]. Based on a review of the available scientific literature, only one previous case of spinal AVM in a patient with PWS has been reported, who suffered from an SAH, while the MR angiography was negative [4]. We believe that the limitation of that case report was the fact that the authors did not perform

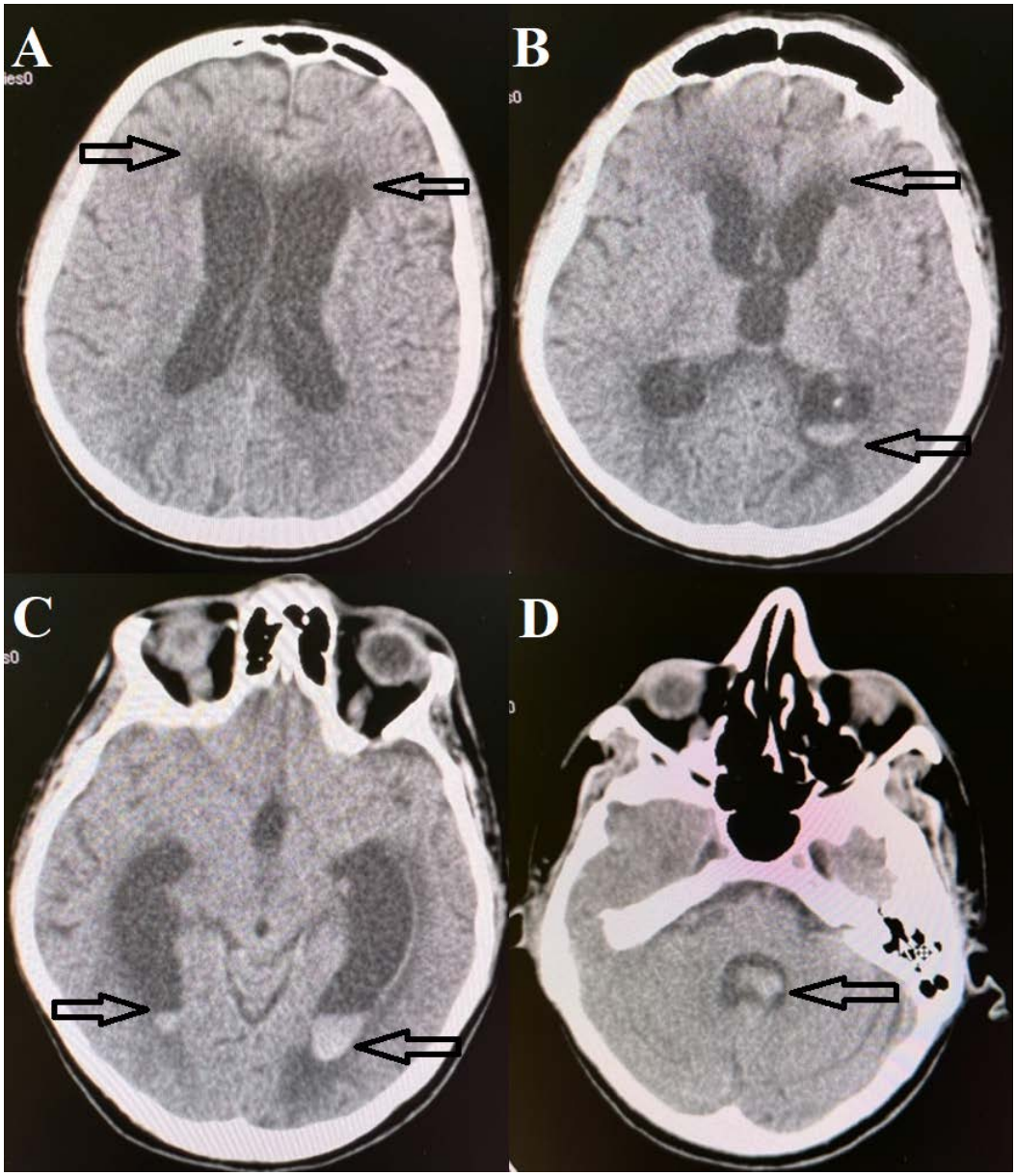

Fig. 5 – Non-contrast axial computed tomography findings indicate the existence of hydrocephalus with periventricular lucency (A, B, C), intraventricular hemorrhage (IVH) in the occipital horns of the lateral ventricles (B, C), and IVH in the fourth (D) ventricle (arrows).

DSA of the brain, which represents the gold standard. Therefore, the existence of a cerebral AVM or aneurysm could not be ruled out with certainty. Furthermore, no genetic evaluation of that patient was performed. In our case, on the other hand, the patient underwent DSA of the brain, and vascular malformations and aneurysms were excluded as potential causes of spontaneous SAH. On the other hand, to the best of our knowledge, this is the only published case of PWS without high-output heart failure, with a history of renal artery aneurysm and subsequent nephrectomy, as well as with intracranial and spinal involvement.

Different studies have shown that predictive parameters for the outcome in patients with SAH are the following: initial neurological status, Fisher score on brain CT, age, history of hypertension, recurrence of SAH, and vasospasm [5, 6]. Some authors suggest that *de novo* hypertension in patients after unilateral nephrectomy can occur due to a reduced total number of nephrons and increased cardiac output, as well as a relative deficiency of 11-beta hydroxylase [7]. Moreover, a clear connection between arterial hypertension and the occurrence of SAH has been previously demonstrated, which is the probable mechanism for the perimesencephalic SAH in our patient [8]. On the other hand, biomarkers that showed prognostic significance in immune dysregulation after SAH, such as C-reactive protein (CRP), interleukins, and neutrophil-lymphocyte ratio, were studied [9, 10]. In cardiovascular diseases, cancer, and sepsis, an elevated neutrophil-lymphocyte ratio has been shown to be a potential indicator of poor clinical outcome [11]. Our patient had verified arterial hypertension after nephrectomy, was middle-aged, and the initial scan showed a modified Fisher score of IV, while his paraparesis was attributed to a spinal AVM instead of SAH. Furthermore, he had relatively low CRP values, low platelet-lymphocyte ratio, and neutrophil-lymphocyte ratio of less than 5. The results of one study showed that a neutrophil-lymphocyte ratio greater than 5.9 predicted as much as a 2-fold greater chance of developing delayed cerebral ischemia after SAH [11]. Although surgical and endovascular treatments were not performed, the patient reached a good recovery, which is in agreement with the aforementioned parameters for predicting the outcome in patients with SAH. Previous studies have shown that angiographically negative SAH, such as perimesencephalic SAH, most often results in good recovery of the patient, and some authors consider it a benign condition because it rarely causes complications such as rebleeding and vasospasm [12]. Moreover, if acute hydrocephalus occurs after a perimesencephalic hemorrhage, it is usually transient and leads to a good recovery of the patient [13], as was the case in our patient.

Surgical treatment of hydrocephalus includes methods such as surgical resolving of the obstruction site, bypassing the obstruction with an alternative route artificially created by endoscopic third ventriculostomy, or by draining cerebrospinal fluid (CSF) from the cerebral ventricles into various absorbent body cavities. Therefore, the CSF drainage procedure can be performed as a ventriculoperitoneal shunt, ventriculoatrial and ventriculopleural shunt, while a lumboperitoneal shunt is rarely used. Various complications can occur after shunt placement surgery, such as obstruction, catheter migration, mechanical blockage, siphoning effect, and infection resulting from colonization of the device by microorganisms [14, 15].

Conservative treatment of hydrocephalus with acetazolamide achieves direct inhibition of carbonic anhydrase in the choroid plexus as well as inhibition of water conductance mediated by aquaporins, which leads to reduced production of CSF. Moreover, for this purpose, acetazolamide is often combined with furosemide. However, the use of corticosteroids in the treatment of hydrocephalus leads to a reduction of inflammation and fibrosis in the subarachnoid compartment. On the other hand, mannitol is occasionally used in the treatment of hydrocephalus in cases of intracranial bleeding [16].

Furthermore, digoxin can be used in the treatment of hydrocephalus, in doses that are not cardiotoxic, as well as urokinase and tissue plasminogen activator, although some previous research indicates that fibrinolytic agents can promote an inflammatory response by rapidly dissolving the clot [16, 17]. Consequently, during the conservative treatment of hydrocephalus in our patient, we opted for the aforementioned therapy, except for digoxin and fibrinolytic agents, because our neurosurgery center lacks experience in their use for this indication.

Surgical treatment of AVM in patients with PWS usually follows unsuccessful conservative treatment and includes amputation of the affected limb, debulking of soft tissues, ligation of AVM feeder, resection of AVM nidus, vein stripping, and epiphyseal stapling for leg length discrepancy. The purpose of treatment is to prevent the occurrence and progression of high-output heart failure, aneurysms, distal ischemia, refractory pain syndrome, ulceration, hemorrhage, and hypertrophy of the affected limb [10, 11]. Moreover, surgical treatment is associated with the occurrence of postoperative complications and has been suppressed, over time, to a significant extent by endovascular procedures [11]. On the other hand, it is impossible to completely cure vascular malformations in PWS due to a tendency for collateral blood vessels to re-appear, as well as for arteriovenous fistulas and AVM relapse as a consequence of the diffuse nature of the disease. That would imply frequent reoperations of the AVM, and for these reasons, embolization is increasingly performed as an initial intervention [10].

The future in the treatment of these patients should be oriented towards targeted molecular therapy, which emphasizes the importance of molecular genetic testing in vascular malformation syndromes. Although there is no official recommendation for targeted molecular therapy for PWS, studies with angiogenesis inhibitors and other agents such as rapamycin, trametinib, thalidomide, and bevacizumab are being conducted to determine their potential efficacy [18].

Considering all this, our findings suggest that this patient was prone to develop intracranial SAH even though he did not have an intracranial vascular malformation as part of PWS, which could be explained by de novo hypertension after unilateral nephrectomy.

Further research comparing the efficiency of different therapeutic modalities for PWS is needed as available data are scarce.

Conclusion

Multidisciplinary assessment and treatment of patients with PWS is necessary due to the complexity of the disease, while their treatment represents a challenge for experienced neurosurgeons and interventional neuroradiologists. To the best of our knowledge, this is the only case report of a patient with PWS who had a spinal AVM and spontaneous perimesencephalic SAH without high-output heart failure and with a history of a previous nephrectomy.

Conflict of interest

The authors declare no conflicts of interest and report no sources of support that require acknowledgment.

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CASE REPORT

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Pectoralis major flap for pharyngocutaneous fistula after total laryngectomy – two different approaches

Upotreba režnja *pectoralis major* za zatvaranje faringokutane fistule posle totalne laringektomije – dva različita pristupa

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Abstract

Introduction. The reconstruction of large postoperative defects after oncologic surgery of the head and neck remains challenging. Regional flaps are considered a less expensive reconstructive option compared to free flaps. The *pectoralis major* flap is one of the most versatile choices for the reconstruction of large head and neck defects. **Case report.** We present technical key points for safe harvesting of *pectoralis major* flap for two cases in a university-affiliated tertiary care medical center. Both patients were male, with an average age of 64 years. The defects that required reconstruction in Case 1 were on the lateral neck region and, in Case 2, on the anterior side of the neck. Flaps were used for covering the pharyngocutaneous fistula after total laryngectomy and irradiation. The donor site was closed primarily. Flaps in both patients healed primarily without complications. **Conclusion.** The *pectoralis major* flap has a constant vascular pedicle and can successfully be used for the reconstruction of large head and neck defects. In order to obtain absolute flap survival, the operative technique must be impeccable.

Key words:
fistula; head and neck neoplasms; laryngectomy; pharynx; plastic surgery procedures; surgical flaps.

Apstrakt

Uvod. Rekonstrukcija velikih postoperativnih defekata posle onkološke hirurgije glave i vrata i dalje predstavlja veliki izazov. Regionalni režnjevi smatraju se prihvatljivijom rekonstruktivnom opcijom u poređenju sa slobodnim režnjevima. *Pectoralis major* režanj je jedna od najčešće primenjivanih opcija u rekonstrukciji velikih defekata glave i vrata. **Prikaz bolesnika.** Prikazujemo ključne tačke u hirurškoj tehnici podizanja *pectoralis major* režnja kod dva bolesnika lečena u jednom od univerzitetskih centara tercijarne medicinske zaštite. Oba bolesnika, prosečne starosti 64 godine, bila su muškog pola. Defekti koji su zahtevali rekonstrukciju nalazili su se kod prvog prikazanog bolesnika na bočnoj strani vrata, a kod drugog bolesnika na prednjoj strani vrata. Režnjevi su korišćeni za pokrivanje faringokutane fistule nastale posle totalne laringektomije i zračne terapije. Donorsko mesto je zatvarano primarno. Kod oba bolesnika režnjevi su zarasli primarno i bez komplikacija. **Zaključak.** *Pectoralis major* režanj ima postojanu vaskularnu peteljku i može biti uspešno iskorišćen za rekonstrukciju velikih defekata glave i vrata. Da bi režanj preživeo, operativna tehnika mora biti besprekorna.

Ključne reči:
fistula; glava i vrat, neoplazme; laringektomija; farinks; hirurgija, rekonstruktivna, procedure; hirurški režnjevi.

Introduction

The reconstruction of large postoperative defects after oncologic surgery of the head and neck remains challenging. Considering different clinical situations, regional pedicle flaps are defined as the "workhorse" compared to free flaps. Microsurgical procedures are demanding and associated with higher complication rates [1]. Pedicle *pectoralis major* musculocutaneous flap (PMMF) is routinely used for head and neck defect reconstruction in centers without proper equipment for free tissue transfer [2]. The flap described by Ariyan in 1979 has been used for more than four decades [3]. It is the most often used flap following laryngectomy and meets the clinical requirements for the treatment of patients with advanced neck disease [4]. This flap exhibits strong resistance to infection and necrosis and also heals rapidly [5]. According to literature data, flap-related complications are classified as major if additional surgical revision is required or minor if

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only conservative wound care is necessary [4, 6]. All patients had given their informed consent prior to their inclusion in the study.

Surgical tips

The crucial point in flap modeling is the definition of the arc of flap rotation. After ablative oncologic surgery, the distance from the upper edge of the defect to the midclavicular point should be measured and transferred inferiorly on the anterior chest wall in the pectoral region. Defect size and shape must be tailored in the parasternal region.

The flap harvesting starts with an incision of the skin and subcutaneous tissue along the medioclavicular line and lateral border of the skin island. The dissection through fat all around the skin island must be divergent, including as many perforators as possible (at least 0.8 cm around the skin in all directions, reaching the pectoral *fascia*). Dermofascial sutures are placed at a distance of 2–3 cm along the edge of the skin island to protect the perforators during manipulation of the flap. The skin and subcutaneous tissue lateral from the incision are elevated, reaching the lateral border of the *pectoralis major* muscle, and blunt dissection is performed in the subpectoral plane, reaching medial insertion to the *sternum* and ribs. The *pectoralis* muscle is incised medially and laterally from the vascular pedicle under continuous, direct visual control of the pedicle. Muscle pedicle is wide 2 to 3 cm. The pedicle of PMMF must be detached from the clavicle on one or both sides. The approach to the pectoral paddle is also possible through the "defensive incision", which preserves perforators from an internal mammary artery, with the preparation of the PMMF performed after raising a deltopectoral flap. That is done to allow future use of the ipsilateral deltopectoral flap [7]. The subplatismal tunnel in the clavicular and cervical region for flap transfer must be four fingers wide, avoiding compression of the pedicle. Flap fixation starts with suspension sutures of the muscle part of PMMF for muscles at the defect with polypropylene non-absorbable 3–0 stitches. After the proper suspension of PMMF, the skin island is sutured for the mucosal layer without the removal of dermo-fascial stitches. This maneuvre protects musculocutaneus perforators.

The case report of two patients with pharyngocutaneous fistula (PCF) after laryngectomy has been approved by the institutional Ethics Committee, and written consent was obtained from both patients. Data collected include demographic data, site of fistula, indication and type of flap, flap complication, and hospital stay.

Case report

Case 1

A 62-year-old male patient was presented to the Otorhinolaryngology Clinic with a transglottic tumor with vocal cord fixation on the right side and a palpable neck mass on the ipsilateral side. After diagnostic imaging was done, which showed the paraglottic space involvement, the patient was scheduled for a biopsy, and a diagnosis of G2 stage squamocellular cancer was obtained. The patient was then scheduled for total laryngectomy and bilateral neck dissection. The pathohistological findings [tumor, node, metastasis (TNM) staging] correlated with clinical findings, and the disease was staged as T3N2bM0. Postoperatively, the patient received 60 Gy irradiation therapy with cisplatin and 5-fluorouracil chemotherapy. The PCF occurred one month after irradiation, following total laryngectomy (Figure 1). Regarding the flap anatomy, we used a musculocutaneous PMMF. The flap was used for secondary reconstruction, more precisely, for skin resurfacing. The pharyngeal wall was closed primarily, and the musculocutaneous flap was used for the reconstruction of skin defects in order to cover the exposed carotid artery. The wound healed primarily without residual fistula. The donor region was closed and healed primarily after flap harvesting; there were no complications. Figure 2 shows the postoperative result after the transposition of the *pectoralis major* flap. The hospital stay lasted for 12 days.

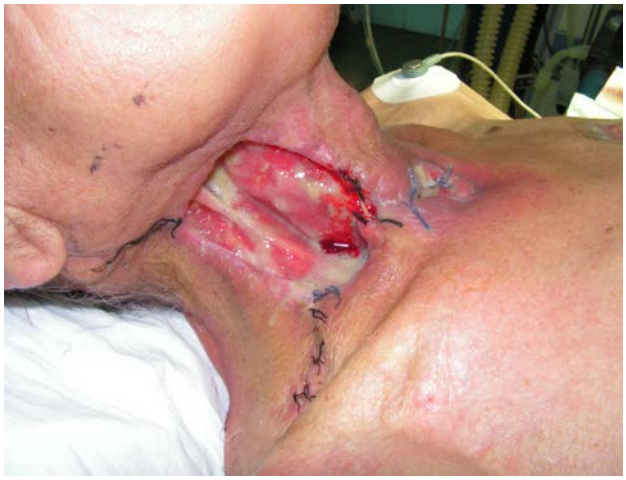

Fig. 1 – Case 1: Pharyngocutaneous fistula occurred one month after irradiation, following total laryngectomy.

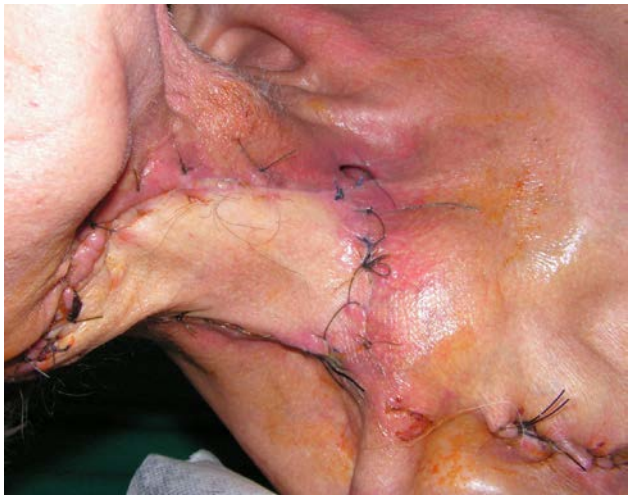

Fig. 2 – Case 1: Definitive postoperative result. The transposition of musculocutaneous *pectoralis major* flap for secondary reconstruction of skin defect in order to cover the exposed carotid artery.

Case 2

A 66-year-old male patient was presented to the Otorhinolaryngology Clinic with dysphagia and hoarseness. Clinical examination revealed a necrotic mass in the left *pyriform fossa* and left aryepiglottic fold with arytenoid infiltration. After the biopsy showed a G3 stage squamocellular carcinoma, the patient opted for low-dose fractionated radiotherapy and chemiotherapy. On the follow-up after oncological treatment, the disease was in partial remission. Therefore, the patient underwent salvage total laryngectomy and partial pharyngectomy with radical neck dissection on the left side and elective lateral neck dissection on the right side. With the preservation of hypopharyngeal mucosa of 5 cm, a decision was made intraoperatively for primary reconstruction of the neopharynx. The pathohistological TNM staging of the carcinoma was T3N2cM0. Five days postoperatively, a PCF occurred in the anterior neck region (Figure 3). PMMF was used for secondary pharyngoplasty. Regarding the flap anatomy, we used a turned-in musculocutaneous flap with a skin island for resurfacing the pharyngeal wall and a partial thickness skin graft for skin reconstruction (Figure 4). The reconstruction was competent without residual fistula, and the skin graft healed primarily. After flap harvesting, the donor region was closed and healed primarily; there were no complications (Figure 5). The hospital stay lasted for seven days.

In both patients, a protective nasogastric feeding tube was in place for three weeks.

Discussion

The primary goal of reconstructive procedures for head and neck defects, especially for PCF, is to restore the patient's premorbid level of functionality and quality of life [8]. The use of PMMF is indicated mostly after oncologic surgery. The mean age of our patients was 64 years. That is in accordance with the studies reporting the use of PMMF in elderly patients aged 57.2 to 76.5 years, on average [9].

There are some controversies regarding the defect closure after pharyngolaryngectomy. The PMMF can be used for reconstruction after a huge resection of the pharyngeal wall and laryngectomy as a patch pharyngoplasty, as a primary procedure, and for secondary reconstruction for postlaryngectomy PCF, or after neck irradiation and subsequent skin loss. Some studies state that the *pectoralis major*

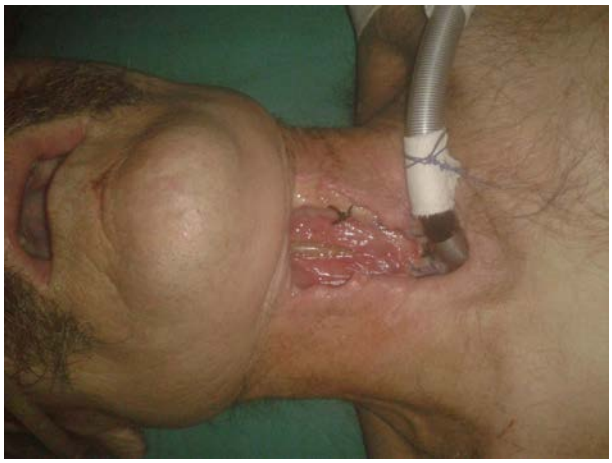

Fig. 3 – Case 2: Pharyngocutaneous fistula occurred in the anterior neck region five days after total laryngectomy.

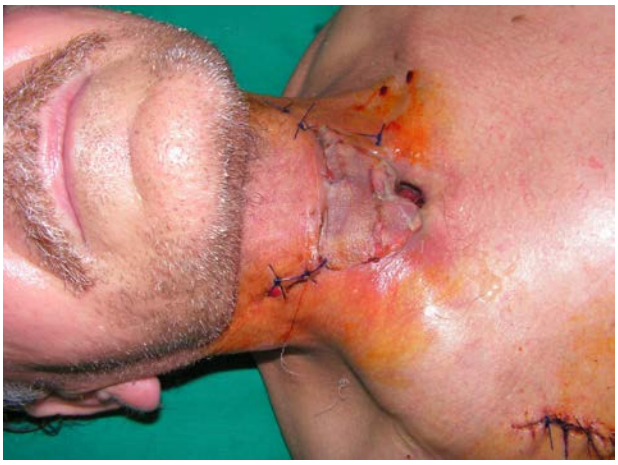

Fig. 4 – Case 2: Definitive postoperative result. A turned-in musculocutaneous *pectoralis major* flap, with skin island (patch pharyngoplasty), was used along with a partial thickness skin graft for skin reconstruction.

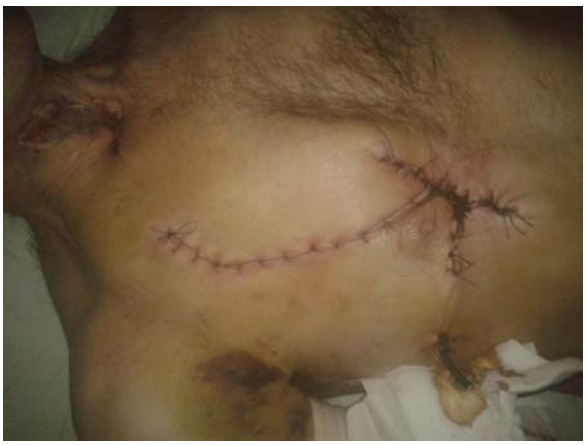

Fig. 5 – Case 2: The donor region of the *pectoralis major* flap, closed and healed primarily.

does not reduce the long-term risk for the development of PCF (27.1% incidence) and is associated with a higher mortality rate (2.1%) [8]. However, recent reports favor the use of PMMF for primary patch pharyngoplasty [10]. In our two patients, pharyngoplasty healed without complications and without late fistula formation. The low complication rate may be attributed to adequate vascularisation by abundant perforators supplying overlaying skin [11]. Therefore, PMMF may be transferable into an infected recipient area and also be used after necrosis of microvascular flaps and in the cases where free flaps are contraindicated (patients not suitable for long procedures, with inadequate recipient vessels or those that underwent high-dose radiotherapy in recipient region) [1, 11]. Radical neck dissection combined with the use of PMMF is feasible for the treatment of giant cervical metastatic cancers that have invaded the skin (salvage surgery), especially for the protection of the carotid axis [12]. Patch pharyngoplasty with skin graft for anterior neck skin appears to be an elegant way of using a PMMF to reconstruct the pharynx. We perform this approach routinely.

The first flap has to be inserted in the defect area to provide a tension-free inset of the flap [13]. Most surgeons transfer the flap to the head and neck through the supraclavicular subplatysmal tunnel without any incision of the overlaying clavicle [14]. In both cases, we performed the same transpositioning of the flap.

Some authors advocate that PMMF may yield unsatisfactory functional and cosmetic results due to donor site morbidity and limitations related to bulk and fibrosis of the proximal muscle stalk [1]. Other key aesthetic factors include the position of the nipple, depression in the upper chest, and the appearance of the chest skin. These are addressed by modifying the incision site and proper reconstruction [15]. We did not register donor site dehiscence, although, in some series, it reaches 8% [16]. Regarding the closure of the donor site, most authors perform the primary closure, but in some cases, the donor site may require coverage with a skin graft [12]. We did not use skin graft for the donor region. Overall complication rates reported vary from 13% to 35.5% [3]. In our series, it was 0%. Despite the high complication rate, the second flap is rarely indicated [14]. Total flap failure ranges from 0% to 2.4% [3, 5, 6, 17]. We did not register either flap loss or partial flap loss, but referred incidence rates ranged from 2.3% to 4.8% [17, 18].

Conclusion

PMMF has passed the test of time and is still considered a valuable tool for PCF reconstruction. Despite the limited number of patients, our study supported the statement that PMMF is a reasonable choice of reconstruction as a salvage procedure after PCF. PMMF can be harvested without any special instrumentation and presents a valuable resource in the armamentarium of reconstructive oncologic surgery.

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Rare primary intrahepatic lithiasis in a young patient

Retka primarna intrahepatična litijaza kod mladog bolesnika

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Abstract

Introduction. Intrahepatic lithiasis (IHL) is a disease that occurs in middle-aged and elderly people. Presentations of IHL in the young are rare, and considerations in the differential diagnosis include primary sclerosing cholangitis, recurrent pyogenic cholangitis, bile acid transporter defect, Caroli's disease, and other known genetic diseases. Treatment is often complex, all in order to prevent complications. In this report, we describe the diagnosis and treatment, with the application of a flexible ureteroscope of 4 Fr, of a younger patient with intrahepatic lithiasis. **Case report.** A 25-year-old man appeared with a known diagnosis of IHL and a recurrent attack of abdominal pain that required medical treatment. Magnetic resonance imaging of the abdomen showed segmental stenosis of the left bile duct and segmental bile duct for the lateral section with intraductal calculi and its proximal dilatation and mild dilatation of the bile ducts for liver segments II and III. During surgery, a cholangiography and ultrasonography of the liver were performed. Through choledochotomy, the bile ducts were flushed, and extirpation of the several calculi was performed. The bile ducts were examined with a choledochscope, and the remaining concrements were removed with a flexible ureteroscope. **Conclusion.** Segmental liver bile ducts may be explored with a flexible ureteroscope without bile duct injury or trauma. In selected cases, with isolated lithiasis in one liver lobe and the absence of concomitant diseases, IHL can be treated surgically without liver resection. This case is unique because we did not perform liver resection but duct stone extraction, which was an appropriate treatment since there was no recurrence during the two-year follow-ups.

Key words:
bile ducts, intrahepatic; choledocholithiasis; diagnosis; surgical procedures, operative; ureteroscopes; treatment outcome.

Apstrakt

Uvod. Intrahepatična litijaza (IHL) je bolest koja se javlja kod osoba srednjeg i starijeg životnog doba. Pojava IHL kod mladih osoba je retka, a diferencijalna dijagnoza uključuje primarni sklerozirajući holangitis, rekurentni piogeni holangitis, defekt transportera žučne kiseline, Karolijevu bolest i druge poznate genetske bolesti. Lečenje je često složeno, sa ciljem da se spreče komplikacije. U ovom radu prikazujemo dijagnozu i lečenje bolesnika mlađeg životnog doba sa IHL korišćenjem fleksibilnog ureteroskopa promera 4 Fr. **Prikaz bolesnika.** Muškarac star 25 godina, sa ranije poznatom dijagnozom IHL i ponavljajućim napadima bolova u stomaku koji su zahtevali medicinski tretman, javio se na pregled. Magnetna rezonanca abdomena pokazala je segmentnu stenozu levog žučnog kanala i segmentnog žučnog kanala za lateralnu sekciju sa intraduktalnim konkrementima, kao i proksimalnom dilatacijom, te blagu dilataciju žučnih puteva za II i III segment jetre. Tokom operacije urađeni su holangiografija i ultrazvuk jetre. Žučni kanali su isprani kroz holedohotomiju i izvršena je ekstirpacija nekoliko konkremenata. Pregled žučnih puteva urađen je holedoskopom, a preostali konkrementi su uklonjeni fleksibilnim ureteroskopom. **Zaključak.** Segmentni žučni kanali jetre mogu se eksplorisati bez traume fleksibilnim ureteroskopom. U odabranim slučajevima, kod izolovane litijaze u jednom režnju jetre i odsustva pratećih bolesti, IHL se može lečiti hirurški, bez resekcije jetre. Prikazani slučaj je jedinstven jer nismo uradili resekciju jetre, već ekstrakciju konkremenata iz žučnih kanala, što je bio odgovarajući tretman, imajući u vidu odsustvo recidiva tokom dvogodišnjeg praćenja.

Ključne reči:
žučni putevi, intrahepatički; holedoholitijaza; dijagnoza; hirurgija, operativne procedure; ureteroskopi; lečenje, ishod.

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Introduction

Intrahepatic lithiasis (IHL) is defined as the presence of gallstones in the bile ducts located proximally from the junction of the main hepatic ducts (left or right hepatic duct, sectional and segmental ducts, and their branches) [1]. Primary IHL is rare in Europe, and difficulties encountered in the etiological classification have led to the absence of generally accepted standard treatment [2]. Therefore, there is a high risk of residual and/or recurrent stones.

The highest incidence of primary IHL is present in Asia, predominantly in China, Japan, and South Korea, while the relative incidence in the Western world is about 1%. The disease occurs in people with lower socioeconomic status [3]. Primary IHL occurs more often in the 5th and 6th decades of life without gender preference. However, concomitant intrahepatic and extrahepatic stones are present in the older age groups (7th and 8th decades) and are found in approximately 70% of all cases of hepatic lithiasis [4]. However, it is not the same entity, and only several cases have been reported of primary IHL patients under 30 years of age.

In this paper, we present steps in diagnosis and our own experience in treatment using a flexible ureteroscope of 4 Fr for successful sparing surgical treatment of primary IHL in a young patient.

Case report

A 25-year-old man was admitted to our hospital for the treatment of previously diagnosed IHL. The patient had abdominal pain, nausea, and vomiting a month prior to hospitalization. Gallstones in the common biliary duct and IHL were diagnosed on abdominal ultrasound. The patient reported no chronic diseases, denied previous surgeries, and suggested allergies to penicillin, metronidazole, and ciprofloxacin. His mother suffered from gallbladder stones, and his father had chronic renal failure. The patient had no surgical interventions in the past.

On admission, he had light tenderness in the upper right quadrant of the abdomen. Increased serum values of direct bilirubin of 15 mmol/L [normal range (NR) 0–5 mmol/L] and indirect bilirubin of 34 mmol/L (NR 2–15 mmol/L) were found in the laboratory findings on admission. Findings of other laboratory parameters were presented in normal ranges. Magnetic resonance (MR) imaging of the abdomen showed segmental stenosis of the left bile duct and segmental bile duct for the lateral section with intraductal calculi and its proximal dilatation and mild dilatation of the bile ducts for liver segments II and III. The gallbladder calculi were also found, as well as the direct confluence of the right anterior sectional duct into the common hepatic bile duct. Extrahepatic bile ducts were of regular contour, width, and lumen without defined calculi.

Upper endoscopy showed no pathologic findings, while endoscopic ultrasonography (US) indicated multiple calculi of the left bile duct and one calculus in the right bile duct.

After preoperative counseling, an open surgery was performed. Intraoperatively, the gallbladder had a thickened wall, and a 10 mm stone diameter was found in its lumen. Following choledohotomy and extrahepatic and intrahepatic bile duct exploration with biliary forceps, a stenosis of the left hepatic duct was found. During surgery, a cholangiography and US of the liver were performed. An IHL has been verified for stenosis of the left bile duct and segmental distal dilatation (Figures 1 and 2). The variation of intrahepatic bile ducts was found with a separate drainage of the anterior right segmental branch into the common hepatic bile duct.

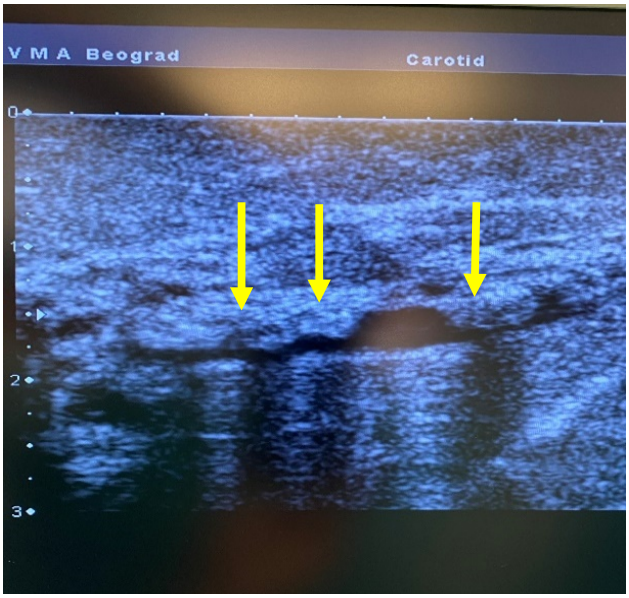

Fig. 1 – Intraoperative ultrasound with visible calculi in the intrahepatic bile ducts.

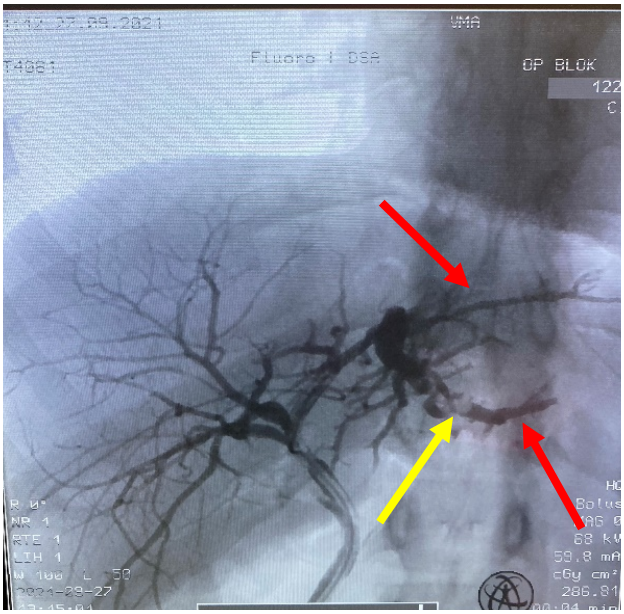

Fig. 2 – Intraoperative cholangiography with intrahepatic calculi in liver segments II and III (yellow arrow) and proximal dilatation of intrahepatic bile duct (red arrows).

The gallbladder was removed first. Through choledochotomy, the bile ducts were flushed with saline, and extirpation of the several calculi was performed (Figure 3). An Olympus (Melville, NY, USA) choledochoscope (URF-P2; outer diameter, 2.8 mm; channel, 1.2 mm) was then introduced into the biliary tree through choledochotomy; however, segmental ducts could not be explored due to the smaller diameter of the ducts themselves than the choledochoscope. A 0 [Flex X2 (FO) (Karl Storz®)] with a diameter of 4 Fr was used to confirm duct clearance (Figure 4). The remaining calculi were extracted subsequently. At the end of the operation, a biliary T-drain was placed.

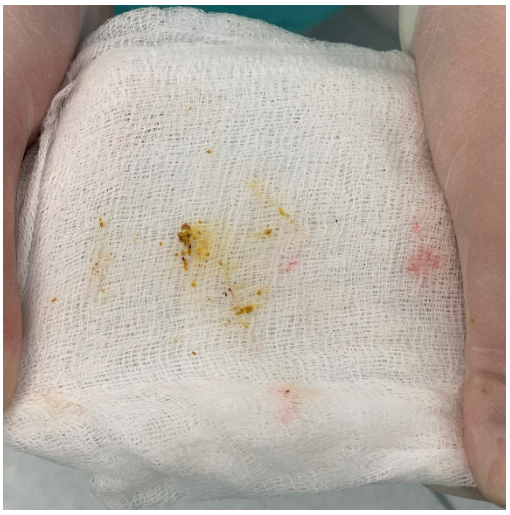

Fig. 3 – Removed bile duct calculi from the left biliary tree.

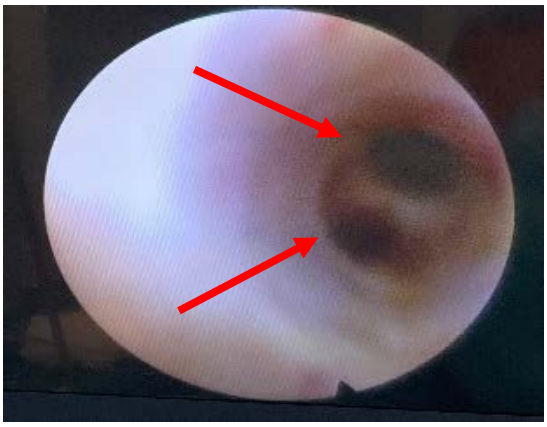

Fig. 4 – Confirmation of bile ducts clearance bile with flexible ureterorenoscope.

The postoperative course was uneventful. Our patient was taken care of for one day in the Intensive Care Unit and was, after that, transferred to the Department of Abdominal Surgery. On the sixth postoperative day, the patient was discharged from the hospital. Biliary T-drain was removed after six weeks following normal cholangiography and a normal level of bilirubin in the blood. During 12 and 24-month follow-ups, the patient was in good condition without rest IHL on repeated MR cholangiopancreatography (MRCP).

Discussion

The highest incidence of IHL occurs between the 5th and 6th decades of life and is usually distributed between 30 and 70 years of age [4]. IHL is more common in younger ages, while both IHL and calculi in the extrahepatic biliary tree occur in older groups. Usually, the first symptoms of IHL occur between the ages of 30 and 50, but sometimes earlier [5].

The differential diagnosis of IHL includes primary sclerosing cholangitis (PSC), recurrent pyogenic cholangitis, bile acid transporter defect, and Caroli's disease [1, 3, 6]. PSC is characterized by biochemical cholestasis, it generally affects the entire biliary tract and is not isolated on one side of the liver [7]. Recurrent pyogenic cholangitis is a chronic infectious process characterized by recurrent attacks of acute bacterial cholangitis in the environment of intrahepatic bile duct dilatation and strictures, which is endemic in Southeast Asia [8]. Bile acid transporter defects, such as ABCB4/MDR3, may occur with IHL at a younger age, but the absence of disease in the right hepatic duct system disputes this as a cause in our patient [6, 9]. Caroli's disease has been described as a congenital malformation of the intrahepatic bile ducts, characterized by the following: segmental cystic dilatation of the intrahepatic bile ducts; increased incidence of biliary lithiasis, cholangitis, and liver abscess; absence of cirrhosis and portal hypertension; association of renal tubular ectasia or similar renal cystic disease. The mode of inheritance is still unclear, but in most cases, it is transmitted in an autosomal recessive manner [10]. However, the MR in our patient and intraoperative US and cholangiography did not support this diagnosis. Finally, the cause of IHL in our patient remains unknown. A cause may be an undiscovered genetic factor due to the positive family history [6].

It is reported that a variation of segmental bile duct drainage may be associated with IHL [11]. Cranial shifting of the right or left sectorial ducts proximal to the hepatic confluence can cause bile stasis and enhance the formation of IHL. Our patient had a separate drainage of the anterior right segmental branch into the common hepatic bile duct. Therefore, the possible mechanism in our patient may be the bile stasis in the left-sided bile ducts; however, the MRCP on follow-up examination 12 months after surgery does not justify this hypothesis.

Primary IHL may be managed surgically and by using nonsurgical alternatives. These conservative or minimally invasive approaches include medical therapy, extracorporeal shock wave lithotripsy, electrohydraulic lithotripsy, transhepatic approach, and endoscopy, but do not eliminate the risk of recurrence neither provides complete clearance of bile ducts [2, 12–14]. On the other hand, endoscopic sphincterotomy and stone removal using endoscopic retrograde cholangiopancreatography may be associated with low rate success and relatively high morbidity, especially in cases with calculi in segmental bile ducts [15, 16]. Alternative to this minimally invasive technique is open surgery or laparoscopy. The main challenge with bile duct exploration is the large impacted stones that cannot be managed using a flexible choledochoscope as it has a narrow working channel and instruments

like graspers cannot be forced through it. In the right or left main bile ducts, a flexible choledochoscope may enter; however, to break stones, it is necessary to apply a holmium laser or an electrohydraulic lithotripter, which are very expensive and not available in most of the developing world [17, 18].

The usage of nephroscope, ureteroscope, and ureterorenoscope in bile duct exploration and removal of bile duct stones has been described in only two reports [19, 20]. In addition, in those reports, the laparoscopic approach used a rigid ureteroscope. A probable reason why a rigid ureteroscope was used lies in the fact that a flexible fiberoptic instrument is composed of thousands of densely packed flexible glass fibers, which is liable to damage [19]. In open surgery, gentle movements allow the safe introduction of fiberoptic instruments into the segmental bile ducts, which is not possible in laparoscopy. In our case, we used a flexible ureterorenoscope of 4 Fr for the exploration of bile ducts for liver segments II and III. The scope could be maneuvered into the distal and proximal bile ducts easily. To our knowledge, this is the first exploration of bile ducts with a flexible ureterorenoscope.

It has been reported that liver resection is the only valid treatment of primary IHL since it removes both stones and the involved area, thus eliminating the risk of recurrence [11, 21, 22]. However, the majority of patients who underwent liver resection for IHL had some concomitant disease (Caroli's syndrome, cholangitis, and/or previous biliodigestive anastomosis) or had recurrent IHL [11, 21]. In such patients, performing a hepatic resection as a treatment of choice is justified. When the etiology of primary IHL is unknown or uncertain, and the absence of cholangitis, hepatectomy, as a first-line treatment, should be avoided whenever possible. In our patient, we performed successful bile duct stone extraction without liver resection, which was an appropriate treatment since there was no recurrence during two-year follow-ups.

Conclusion

Primary IHL is rare at a young age, and as such, it needs additional diagnostic tools to illuminate possible etiological factors. Segmental liver bile ducts may be explored successfully with a flexible ureteroscope, which provides adequate visualization and maneuvering without bile duct injury or trauma. In selected cases with unknown etiology of IHL, isolated lithiasis in one liver lobe, and absence of concomitant diseases, IHL can be treated surgically but without liver resections.

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Neonatal multisystem inflammatory syndrome during acute SARS-CoV-2 infection

Multisistemski zapaljenski sindrom kod novorođenčadi tokom akutne infekcije SARS-CoV-2

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Abstract

Introduction. During the development and spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic, a new inflammatory response syndrome arose in newborns, defined as the multisystem inflammatory syndrome (MIS) in neonates (MIS-N). **Case report.** A term infant girl with a fever diagnosed with SARS-CoV-2 infection was admitted to the hospital. In laboratory findings, the values of lactate dehydrogenase, ferritin, interleukin-6, and D-dimer were elevated. Upon admission, dual parenteral antibiotic therapy (ceftazidime, vancomycin), and one day later, low molecular weight heparin (LMWH) therapy, was commenced. After five days of hospitalization and febrility, with negative results of microbiological analyses and further deterioration of laboratory findings, intravenous immunoglobulin (IVIg) was administered at a dose of 2 g/kg for one day and methylprednisolone at a dose of 1 mg/kg/day for four days, after which the reduction of corticosteroid therapy was continued with prednisone. One day after IVIg dministration, the newborn became afebrile, with the gradual normalization of laboratory findings. The newborn was discharged after 16 days of hospitalization. Ten days after discharge, prednisone therapy was discontinued. Two weeks after discharge, the administration of heparin was discontinued. Seven days later, the D-dimer value increased significantly, and the anticoagulant therapy was reinstated. After one month, the D-dimer value completely normalized, and the LMWH therapy was discontinued. **Conclusion.** After the applied therapy for MIS in children, there was a cessation of febrility and gradual normalization of values of the laboratory parameters. This confirms that the newborn, in this case, probably had MIS-N. The prolonged elevated D-dimer value was most probably a consequence of the MIS.

Key words:
covid 19; diagnosis; fibrin fragment d; heparin, low-molecular-weight; immunoglobulins, intravenous; infant, newborn; inflammation; syndrome.

Apstrakt

Uvod. Tokom razvoja i širenja epidemije izazvane *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2), pojavio se novi sindrom zapaljenskog odgovora kod novorođenčadi koji je označen kao multisistemski zapaljenski sindrom (MZS) kod novorođenčadi (MZS-N). **Prikaz bolesnika.** Terminsko novorođenče, devojčica, primljeno je u bolnicu zbog povišene telesne temperature i dijagnostikovane infekcije SARS-CoV-2. U laboratorijskim nalazima nađene su povišene vrednosti laktat dehidrogenaze, feritina, interleukina-6 i D-dimera. Po prijemu, započeta je dvojna antibiotska terapija (ceftazidim, vankomicin) parenteralno, a dan kasnije, i terapija heparinom niske molekulske mase (HNMM). Nakon pet dana hospitalizacije i febrilnosti, pri čemu su nalazi mikrobioloških analiza bili negativni, a zbog daljeg pogoršanja vrednosti laboratorijskih nalaza, primenjena je terapija intravenskim imunoglobulinom (IVIg) u dozi od 2 g/kg jedan dan i metilprednizolonom u dozi od 1 mg/kg/dan četiri dana, nakon čega je nastavljeno postepeno smanjenje terapije kortikosteroidima primenom prednizona. Jedan dan nakon primene IVIg-a, novorođenče je postalo afebrilno, uz postepenu normalizaciju laboratorijskih nalaza. Dete je otpušteno posle 16 dana hospitalizacije. Deset dana posle otpusta, prekinuta je terapija prednizonom. Dve nedelje nakon otpusta, prekinuto je davanje heparina. Sedam dana kasnije, vrednost D-dimera je značajno porasla i terapija

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antikoagulansom je ponovo započeta. Mesec dana kasnije, vrednost D-dimera se potpuno normalizovala, i terapija HNMM-om je prekinuta. **Zaključak.** Nakon primenjene terapije za MZS kod dece, došlo je do prestanka febrilnosti i postepene normalizacije vrednosti laboratorijskih parametara, što ukazuje da je novorođenče u ovom slučaju imalo MZS-N. Povišena vrednost D-dimera tokom produženog vremenskog perioda je najverovatnije bila posledica MZS.

Ključne reči:
covid 19; dijagnoza; d dimer; heparin, niskomolekulski; imunoglobulini, intravenski; novorođenče; zapaljenje; sindrom.

Introduction

In the latter half of April 2020, a new syndrome in children and adolescents associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was described for the first time and was named the multisystem inflammatory syndrome (MIS) in children (MIS-C) [1, 2]. The World Health Organization has developed a case definition for MIS-C, which includes children up to 19 years of age with clinical signs of elevated body temperature that persists for more than three days, involving two or more organ systems (digestive system, skin and mucous membranes, cardiovascular system), elevated markers of inflammation, and evidence of the existence of SARS-CoV-2 infection without another microbiologically proven cause of inflammation [3]. This syndrome is relatively rare but potentially a severe and life-threatening complication of SARS-CoV-2 infection [4, 5]. This syndrome also occurred in newborns and was named MIS in neonates (MIS-N). There is no clear agreed definition of MIS-N, as well as no guidelines and protocols for the diagnosis and treatment of this complication [5].

Case report

A term infant girl became ill on the day of admission to the hospital, on day eight after birth, with the appearance of elevated body temperature and secretion from the nose. This was a child from normal pregnancy, which ended with a vaginal delivery without complication, Apgar score 9/10. The newborn was breastfed since birth.

On admission to the hospital, the baby was awake, eupneic, and tachycardic. Body temperature measured rectally was 38.2 °C (normal temperature measured rectally is 36.5 °C–37.5 °C). In the physical findings, only the yellow discoloration of the skin stood out. Upon admission, a nasopharyngeal swab was performed: the reverse transcriptase-polymerase chain reaction test for SARS-CoV-2 for the child and the mother was positive. The mother had suffered from nasal discharge, cough, and fever, which started two days before the child was admitted to the hospital. Until then, the mother had no confirmed COVID-19 infection and did not receive the vaccine against COVID-19. Initial laboratory analysis was performed (Table 1). Chest X-ray, ultrasound of the central nervous system and abdomen, and echocardiographic findings were normal. Furthermore, upon admission to the hospital, blood culture, urine culture, and stool were sampled; after that, dual empirical parenteral antibiotic therapy (ceftazidime, vancomycin) was commenced. Dual empirical antibiotic therapy was started because of suspected late neonatal sepsis and was applied until sepsis was ruled out. Due to elevated D-dimer value (> 2,500 ng/mL, reference range 0–230 ng/mL), low molecular weight heparin (LMWH) was introduced at a dose of 100 IU/kg/day. Febrility persisted with a further increase in lactate dehydrogenase, ferritin, interleukin-6 (IL-6), and persistence of elevated D-dimer values (Table 1) without the involvement of other organ systems.

After five days of hospitalization and febrility, with negative results of the blood and urine culture and further deterioration of laboratory findings, intravenous immunoglobu-

Table 1

Laboratory results

Parameter	Reference range	Days of hospitalization				
		1	3	5*	11	18
C reactive protein (mg/L)	0–5	0.72	0.78	0.76	0.46	0.3
Albumin (g/L)	38–60	/	33	/	29.79	33.85
GGT (IU/L)	13.8–132	184.8	119.88	125.04	76.2	78.6
LDH (IU/L)	180–433.2	544.2	522	696	683.4	381
Interleukin-6 (pg/mL)	0–6.4	/	64.9	104	23.7	3.0
D-dimer (ng/mL)	0–230	/	>2,500	>2,500	1,620	540
Ferritin (μg/L)	5–204	/	863	1,582	/	781
High-sensitivity troponin I (ng/L)	**	/	35.6	41.5	/	28.3
CK-MB (ng/mL)	**	/	10.7	7.5	/	2.7
Bilirubin (mmol/L)	0.01–21	256	/	82.84	/	32
Indirect bilirubin (mmol/L)	0.01–16	242.9	/	74.64	/	29

GGT – gamma-glutamyl transpeptidase; LDH – lactate dehydrogenase; CK–MB – creatine kinase- myoglobin binding.
***On this day, a decision was made to begin therapy with intravenous immunoglobulins due to further deterioration of the values in the laboratory findings.**
****In the laboratory where CK-MB and high-sensitivity troponin I analyses were performed, reference values for the pediatric population were not specified.**

lin (IVIg) therapy was administered at a dose of 2 g/kg for one day. Then, parenteral corticosteroid therapy (methylprednisolone) was started at a dose of 1 mg/kg/day for four days, after which the reduction of corticosteroid therapy was performed with oral corticosteroid (prednisone). One day after IVIg administration, the newborn became afebrile, with gradual normalization of laboratory findings (Table 1). The newborn was discharged after 16 days of hospitalization to continue treatment with prednisone and LMWH at home. Ten days later, prednisone therapy was discontinued. Two weeks after the discharge, the administration of LMWH was stopped (at the D-dimer value of 410 ng/mL). Seven days later, the D-dimer value increased significantly (2,358 ng/mL). The child was hospitalized, and anticoagulant therapy was reinstated. Results of additional laboratory analyses (C-reactive protein, complete blood count, fibrinogen, indicators of kidney and liver function, IL-6, ferritin, and hemostasis parameters) were normal, as well as the findings of radiological and cardiovascular examinations (abdominal and cranial ultrasound, echocardiographic examination). After one month, the D-dimer value completely normalized, and the LMWH therapy was stopped.

Discussion

There is limited knowledge about cases of MIS in the neonatal population. Although the incidence of MIS-N is not known, this entity is rare. In a systematic review that included 27 studies, only 104 cases of neonates with MIS-N were described [5]. The pathophysiological mechanisms of MIS-N have not been fully elucidated. Maternal SARS-CoV-2 infection during pregnancy can cause a hyperinflammatory response in the newborn by transplacental transfer of IgG that binds to the spike protein of the virus. These antibodies cross the placenta to provide passive immunity to the newborn. The patient's mother also had a current COVID-19 infection. As it had happened during the pandemic, some people had COVID-19 infection several times. We had no information if the mother initially had an infection during pregnancy. For technical reasons, an IgG test was not performed for the mother. Postnatally, IgA antibodies are transmitted to the newborn *via* breast milk, which may also play a role in the body's defense against infection [6–9]. In children with a genetic predisposition, antibodies bind to receptors on neutrophils and macrophages, causing the activation and secretion of proinflammatory cytokines responsible for the development of MIS [10, 11]. According to More et al. [12], MIS-N can be divided into early MIS-N, which occurs in the first 72 hours of life due to the transplacental transfer of maternal antibodies, and late MIS-N, which occurs after 72 hours of life as a consequence of the secondary production of antibodies, as part of the current infection of the newborn, but it can also be caused by transplacental transfer of maternal antibodies. The diagnosis of MIS-N in our patient was made on day 12 of life, which, according to these criteria, would correspond to late MIS-N. Given the specificity of the neonatal age, the question arises of the possibility of applying the proposed MIS-C criteria [13]. In our case, the newborn met the following criteria for the diagnosis of MIS-C: febrile for longer than five days without other signs of infection and pathological values of laboratory findings. Despite negative microbiological analyses (blood, urine, and stool culture for bacteria and fungi) and the applied broad-spectrum empirical antibiotic therapy, the parameters of inflammation continued to increase, which made us consider the MIS-N diagnosis in our case.

The American College of Rheumatology has issued guidelines for MIS-C diagnosis and treatment without special recommendations for the treatment of MIS-N [14]. The treatment strategy mainly follows the treatment standards for Kawasaki disease due to the overlapping features. Although both diseases are systemic inflammatory diseases, they differ in their diagnostic criteria. For the treatment of MIS-C, high doses of IVIg (1–2 g/kg) and corticosteroids 1 mg/kg are generally recommended, which was applied during the treatment of the newborn in our case summary [15–17].

Conclusion

After the applied therapy, there was the cessation of febrility and gradual normalization of laboratory parameters, which implies that the newborn in question probably had MIS-N. In this case, prolonged administration of LMWH was required. Since the presence of deep vein thrombosis was ruled out by supplementary testing, we believe that the prolonged elevated D-dimer value is a consequence of inflammation as part of a multisystem inflammatory response. Currently, there is no widely accepted standard for the treatment of MIS-N, but only recommendations based on case series, reports, and experience in the treatment of MIS-C. There is a need for further research in this field because as much as MIS-N resembles MIS-C, it remains significantly different.

Conflict of interest

The authors declare no conflict of interest.

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

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

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

Literatura

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

Primeri referenci:

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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.

Skraćenice i akronimi

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

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

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