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Janus Kinase Inhibitors in the Treatment of Alopecia Areata

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Abstract: Alopecia areata is a disease of autoimmune origin which causes non scarring hair loss. The extent of alopecia varies from a small patch to complete scalp and body hair loss, which can have huge psychosocial impact for those affected. Treatment modalities which have been used so far included nonspecific immunosuppressive medications, such as corticosteroids, cyclosporine, and methotrexate, or topical immunomodulators, such as diphencyprone, dithranol, and squaric acid dibutylester. The recognition of the importance of Janus kinase pathway in alopecia areata pathogenesis enabled more specific approaches in treatment. Positive outcomes of Janus kinase inhibitors in several trials granted approval for baricitinib which became the first on-label treatment for alopecia areata. The aim of this review is to summarize the role, efficacy and safety of several Janus kinase inhibitors in alopecia areata.

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Introduction

Alopecia areata is an autoimmune disease which causes reversible hair loss. It affects around 2% of population worldwide regardless of ethnicity and gender (Darwin et al., 2018). Autoantibodies are formed and attack the follicular hair cells of the bulb disturbing the anagen phase and causing hair loss (Darwin et al., 2018). Any hair-bearing region can be attacked, mainly the scalp but also facial and body hair. Hair loss can vary, from a small patch (patchy alopecia) to total scalp hair loss (alopecia totalis) or loss of all body hair (alopecia universalis) (Figure 1) (Villasante Fricke and Miteva, 2015). Even though the loss is described as temporary, only around 65% of patients will demonstrate complete hair regrowth within 5 years and almost all of them will experience one or more relapses within 20 years from the first incident (Trueb et al., 2018). Alopecia areata is associated with atopic diseases, such as atopic dermatitis, rhinitis and asthma, and other autoimmune diseases, namely autoimmune thyroid disorders, vitiligo, pernicious anaemia, and diabetes mellitus 1 (Goh et al., 2006).

Diagnosis is nowadays based on dermoscopic identification of black and yellow dots, exclamation mark hairs, broken hairs, short vellus hairs and tapered hairs (Waśkiel et al., 2018). In doubtful cases, a biopsy taken from the margins of an active lesion typically shows the presence of lymphocytes surrounding and invading hair bulbs (Ohyama, 2018).

Damage to the hair follicle occurs when there is a disruption of the protective shield of growing hair, resulting in formation of autoreactive $CD8^+$ T-cells directed against the follicular cells. In healthy individuals, growing hairs enjoy a state of immune privilege which is a result of several mechanisms (Paus et al., 2003). These include the downregulation of major histocompatibility complex class I (MHC) antigens by the follicular bulb cells, local synthesis of cytokines with potent

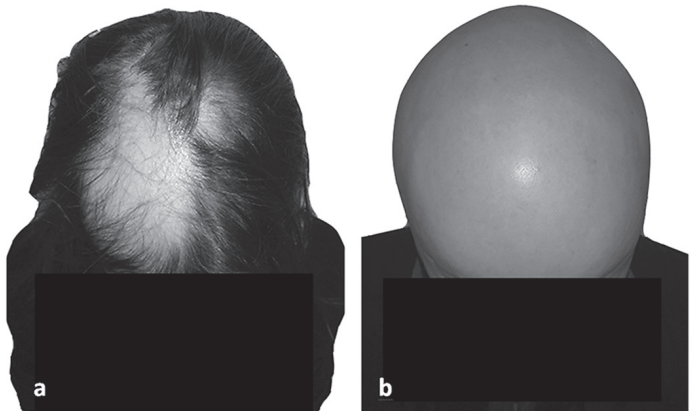

Figure 1 – Clinical manifestation of alopecia areata. a) One large and one smaller patch without hair on the frontoparietal region. b) Alopecia totalis: total loss of scalp hair.

anti-inflammatory activity, such as interleukin 10 (IL-10) and tissue growth factor β1 (TGFβ1), and expression of Fas Ligand (FasL) which causes lysis of autoreactive T-cells (Paus et al., 2003). Genetic mutations in genes encoding MHC antigens as well as environmental factors, such as viral infections, vaccines, low vitamin D blood levels, have been identified as possible triggers for the collapse of follicular protection (Anzai et al., 2019).

In order to suppress the immune reaction and initiate hair growth in patients with alopecia areata, topical or intralesional corticosteroids are prescribed with varying degrees of success. Alternatively, topical immune modulators, namely difencyprolone or anthralin, and topical minoxidil can be applied. In resistant cases, systemic immunosuppressants, such as cyclosporine and methotrexate or oral corticosteroids, are used but are not very effective and may cause several serious side-effects (Alkhalifah et al., 2010).

Until June, 2022, no treatment was officially approved worldwide for the treatment of alopecia areata. On the 13th of June, 2022, the U.S. Food and Drug Administration (FDA) approved Olumiant (baricitinib), a Janus kinase (JAK) inhibitor, for the treatment of adult patients with severe alopecia areata. The aim of this article is to examine the mechanism of action of JAK inhibitors and the current evidence on the safety and efficacy of currently existing drugs of this class in the treatment of alopecia areata.

JAK/STAT pathway

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway plays a key role in maintaining the innate and adaptive immunity. It is an intracellular signalling pathway which controls and affects several hormones and cytokines, including interleukins (IL), interferons (IFNs), growth factors, and colony-stimulating factors (Fragoulis et al., 2019).

The JAK/STAT signalling pathway consists of three components: the receptor, the JAK, which is connected to the intracellular side of the receptor, and the STAT (Fragoulis et al., 2019). JAKs belong to the Janus family of tyrosine kinases (Rochman et al., 2009). There are four members of this group, TYK2, JAK1, JAK2 and JAK3 with each one being expressed in varying concentrations in cells of hemato/lymphopoietic system. STATs are transcription factors with seven members: STAT 1, STAT 2, STAT 3, STAT 4, STAT 5A, STAT 5B and STAT 6 (Rochman et al., 2009).

Binding of a ligand, for example interleukin, to the receptor causes its dimerization, resulting in activation of JAK proteins by transphosphorylation. JAKs phosphorylate tyrosine molecules on the intracellular domain of the receptor which allows STATs to bind. JAK phosphorylate STATs causing their dissociation and translocation to the nucleus in order to regulate expression of genes via DNA transcription. Each receptor is associated with a particular JAK or combination of JAKs which activate a specific STAT leading to transcription of the respective genes. Suppressors of

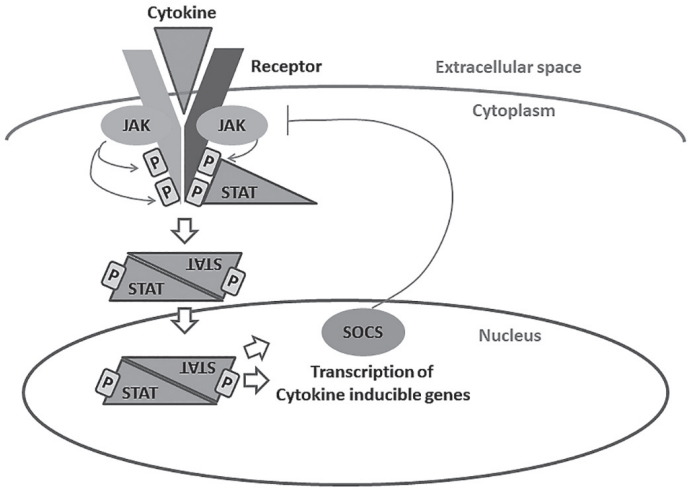

Figure 2 – The JAK/STAT pathway: there are three major components: cell-surface receptors, JAKs and STATs. When a molecule, such as cytokine binds to receptor, JAK prosphorylates the receptor (P molecules). This enables two STATs to bind to the receptor (here only one is shown) which are subsequently prosphorylated by JAK and form a STAT-STAT dimer. The activated dimer enters the nucleus and activates the transcription of target genes, such as cytokine-inducible genes. Suppressors of cytokine signalling (SOCS) are also produced and act as negative feedback.

cytokine signalling (SOCS) act as negative feedback and are responsible for the termination of the cascade. The key components of the pathway are presented in Figure 2.

The types of cytokines produced via the JAK/STAT pathways depend on the ligand and the different combinations of JAKs linked to the receptor. For example, interleukin 15 (IL-15) signals through JAK1 and JAK3 whereas interferon γ (IFNγ) through JAK1 and JAK2. In alopecia areata autoreactive $CD8^+$ effector T-cells produce IFNγ which binds to the IFNγ receptors on follicular epithelial cells and via JAK1/2-STAT pathway induces the production of IL-15. IL-15 binds to receptors on $CD8^+$ T-cells and via JAK1/3-STAT pathway signals the production of more IFNγ. This positive feedback loop potentiates the inflammatory response and leads to disruption of anagen phase with consequent hair loss (Zhou et al., 2021).

JAK inhibitors

JAK inhibitors are small molecules which bind to Janus kinases and disrupt the signalling cascade. Because of their immunomodulatory action, they have been studied and used in the treatment of several myelodysplastic and inflammatory diseases, namely polycythaemia vera, essential thrombocytopenia, rheumatoid arthritis, Crohn's disease, alopecia areata and atopic dermatitis (Kerschbaumer et al., 2020). Ruxolitinib, tofacitinib and baricitinib belong to the first generation of JAK

inhibitors which is not very selective and is able to block more than one type of JAK. With increasing knowledge on the function and importance of each JAK type, more selective JAK blockers have been developed and are tested for their safety and efficacy with various results.

In contrast to biologic therapy which uses large molecules-antibodies administered usually subcutaneously, JAK inhibitors are orally administered, rapidly absorbed and have short half-lives mostly between 4–8 hours. They undergo hepatic metabolism and get excreted in urine and faeces at varying degrees (Ma et al., 2019; Ramírez-Marín and Tosti, 2022).

JAK inhibitors in alopecia areata

As alopecia areata is an autoimmune T-cell mediated disorder, disruption of downstream signalling initiated by pro-inflammatory cytokines, such as IFNγ, could hinder leucocyte recruitment to the hair follicle and block the release of cytotoxic granzymes which are responsible for hair loss. After their approval for other diseases, JAK inhibitors were tried off-license in a small number of patients with alopecia areata with satisfactory outcomes. As a result, several phase 2 and phase 3 clinical trials were designed for patients with this type of autoimmune hair loss.

1) Tofacitinib

This JAK1/3 inhibitor was the first drug of its category to be produced. It is licensed in European Union for the treatment of ulcerative colitis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and juvenile idiopathic arthritis (European Medicines Agency, 2017). Its effect on alopecia areata was first noted in 2014 in a young male patient with alopecia universalis and psoriasis (Craiglow and King, 2014). Complete regrowth was achieved after 8 months of therapy with tofacitinib. Since then, several studies have been published in patients with moderate-to-severe alopecia with growth rates ranging between 50–90% after 6–12 months of treatment (Kennedy et al., 2016; Liu et al., 2017). Topical tofacitinib has been also formulated and tested but appears to be less effective (Bayart et al., 2017). Side effects after oral administration were limited to grade I and II infections (upper respiratory and urinary tract infections, zoster, folliculitis, conjunctivitis), with rarer occurrences of liver toxicity, thrombocytopenia, neutropenia, hypercholesterolemia and acneiform eruptions (Dillon, 2021). Most side effects were either short-lived or disappeared after drug discontinuation.

However, based on the results of a recent trial that compared the safety and efficacy of tofacitinib with TNF inhibitors in patients with rheumatoid arthritis, the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) concluded that tofacitinib carries an increased risk of serious heart-related events such as heart attack or stroke, cancer, blood clots, and death (Liu et al., 2022). This could potentially apply for other JAK inhibitors but extensive safety data are lacking.

2) Ruxolitinib

Ruxolitinib is a JAK1/2 inhibitor approved for the treatment of myelofibrosis, polycythaemia vera and graft-versus-host reaction (Triyangkulsri and Suchonwanit, 2018). Several case reports on the use of ruxolitinib in patients with alopecia areata have been published (Dillon, 2021). Notably, in an open label trial, Mackay-Wiggan et al. (2016) administered ruxolitinib 20 mg tablets twice daily to 12 patients with severe alopecia for 6 months. By the end of the trial, more than 50% of them achieved nearly complete hair regrowth. Similar to tofacitinib, side effects were limited to upper respiratory infections. No reports of hospitalisation or malignancy were reported.

3) Baricitinib

Baricitinib is a newer potent JAK1/2 inhibitor but can also block JAK3 (Triyangkulsri and Suchonwanit, 2018). In Europe is licensed for the treatment of severe resistant rheumatoid arthritis and atopic dermatitis (European Medicines Agency, 2017). As of 13th of June, 2022, it is also the first drug which is licensed for the treatment of alopecia areata in the United States and has pending approval in the European Union (U.S. Food and Drug Administration, 2022). The decision was based on the results of two randomized phase 3 clinical trials (BRAVE-AA1 and BRAVE-AA2) involving patients with more than 50% hair loss (U.S. Food and Drug Administration, 2022). Around 40% of patients who received baricitinib 4 mg tablets had at least 80% scalp covered by hair in contrast to only 6% of the placebo group after 36 weeks.

Side effects include upper respiratory and urinary tract infections, acne, increased low-density cholesterol and creatine kinase levels, and herpesvirus infections (European Medicines Agency, 2017).

4) Ritlecitinib

JAK3 is exclusively associated with the common gamma chain receptor; therefore, it is involved in the functions of T-cells, B-cells and natural killer (NK) cells but does not interfere with metabolic and hematopoietic pathways (Ramírez-Marín and Tosti, 2022). Ritlecitinib is a highly selective and irreversible JAK3 inhibitor. It also blocks another family of tyrosine kinases found in hematopoietic cells, called tyrosine kinase expressed in hepatocellular carcinoma (TEC), because they share similar chemical structure with JAK3 (Ramírez-Marín and Tosti, 2022). Inhibition of JAK3 and TEC blocks the function of $CD8^+$ T-cells, $CD4^+$ T-cells, B-cells, Tregs and NK cells which are involved in the pathogenesis of alopecia areata.

Data for the efficacy of ritlecitinib in alopecia areata were extracted from various ALLEGRO trials. A phase 2b/3 randomised, placebo-controlled double-blind trial involved 718 patients older than 12 years from around the world, including Czech Republic (Pfizer, 2021). Scalp hair loss was required to be greater than 50% persisting for more than 6 months but less than 10 years. After 24 weeks, at least 80% of scalp was covered with hair in a "statistically significant" proportion of patients receiving

ritlecitinib in comparison to placebo. ALLEGRO-LT is a global phase 3 trial in patients older than 12 years with more than 50% hair loss which is currently running and expected to finish by 2026 (ClinicalTrials.gov, 2022).

5) Brepocitinib

TYK2 is an important regulator of IFNα, IFNγ, IL12/23, IL-6 and IL-10 signalling pathways (Winnette et al., 2022). These cytokines are crucial in the pathogenesis of several autoimmune diseases, including chronic plague psoriasis and alopecia areata. Brepocitinib, a TYK2/JAK1 inhibitor, was developed and tested against ritlecitinib and placebo in a randomised study involving 142 adults with more than 50% hair loss persisting for at least 6 months (Winnette et al., 2022). At the end of the 24-week period, the proportion of patients who managed to regain at least 70 of their scalp hair was 64% with brebocitinib, 50% with ritlecitinib and only 2% with placebo.

Safety profile of JAK inhibitors

JAK inhibitors have been only recently used for the treatment of alopecia areata. As a result, the majority of safety data are pooled from studies and reports from patients with different background than individuals with alopecia areata. A recent retrospective study analysed almost 127,000 safety reports from the pharmacovigilance database of World Health Organization (WHO) concerning the use of ruxolitinib, tofacitinib and baricitinib in patients with haematological and autoimmune disorders (Hoisnard et al., 2022). The median age of patients with adverse events was 71 years for ruxolitinib and 61 years for baricitinib and tofacitinib. Ruxolitinib was mainly used for the treatment of oncological and haematological disorders, tofacitinib for the treatment of rheumatoid arthritis and psoriasis, and baricitinib for the treatment of psoriasis. Most reports pertained to higher doses of drugs. JAK inhibitors were associated with herpetic infections, upper respiratory tract infections, musculoskeletal and connective tissue disorders, embolism and thrombosis and neoplasms, such as benign and malignant skin tumours and benign soft tissue neoplasms. Tumours were mostly associated with ruxolitinib. Patients receiving high doses of tofacitinib were in increased risk of gastric perforation.

The safety and efficacy of tofacitinib in alopecia areata was documented in several small retrograde studies and case series. After oral administration, the prevailing adverse events were upper respiratory tract infections, urinary tract infections, acne, and headache, followed by nausea, liver enzyme abnormalities and leukopenia (Kennedy et al., 2016; Liu et al., 2017; Jabbari et al., 2018). Side effects were inconsistent amongst studies, mostly mild and transient. No malignancy was noted. When given topically, tofacitinib was associated with scalp irritation and folliculitis according to one open label study (Liu et al., 2018).

Data on ruxolitinib are also based on case reports and small studies. In one open label study with 12 patients, oral intake of ruxolitinib 20 mg tablets twice daily for

3–6 months resulted in seven patients contracting upper respiratory tract infections, one contracting urinary tract infection, one reporting mild gastrointestinal symptoms and one developing anaemia (Mackay-Wiggan et al., 2016). Adverse events after local administration of ruxolitinib were limited to scalp irritation and folliculitis in a minority of patients (Olsen et al., 2020).

Currently, the only JAK inhibitor registered for the treatment of alopecia areata is baricitinib. Reported side effects from the two phase 3 BRAVE-AA trials, were limited to acne and increased levels of creatine kinase, high-density, and low-density lipoproteins (King et al., 2022). A study on healthy volunteers also reported reduced levels of reticulocytes and neutrophils in some patients (Shi et al., 2014).

Results on the safety of ritlecitinib, a selective JAK3 inhibitor, were reported after 24 weeks of the ALLEGRO phase 2a trial (Ramírez-Marín and Tosti, 2022). Adverse events were reported by almost two thirds of patients. About 20% developed herpes zoster and 10% reported headache, acne, nasopharyngitis and upper respiratory tract infections. Two of the 48 participants were diagnosed with breast cancer and were withdrawn from the study.

The use of JAK inhibitors in pregnant and lactating women is contraindicated (Kerschbaumer et al., 2020). Even though there are no sufficient data in humans, animal studies showed that baricitinib and tofacitinib can be toxic to fetus and are excreted in milk (Jorgensen et al., 2022).

Drug interactions

JAK inhibitors are metabolised in the liver by the cytochrome P450 enzymes (CYP450) but the degree of metabolism as well as the extent or renal excretion varies greatly amongst group members (European Medicines Agency, 2017). More than 99% of ruxolitinib and 70% of tofacitinib undergo first-pass metabolism necessitating dosage adjustment of the drug when strong CYP450 inhibitors or inducers are co-administered (European Medicines Agency, 2017; National Center for Biotechnology Information, 2022).

Studies demonstrated that only 6% of baricitinib is metabolised by C450 enzymes (National Center for Biotechnology Information, 2022). Co-administration of strong CYP450 inhibitors or inducers, such as ketoconazole or rifampin did not affect significantly drug concentrations. However, because baricitinib binds to several transporters, co-administration with strong inhibitors, such as probenecid decreased renal clearance and increased plasma concentrations (European Medicines Agency, 2017). According to labelling, baricitinib dose should be reduced if probenecid is co-prescribed (European Medicines Agency, 2017).

Caution should be also exercised when combining JAK inhibitors with other immunosuppressive agents, such as azathioprine, methotrexate, and cyclosporine, because of the additive immunosuppressive effect.

Relapse after drug withdrawal

Alopecia areata is typified by relapses and remissions. The main concern with the disease management is whether and when will hair falls after drug withdrawal. Several small retrospective and prospective studies involving oral tofacitinib and ruxolitinib reported hair loss in at least 90% of patients three-to-six months after treatment cessation (Ramírez-Marín and Tosti, 2022).

These results demonstrate the importance of continuing the treatment with JAK inhibitors after achieving hair growth at least with some maintenance dose. In BRAVE-AA2 study, patients successfully treated with oral baricitinib 4 mg for a year, were randomized to receive either 4 mg or 2 mg of baricitinib for six months (King et al., 2022). Almost all patients in high dose group and around 75% of patients in low dose group maintain their hair at the end of study.

In a study following the ALLEGRO trial, patients who were successfully treated with oral ritlecitinib for 52 weeks, lost at least 30% of their scalp hair within 4 months after drug withdrawal (Ramírez-Marín and Tosti, 2022). Reintroduction of ritlecitinib for 24 weeks resulted in 70% hair regrowth in only 57% of previously successfully treated patients. This study demonstrated that ritlecitinib loses its efficacy after withdrawal and retreatment.

Conclusion

After the recognition of the importance of JAK/STAT pathway in the pathogenesis of alopecia areata, several JAK inhibitors have been tested in the treatment of this disease with positive outcomes. Baricitinib has become the first representative of its class to obtain registration and is currently the only on-label option for the treatment of alopecia areata. The selective inhibition of JAK3, the key JAK in alopecia areata, appears to provide higher efficacy with more favourable safety profile. Studies to demonstrate the long-term safety of JAK inhibitors are necessary because patients may need to receive maintenance treatment in order to prevent relapse.

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Identification of Pathogenic Microflora and Its Sensitivity to Antibiotics in Cases of the Odontogenic Purulent Periostitis and Abscesses in the Oral Cavity

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Abstract: Odontogenic infections are the most common infectious and inflammatory diseases of the maxillofacial area and problem of the causative pathogen identification is an actual task, part of a permanent process of updating and modernization of treatment and diagnostic protocols and standards. In presented study a purulent exudate from 13 patients with acute purulent odontogenic intraoral lesions was studied by bacteriological method with detection of sensitivity to antibacterial agents. Bacteriological studies showed that genus *Streptococcus* predominated in 69.23% cases. Pathogenic microorganisms in clinically significant concentrations (10^5 per 1 ml and above) (*Streptococcus* and *Staphylococcus*) were resistant to Tetracycline and Doxycycline, had moderate sensitivity to macrolides in 22.22% and resistance in 77.78%. Amoxicillin/clavulanate caused effective growth retardation in 22.22% cases and moderate delay – in 77.78% without cases of resistance. Sensitivity to cephalosporins was detected in 50.00% cases, moderate sensitivity – in 38.89%, resistance – in 11.11%. Fluoroquinolones were the most effective – sensitivity in 72.22% cases, moderate sensitivity – in 22.22%, resistance – in 5.56%. The most effective fluoroquinolones were Moxifloxacin and Ciprofloxacin.

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The highest resistance to antifungal agents was shown by genus *Candida*, antifungal susceptibility was observed only in 20.00% cases. The microbiota of purulent odontogenic inflammation in the oral cavity was identified in clinically significant concentrations in only 61.54% cases with predominance of *Streptococcus*. The most effective antibacterial agents for odontogenic purulent process may be considered among cephalosporins and fluoroquinolones. There is a need to repeat similar studies in other regions of Ukraine and at other times of the year.

Introduction

Odontogenic infections are the most common infectious diseases of the maxillofacial area affecting the human population for a long time. Their nosological forms include a whole number of diseases ranging from periapical abscesses of the jaws to phlegmons of the deep cervical areas and mediastinitis. The success of treatment of abovementioned diseases depends on the timeliness of care (decompression of foci of bone inflammation, tooth extraction and dissection the foci of purulent inflammation in soft tissues), elimination of etiological factors and administration of a rational antibacterial chemotherapy (Connors et al., 2017; Dave et al., 2021). This problem continues to be studied at many scientific and clinical centers around the world and to date a significant amount of scientific and medical information on infectious and inflammatory complications of dental diseases has been collected (Schmidt et al., 2021).

Oral microbiome and odontogenic inflammation of maxillofacial area

The rapid spread of odontogenic purulent infection in the maxillofacial area tissues is determined by a range of factors – both anatomical (close location of large blood vessels, venous plexuses and lymphatic ducts) and immunological response, infectious agent's virulence and its resistance to antibiotics (Heim et al., 2017). Even simple damage of the oral mucosa may be a gateway for the penetration of certain genera of bacteria – *Actinomyces*, *Streptococcus mitis*, *Streptococcus mutans* and *Streptococcus sanguinis* (they normally colonize a tooth enamel), *Streptococcus salivarius* and *Veillonella spp.* (they often inhabit oral mucosa and tongue surface), *Porphyromonas*, *Prevotella* and *Spirochetes* colonize gum surface and gingival sulcus.

The problem of pathogen's identification is part of a permanent process of updating and modernization of treatment and diagnostic protocols and standards for the treatment of infectious and inflammatory diseases of the maxillofacial area. Nowadays, different methods of pathogenic microorganisms' identification may be used (Zhang et al., 2020; Böttger et al., 2021b).

The study of Böttger et al. (2021a) showed that odontogenic abscesses of the maxillofacial area are mainly caused by bacteria involved in the oral microbiome. When using the standard cultural method of pathogen's identification, a causative agent may be identified among 85.50% of patients. Associations of microorganisms were determined in 96.00% of cases, monoculture – 4.00%. Moreover, the

maximum information may be obtained by using molecular genetic methods of identification, especially for anaerobes. In the cases of standard cultivation only the families *Streptococcus*, *Staphylococcus*, and *Prevotella spp*. were recognized. According to the next work by Böttger et al. (2021b), the oral microbiome among patients with odontogenic maxillofacial area abscesses is quite diverse, even compared to healthy patients. Moreover, healthy people often have enough amount bacteria in the mouth with germs with high pathogenic potential. Mostly such infections were caused by anaerobic microorganisms, aerobes and facultative anaerobes play a smaller role. *Prevotella*, *Porphyromonas* and *Fusobacterium* combined with *Veillonella*, *Parvimonas*, *Streptococcus*, *Mogibacterium* and *Filifactor* were the most commonly identified in the purulent exudate. Pathogenic microorganisms of purulent exudate had a higher pathogenic potential compared to those obtained from saliva. Today, molecular genetic diagnosis has become a more accurate method of identifying pathogens in purulent inflammation given the predominance of anaerobic flora in the etiology of such diseases.

As shown in the work of Kang and Kim (2019), odontogenic inflammatory diseases of the maxillofacial area are also most often caused by bacteria from the residents of the oral cavity. Among such strains are more common *Streptococci* – *S. anginosus*, *S. viridans* and others. The data of Plum et al. (2018) showed that in purulent exudate the different microbial associations were identified in 60.00% cases and monocultures in 34.00%. Among the identified pathogens, the most common were α-hemolytic streptococci – *Streptococcus milleri* (32.10%), *Prevotella* (16.80%), and coagulase-negative staphylococci (14.50%). *Candida* and *Morganella spp.* were more common among pediatric patients. Sweeney et al. (2004) showed that the results of studies of the microbiome of purulent foci emphasize regional, age and geographical differences in the microbiological profile of inflammation, susceptibility of pathogenic microorganisms to antibacterial and antimicrobial agents, which affects the development of national and regional protocols for medical care.

The results of studies performed by Zhang et al. (2020) showed that purulent exudate in periapical abscesses revealed the presence of 125 species of bacteria (mostly anaerobes) among which were *Firmicutes*, *Proteobacteria*, *Fusobacteria*, *Bacteroides*, *Actinobacteria*, *Tenericutes*, *Deinococcus-Thermus* and *Spirochaetes*. The most dominant species were *Streptococcus* (13.30%), *Fusobacterium* (11.80%), *Parvimonas* (7.80%), *Prevotella* (6.70%), *Sphingomonas* (5.80%) and *Hafnia* (5.20%). Among the *Fusobacteriae* the most common was *Fusobacterium nucleatum*.

Studies of Turchina and Pinelis (2016) were performed on purulent exudate from abscesses and phlegmons of maxillofacial area. The results showed that patients were more likely to have hemolytic and non-hemolytic *Streptococcus*, *Staphylococcus aureus*, Gram-negative bacilli, bacteroides, diphtheroides, moderately pathogenic *Staphylococcus*, *Micrococci*, *Peptostreptococcus* and *Candida*. According to literature data the microbial landscape of purulent wounds of patients-residents of different inhabitant areas may differ significantly. Hemolytic *Streptococci* and

bacilli or hemolytic *Streptococci* and *Staphylococcus aureus* were more common among urban residents. The microbial flora of rural residents was inferior to urban residents in the number of pathogenic strains. Analysis of microbiota species revealed that purulent secretions from wounds were dominated by pathogenic *streptococci* (α- and β-haemolytic) which had hemolytic activity and *staphylococci* (in particular, *Staphylococcus aureus*) capable of coagulating citrate plasma, also they had anti-lysozyme activity. The study found that leading associations of bacteria include *Staphylococci*, pyogenic *Streptococci*, *Peptostreptococci*, *micrococci*, *Veilonella*, diphtheroides, bacilli, bacteroides, *Escherichia coli* and *Candida*. The study found 100.00% susceptibility of all microorganisms isolated from periostitis (odontogenic subperiosteal abscesses) to cefotaxime, moderate susceptibility of most microorganisms to ampicillin, gentamicin and rifampicin and no susceptibility of most presented microorganisms to Lincomycin and Tetracycline.

According to the analysis of the specialized literature, early detection of pathogens of purulent inflammation and determination of their properties are necessary measures for the successful functioning of health care systems in many countries. This is the basis of infection control and monitoring of the evolution of human pathogens. The constant use of antibacterial agents in outpatient dental practice makes a small contribution to deepening the problem of antibiotic resistance in health care. According to Kabanova (2017), the prevalence of highly pathogenic and resistant to antibacterial drugs microorganisms leads to the development of severe inflammatory processes in maxillofacial area characterized by severe intoxication and impaired immune status. Insufficient effectiveness of treatment of these complications partially may be explained by the presence among microorganisms of some effective protection mechanisms against external damaging factors.

Widespread use of antiseptics and disinfectants in health care facilities, specialized laboratories of biotechnology and food production, as well as in everyday life, provides a pronounced selective effect on populations of microorganisms. This process promotes the selection of resistant strains among microorganisms and requires new tools and approaches to save an antibiotics' curative effect (Kryvtsova et al., 2018; Ivanova et al., 2019). Microorganism resistance to antimicrobial substances may be natural or acquired. The first type of resistance is characterized by the absence of target microorganisms for the action of antimicrobial drugs or its unavailability. Acquired resistance is due to the effects of antimicrobial drugs on microorganisms, especially in low concentrations. In recent decades, the study of the mechanisms of bacterial survival had an especial importance. The widespread of antibiotic resistance among infectious agents poses a serious threat to the health care systems of most countries. The next statements should be minded: clinical strains of *streptococci* are the most commonly identified bacteria resistant to antibiotics, especially to Penicillin and Clindamycin (Heim et al., 2021).

According to Tent et al. (2019), from 7.00 to 10.00% cases of antibiotics usage in clinical practice accounted for cases of infectious lesions of the head and neck. Since

the introduction of the first antibiotics into clinical practice in the human population the strains of pathogenic microorganisms that are not sensitive to antibacterial agents have begun to appear and circulate. That is explained by the evolution of microorganisms (genetic mutations, exchange of genetic material, changes in gene expression and metabolic adaptation).

As shown by Sweeney et al. (2004), today, aminopenicillins are one of the most commonly prescribed groups of antibacterial drugs in dental practice. Since the mid-1980s, strains of beta-lactamase-positive pathogenic and opportunistic bacteria that are resistant to semisynthetic penicillins have been periodically isolated. Although beta-lactamase production is not a characteristic of *streptococci*. Some strains of *Streptococcus salivarius*, *Streptococcus oralis* and *Streptococcus mitis* (except *Streptococcus mutans*) have been shown to be resistant. It has been shown that resistance to penicillin antibiotics may be transmitted between bacteria found in the oral cavity – especially between *S. pneumoniae* and other α-hemolytic *streptococci*. Resistance of oral bacteria to tetracyclines is a new phenomenon, especially for *Streptococci*, and this quality is encoded by a mosaic of 27 *tet-* genes. Along with penicillins, α-hemolytic *Streptococci* may have a high resistance to cephalosporins, especially first- and second-generation cephalosporins (especially Cefotaxime). High resistance to cephalosporins was determined among *Enterococcus spp*. obtained from root canals. Bacterial resistance to Erythromycin has been linked to the acquisition of one of the 21 *erm* genes or by inactivation of the substance by enzymes encoded by the *mph-* and *efflux-* genes (among *Staphylococci*). Resistance to Erythromycin in 38.50% cases was described for α-hemolytic *streptococci* of the oral cavity of healthy patients and in 33.50% cases to Clarithromycin. About 50.00% of oral *streptococci* were resistant both to erythromycin and clarithromycin. This situation is considered to be a consequence of the widespread use of macrolides in the treatment of inflammatory periodontal lesions in the world. The problem of increased resistance of microorganisms to the most commonly used antibiotics affects the effectiveness of treatment of major dental diseases (Kryvtsova and Kostenko, 2020).

Thus, according to Hatilo et al. (2016), cases of ineffective drug treatment of acute and exacerbated chronic apical periodontitis may be explained due to the resistance of odontogenic microflora (*Streptococci* and *Staphylococci*) to the antibiotic used and the state of hypersensitivity to that germs. Therefore, measures to prevent complications in the treatment of acute apical periodontitis with antibiotics should include the use of those antibacterial agents to which the odontogenic microbial flora is most sensitive, in parallel with the administration of antiallergic medicines.

Antibiotic resistance of pathogenic microorganisms that cause odontogenic abscesses of the maxillofacial area has distinct regional and age differences. Although protocols for the treatment of odontogenic abscesses and phlegmon of the maxillofacial area contain mostly recommendations for the use of penicillin antibiotics, it seems more rational to use third-generation cephalosporins, Clindamycin or fluoroquinolones (Kang and Kim, 2019).

From the work of Sobottka et al. (2012) it is seen that in odontogenic abscesses of the maxillofacial area the main etiological factors may be considered the group of *S. anginosus* and hemolytic *Streptococci*, and in inflammatory infiltrates of odontogenic origin the group of *S. mitis* and *Neisseria spp*. Also, the identified strains were found to be highly sensitive to Moxifloxacin.

According to the results of studies performed by Kabanova (2008), for patients with periostitis of the jaws in dental clinics, including stepwise antibiotic therapy, may be recommended the penicillins (Amoxicillin), fluoroquinolones (Ofloxacin, Cyprofloxacin, Pefloxacin), lincosamides (Clindasole). Penicillins (Amoxicillin), fluoroquinolones (Ciprofloxacin, Ofloxacin, Pefloxacin) and sulfanilamides (Co-trimaxosol) are used for outpatient treatment of maxillofacial lymphadenitis. Fluoroquinolones Ofloxacin, Norfloxacin and Ciprofloxacin may be used for the treatment of maxillofacial furuncules at outpatient clinics.

Therefore, odontogenic infectious-inflammatory diseases of the maxillofacial area are the most common complications of dental caries which may occur in clinical practice. Problem may develop due to untimely dental treatment, the lack of a system of medical examination of dental patients and changes in the pathogenic properties of the causative agents of odontogenic inflammatory diseases. Control of etiological agents and their properties, especially the sensitivity of pathogenic microorganisms to antibacterial and antimicrobial agents is an urgent task of the health care system which has become the purpose of this study partly.

The aim of this study was to determine the microbiota of the purulent inflammation focus of odontogenic origin using a bacteriological method followed by a study of the sensitivity of detected pathogenic microorganisms to antibacterial agents.

Material and Methods

The study group included 13 patients who applied for emergency care in the surgical ward of the municipal city dental clinic from March till May of 2021. Patients were aged from 26 to 82 years with average age of 43.54 ± 13.66 (median = 42.00) years. Among the patients there were 8 women (61.54%) and 5 men (38.46%). 11 cases of acute purulent odontogenic periostitis of the jaw (84.62%) and one case of odontogenic abscess of the palate and purulent radicular cyst of the jaw (7.69%, respectively) were identified. All patients were treated with the next standard protocol – conductive and infiltrative local anesthesia with articaine hydrochloride, removal of the causative tooth, hemostasis, dissection and emptying a zone of purulent inflammation, drainage of purulent wound (if possible) for 3–5 days with rinsing with antiseptic solutions, prescribing of general and local anti-inflammatory therapy. Anti-inflammatory therapy included the empirical use of wide-spectrum antibacterial agents, histamine receptor blockers (antiallergic), non-steroid anti-inflammatory drugs (non-selective cyclooxygenase-2 blockers seu pain-killers), local – mouth washing with light alkaline-salt water solutions. Patients were observed

daily – until the removal of the draining latex tape. During each examination revision of the purulent wound was performed together with its rinsing (using a solution of decamethoxin or chlorhexidine bigluconate). All patients reported an improvement in general condition from the second day of treatment, while maintaining a slight asymmetry of the face, resulting from swelling of soft tissues around the affected area.

The samples of the purulent exudate were obtained during dissection of abscess (or accidental damage of its capsule). The samples were placed in a sterile transport system (plastic tube with AMIES gel and applicator for biological fluids). All tubes were transported to the bacteriological laboratory within 24 hours without freezing and over-heating. At the laboratory a passage of the material was performed on the different nutrient media: "Sabouraund Dextrose Agar" ("HiMedia") for the cultivation of microscopic fungi of the genus *Candida*; for hemolytic microflora, in particular bacteria of the genus *Streptococcus* and *Neisseria* – bloody agar, *Enterobacteriaceae* – medium Endo and Ploskirev's agar ("HiMedia"), bacterias of the genus *Staphylococcus* were cultivated on "Mannitol Salt Agar" ("Biolif-Italia") bacteria of the genus *Enterococcus* – on "Bile Esculin Agar" ("Biolif-Italia"). Pure culture of microorganisms was isolated by the method of sector seeding by Gold. Identification of microorganisms was performed due to the morphological, tinctorial, cultural and biochemical properties of bacteria using "ENTEROtest 24", "STREPTOtest 16" and "STAPHYLOtest 16" produced by "Erba Lachema" (Czech Republic).

Susceptibility to antibacterial agents was determined using the method of discs due to the diameter of the growth retardation of the culture on a nutrient medium. Susceptibility was determined to the following antibacterial agents – Doxycycline, Tetracycline, Erythromycin, Azithromycin, Amoxicillin/clavulanate, Ceftriaxone, Cefuroxime, Ciprofloxacin, Norfloxacin, Moxifloxacin and Ofloxacin ("HiMedia"). Itraconazole, Fluconazole, Ketoconazole and Nystatin were selected as antifungals. All measurements were performed three times; in the intermediate spreadsheets the average value was entered. The statistical analysis was performed in the software package Microsoft Excel 2016. All stages of bacteriological studies were performed at the bacteriological laboratory of the Department of Genetics, Plant Physiology and Microbiology, Faculty of Biology, Uzhhorod National University, Uzhhorod, Ukraine (Head of bacteriological laboratory – Professor Maryna Kryvtsova, DrBiolSc.).

Results

Bacteriological studies showed that from the vast majority of patients a monoculture of pathogenic microorganisms was cultivated – 7 out of 13 cases. The following microorganisms were identified in samples of purulent exudate obtained from patients: *Streptococcus agalactiae*, *Streptococcus viridans*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Acinetobacter spp.* and *Candida albicans*.

Among the identified genera of microorganisms, the genus of *Streptococcus* (*S. agalactiae*, *S. viridans*, *S. pyogenes*, *S. pneumoniae*) predominated. Such bacteria were detected in 69.23% cases; the most common was *S. agalacticae* – 30.77%, *S. viridans* and *S. pneumoniae* were found in 15.39% cases each. Bacteria of the genus *Enterococcus* were detected in three cases, which was 23.08% of the total. In one case, a monoculture of the genus *Enterococcus* was detected – 15.39% of the total number of patients – one case of *E. faecalis* and *E. faecium*.

Bacteria of the genus *Staphylococcus* were identified in two cases out of thirteen – 15.39% of the total. In both cases, the culture was defined in the association. In one case *S. aureus* was detected and in another – *S. epidermidis* (which can be considered a representative of the transient microflora, not characteristic of the oral microbiota).

The genus *Acinetobacter* was identified in one case (7.69%) in the association, no further species identification was obtained (Figure 1).

In five cases (38.46%) associations of microorganisms were found that did not recur:

1) *S. viridans* and *S. aureus*
2) *S. agalactiae* and *S. epidermidis*
3) *S. viridans* and *C. albicans*
4) *E. faecalis* and *C. albicans*
5) *Acinetobacter spp.* and *C. albicans*

In the most common association of microorganisms *C. albicans* was presented (three cases out of five).

Analysis of the amount of colony-forming units (CFU) in 1 ml of the isolated bacteria showed that pathogenic microorganisms in the clinically significant

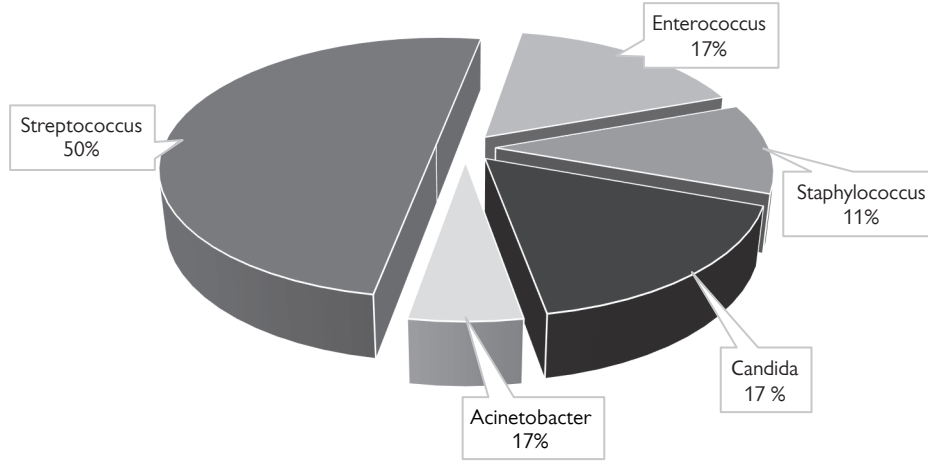

Figure 1 – Representation of detected microorganisms genuses.

Table 1 – Identified microorganisms with the highest resistance to antibacterial agents

Zone of growth retardation of the culture around the disc with the antibiotic, mm	Microorganism	
	Enterococcus faecalis	*Acinetobacter spp.*
Ofloxacin	8.00 ± 0.15	0.00
Moxifloxacin	11.00 ± 0.10	8.00 ± 0.15
Norfloxacin	0.00	0.00
Ciprofloxacin	10.00 ± 0.12	0.00
Cefuroxime	0.00	0.00
Ceftriaxone	0.00	0.00
Amoxicillin/clavulanate	0.00	0.00
Azithromycin	0.00	0.00
Erythromycin	0.00	0.00
Tetracycline	0.00	0.00
Doxycycline	0.00	0.00

concentrations (CFU in the order of 10^5 per 1 ml and above) were found in 8 cases – 61.54% of the total. In such cases, the predominance of the genus *Streptococcus* was observed – in 8 of 13 (61.54%), including one case of association with *S. aureus* (2.1×10^5 CFU/ml), which was 7.69% of the total number of cases. All cases of detection bacteria of the genus *Enterococcus* were marked by clinically insignificant concentrations of microorganisms. Fungi of the *Candida* genus were also found in associations and in clinically insignificant concentrations (10^2–10^4 CFU/ml).

The analysis of the sensitivity of isolated microorganisms' cultures to antibacterial agents revealed that the most resistant bacteria were presented in two clinical cases, they were isolated in clinically insignificant concentrations. *E. faecalis* and *Acinetobacter* were resistant to the whole set of antibacterial agents in this study (Table 1).

Analysis of the level of growth retardation of isolated bacterial colonies by groups of antibiotics showed that the use of medicines from the tetracyclines group (Tetracycline and Doxycycline) was ineffective – all detected bacteria were resistant. The results of the macrolide antibiotics (Erythromycin and Azithromycin) usage were also questionable. Only in 3.57% cases this group was effective, in 14.30% the bacteria were moderately sensitive and in 85.71% the resistance of isolates was determined. Only the one culture of *E. faecium* was sensitive to Azithromycin. The cultures of isolated bacteria were sensitive to semi-synthetic penicillins (Amoxicillin/clavulanate) in 28.57% cases, moderately sensitive – in 57.14% cases and resistant – in 14.29% cases. The cephalosporines group caused an effective growth retardation of microorganisms in 42.86% cases, in 35.71% cases there was a moderate growth retardation, and in 25.00% the resistance was determined. The group of fluoroquinolones was the most effective – effective growth retardation of

Table 2 – Sensitivity of all isolated bacterial isolates to antibiotics, % of cases

Group of antibacterial agents	Sensitive	Moderately sensitive	Resistant
Tetracyclines	0.00	0.00	100.00
Macrolides	3.57	14.29	85.71
Semi-synthetic penicillins	28.57	57.14	14.29
Cephalosporins	42.86	35.71	25.00
Fluoroquinolones	53.57	21.43	25.00

Table 3 – Sensitivity of isolated bacteria of the genus *Streptococcus* to antibiotics, % of cases

Group of antibacterial agents	Sensitive	Moderately sensitive	Resistant
Tetracyclines	0.00	0.00	100.00
Macrolides	0.00	22.22	77.78
Semi-synthetic penicillins	33.33	66.67	0.00
Cephalosporins	38.89	50.00	11.11
Fluoroquinolones	66.67	22.22	11.11

microorganisms was found in 53.57% cases; moderate effect – in 21.43% cases and resistance was found in 25.00% cases (Table 2).

Given that the most common pathogenic bacteria isolated from purulent exudate belonged to the genus *Streptococcu*s the results of determining the sensitivity to antibacterial agents for this subgroup of microorganisms were calculated separately. It was found that the group of tetracyclines does not affect the growth of isolated *Streptococci* – there was 100.00% resistance of cultures. Macrolides caused moderate growth retardation in 22.22% cases and resistance was observed in 77.78% cases. No sensitive bacteria were isolated. Amoxicillin/clavulanate caused effective growth retardation in 33.33% cases, in 66.67% moderate growth retardation was observed, but no resistant isolates were detected. Higher sensitivity to cephalosporines was observed – in 38.89% cases sensitivity was determined, in 50.00% there was moderate sensitivity and in 11.11% – resistance. The medicine for parenteral administration (Ceftriaxone) was more effective than oral Cefuroxime. To Ceftriaxone microorganisms were sensitive in 55.56% cases, moderately sensitive – in 33.33% cases and resistant – in 11.11%. Fluoroquinolones were the most effective in influencing on the genus *Streptococcus*. Sensitivity to fluoroquinolones was determined in 66.67% cases, moderate sensitivity – in 22.22% cases and resistance – in 11.11% cases. The highest sensitivity among the isolated bacteria was observed to Moxifloxacin (88.89% cases and no resistant strains

were detected) and to Ciprofloxacin (77.78% cases and also without the resistant microorganisms). Ofloxacin was found to be the least effective – the amount of sensitive, moderate sensitive and resistant cultures divided equally into three parts (Table 3).

Analysis of properties of the group of pathogenic microorganisms isolated in clinically significant concentrations (CFU of 10^5 per 1 ml and above) which included bacteria of *Streptococcus* and *Staphylococcus*, showed strengthening of previously identified trends in determining the sensitivity of cultures to antibacterial agents. Thus, all isolated microorganisms were resistant to the group of tetracyclines (Tetracycline and Doxycycline). Only moderate sensitivity to macrolides was determined in 22.22% cases, and resistance in 77.78%. Semi-synthetic penicillins

Table 4 – Sensitivity of isolated pathogenic bacteria in clinically significant concentrations to antibacterial agent's groups, % of cases

Group of antibacterial agents	Sensitive	Moderately sensitive	Resistant
Tetracyclines	0.00	0.00	100.00
Macrolides	0.00	22.22	77.78
Semi-synthetic penicillins	22.22	77.78	0.00
Cephalosporins	50.00	38.89	11.11
Fluoroquinolones	72.22	22.22	5.56

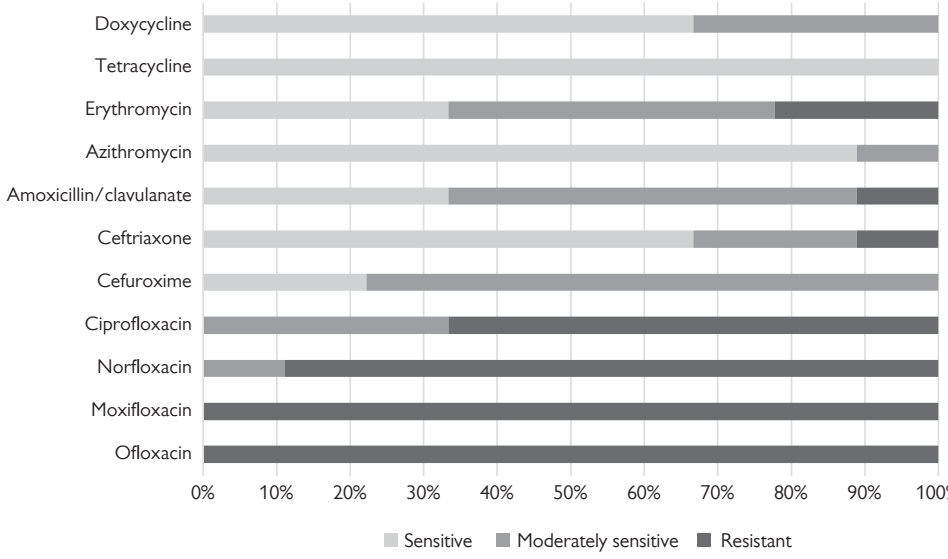

Figure 2 – Sensitivity of pathogenic microorganisms isolated in clinically significant concentrations to antibacterial agents.

Table 5 – Sensitivity of isolated pathogenic bacteria in clinically significant concentrations to Cephalosporins and Fluoroquinolones, % of cases

Antibacterial agents	Sensitive	Moderately sensitive	Resistant
Ceftriaxone	66.67	22.22	11.11
Cefuroxime	33.33	55.56	11.11
Ciprofloxacin	88.89	11.11	0.00
Norfloxacin	33.33	44.44	22.22
Moxifloxacin	100.00	0.00	0.00
Ofloxacin	66.67	33.33	0.00

(Amoxicillin/clavulanate) caused effective growth retardation in 22.22% cases, and moderate delay – in 77.78% cases, resistant microorganisms were not detected. Sensitivity to cephalosporins was detected in 50.00% cases, moderate sensitivity – in 38.89%, resistance – in 11.11%. Fluoroquinolones were the most effective. Sensitivity to them was noted in 72.22% cases, moderate sensitivity – in 22.22%, resistant bacteria – in 5.56% cases (Table 4).

In the group of fluoroquinolones, the greatest effect on microorganisms was caused by Moxifloxacin – 100.00% susceptibility. Ciprofloxacin caused effective growth retardation in 88.89% cases and moderate delay in 11.11 cases. 66.67% of cultures were sensitive to Ofloxacin, 33.33% were moderately sensitive, and no resistant ones were observed. The least effective among the group of fluoroquinolones was Norfloxacin – 33.33% of cultures were sensitive, 44.44% were moderately sensitive and 22.22% of bacterial cultures were resistant (Figure 2).

It should be noted that isolated in clinically significant concentrations of pathogenic microorganisms were not resistant to four used antibacterial agents – Amoxicillin/clavulanate, Ofloxacin, Ciprofloxacin and Moxifloxacin (Table 5).

A study of susceptibility to antifungal agents in selected from associations of fungi of the genus *Candida* revealed a higher resistance of isolated cultures to specific agents. In one case the selected culture of fungi was not sensitive to any of the selected antifungal agents, there was only moderate sensitivity and resistance. In general, antifungal susceptibility was observed in 20.00% of the study cases, moderate susceptibility was determined in 46.67%, and resistance in 33.33%. There were no cultures sensitive to Itraconazole and Fluconazole, all were either moderately sensitive or resistant.

Discussion

Despite improved diagnostic and treatment methods in dentistry, the availability of antimicrobial and antibacterial agents the problem of treatment of acute and chronic inflammatory diseases of the maxillofacial area which occur as a complication of dental caries is relevant to the modern health care system in Ukraine. It is generally

accepted in maxillofacial surgery and general surgery that there is a significant predominance in the etiology of odontogenic abscesses and phlegmones of the maxillofacial area of bacteria of the genus *Staphylococcus* and their associations (Bali et al., 2015; Bertossi et al., 2017; Dregalkina et al., 2020).

In our study the obtained results indicate the predominance of *Lactobacilli* in the etiology of acute odontogenic purulent diseases that occur in the oral cavity as a complication of dental caries. The revealed fact is the basis for revision and clarification of generally accepted approaches to complex treatment of the abovementioned diseases because in the presence of a significant mass of saprophytic, transient and opportunistic microorganisms of the *Lactobacilli* family in the oral microbiota there are reasonable grounds for potentially high risk of biofilms exchange of genetic information between bacteria of the same family which increases the pathogenicity of associations of microorganisms and increases antibiotic resistance in particular (Siqueira and Rôças, 2017). All the identified facts suggest a special attention to the abovementioned contingent of patients and in the choice of rational therapy in particular.

By the way, the biofilming properties of pathogenic microorganisms' isolates were not studied in the current study. And what types of bacterial cultures were received (planktonic or biofilming) remains as an unknown fact. The study of these properties requires sequencing of the bacterial genome in order to identify biofilm-making genes.

The problem of antibiotic resistance increasing among pathogenic microorganisms is relevant for many countries in the world and Ukraine is not an exception. Given the progressive decline in the availability of dental care for the country's population as the health care reform continues which has been affected by the COVID-19 pandemic, it can be argued that untimely visits to the dentist in Ukraine are becoming more frequent, and quite often such patients are treated urgently, in the presence of either acute pain or already acute inflammatory complications of dental caries – such as acute periostitis of the jaws, periodontal abscesses and purulent radicular cysts of the jaws. At the same time, with the availability of antibacterial medicines in the pharmacy network in some cases such drugs are prescribed independently, uncontrolled without following the accepted recommendations for dosage and duration of the course (Palmer, 2016; Koyuncuoglu et al., 2017). In addition, antibacterial agents are widely used in industry and food production, all this contributes to improper selection of antibiotic-resistant microorganisms and in the absence of new available compounds with antibacterial and antimicrobial properties the risks of malignant duration of infectious diseases and their complications may increase significantly (Jagadish Chandra et al., 2017; Sideris et al., 2022).

With regard to dental practice, antibacterial agents are prescribed to patients regularly and it is common practice to empirically select antibacterial agents without identifying the causative infectious agent and due to their sensitivity to antibiotics and antimicrobials compositions. If you open the current local protocols

of dental health institutions which are used to treat acute odontogenic periostitis of the jaws, odontogenic abscesses of maxillofacial soft tissues in an outpatient conditions you can find recommendations for the appointment of complex anti-inflammatory therapy with predominant tetracyclines, macrolides, macrolides, semi-synthetic penicillins and cephalosporins in oral forms. Protocols for the treatment of such diseases in the hospitals contain more recommendations for the use of cephalosporines parenterally and fluoroquinolones for oral administration (Hatilo et al., 2016).

The results obtained in the current study showed that mostly outpatients are patients with dental caries complications where the etiological agent is bacteria of the genus *Streptococcus*. The identified number of cases where the concentration of microorganisms was lower than level of clinical significance may indicate on possible participation in associations of pathogenic bacteria caused the current disease of the other members of the micro-world that were not cultured. They also might include the obligate anaerobes or microorganisms that are difficult to distinguish in standard conditions. Also, such cases may be explained by self-medication of patients and presenting not full information to doctor during visits to the clinic. Therefore, the results obtained on the level of sensitivity of detected pathogens are alarming and are objective prerequisites for qualitative changes in local protocols of empirical antibacterial therapy of acute inflammatory diseases of the maxillofacial area or expanding indications for other antimicrobials of artificial and natural origin.

The obtained results showed that an etiological agent of the inflammatory process was identified in only 61.54% cases with further determination of sensitivity to antibacterial agents. And we can assume that in almost 40.00% cases of odontogenic purulent inflammatory processes in the oral cavity the effect of standard antibacterial therapy remains unknown. That's why detected absence of effective inhibition the pathogenic microorganism's growth by the tetracyclines may not be total. The next hypotheses may be suggested: tetracyclines may be effective in the range from 0.00 to 35.71% cases, moderate sensitivity to this group can also be found in the

Table 6 – Probable ranges of sensitivity to antibacterial agents of pathogenic bacteria caused purulent inflammatory diseases of the oral cavity, % of cases

Group of antibacterial agents	Sensitive		Moderately sensitive		Resistant	
	min.	max.	min.	max.	min.	max.
Tetracyclines	0.00	35.71	0.00	35.71	64.29	100.00
Macrolides	0.00	35.71	14.29	50.00	50.00	85.71
Semi-synthetic penicillins	14.29	50.00	50.00	85.71	0.00	35.71
Cephalosporins	37.50	67.86	25.00	60.71	7.14	42.86
Fluoroquinolones	46.43	82.14	14.29	50.00	3.57	39.29

range from 0.00 to 35.71% cases, similarly, antibiotic resistance may range from 64.29 to 100.00%.

When using the macrolides, the sensitivity of the microflora may also be in the range from 0.00 to 35.71% cases, moderate sensitivity may be determined in the range from 14.29 to 50.00%, antibiotic resistance – from 50.00 to 85.71% cases. Regarding the sensitivity to semi-synthetic penicillins we can assume that it is in the range from 14.29 to 50.00%, moderate sensitivity – from 50.00 to 85.71%, and resistance to this group – from 0.00 to 35.71%.

As for sensitivity to cephalosporines, it may range from 37.50 to 67.86%, moderate sensitivity may be determined from 25.00 to 60.71%, and resistance to this group of drugs – from 7.14 to 42.86%. Regarding the sensitivity to fluoroquinolones, it can range from 46.43 to 82.14%, moderate sensitivity can be outlined in the range of 14.29–50.00%, and resistance – 3.57–39.29% cases (Table 6).

Conclusion

Our study of the microbiota in foci of purulent inflammation of odontogenic origin in the oral cavity showed that the pathogenic microflora was identified in clinically significant concentrations in only 61.54% of cases. Bacteria of the genus *Streptococcus* predominated in the studied samples of purulent exudate. The study of the sensitivity of the detected pathogens to the most widely used antibiotics showed a high level of resistance of such microflora to tetracyclines to macrolides. The most effective antibacterial agents for the treatment of purulent inflammatory diseases of the oral cavity which are caused by *Streptococci* and microbial associations with their inclusion may be considered groups of cephalosporins and fluoroquinolones. Given the common approaches to infection control implemented in many countries around the world, there is a need to repeat similar studies in other regions and at other times of the year to offset regional and circadian effects on macro-, microorganisms and their associations.

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Screening for SARS-COV-2 Using RT-qPCR in Patients with Hematologic Neoplasms Receiving Chemotherapy

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Abstract: It has been recommended that patients with leukaemias and lymphomas undergo universal screening for SARS-COV-2 using RT-qPCR before each treatment on the grounds of their high risk of experiencing severe forms of COVID-19. This raises a conflict with different recommendations which prioritise testing symptomatic patients. We found that among 56 RT-qPCR obtained in asymptomatic patients with hematologic neoplasms before chemotherapy administration, 2 (3.5%) were positive. A negative result did not exclude SARS-COV-2 infection in 1 patient (1.8%). It is unclear what the benefit of screening for SARS-COV-2 using RT-qPCR in patients with hematologic neoplasms who receive chemotherapy is.

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Introduction

The unprecedented sanitary, economic, and social consequences caused by the novel coronavirus SARS-COV-2 and its disease (COVID-19) are widely known, including over 500,000,000 confirmed infections and more than 6,000,000 deaths since the first case was notified in December 2019 (World Health Organization).

A retrospective study carried out in the United Kingdom with 1,044 patients with cancer who contracted COVID-19 determined that the subgroup of patients with hematologic malignancies (leukaemias, lymphomas and myelomas) had a 1.5-fold increased risk of experiencing severe disease, compared with those with solid tumours. Moreover, the former had a greater lethality (OR [odds ratio] = 2.25), and recent chemotherapy was independently associated with a greater mortality (OR = 2.09) (Lee et al., 2020).

On these grounds, different worldwide scientific societies have recommended universal screening for SARS-COV-2 using reverse-transcriptase polymerase chain reaction (RT-PCR) in nasal swabs obtained from asymptomatic patients with leukaemias who are to receive chemotherapy, before each treatment, and screening according to resource availability and the epidemiologic status in the case of Hodgkin lymphomas, with special concern for the lung injury bleomycin may cause (American Society of Hematology, European Hematology Association). Nevertheless, testing in asymptomatic populations, except for a few exceptions, conflicts with the recommendations issued by the World Health Organization, based on the lack of evidence of health impact and cost-effectiveness, and the prioritisation which should be given to symptomatic patients (World Health Organization).

In addition, the decisions made upon a positive test, such as delaying or suspending a chemotherapy, may result in devastating consequences in life-threatening diseases, as in the case of acute leukaemias.

It should not be forgotten that the sole detection of SARS-COV-2 genome in a RT-PCR test does not differentiate between an active infection and the excretion of non-infective viral particles. A determination which can be retrieved in a qualitative RT-PCR (RT-qPCR) is the cycle threshold (Ct), understood as the number of PCR cycles needed to amplify the viral DNA sequences up to a detectable level.

This value maintains an inverse relation with the viral load in the analysed sample. Even though it may be of use in certain selected cases, it has not been formally validated as a prognostic marker (Infectious Disease Society of America).

We aimed to determine the prevalence of asymptomatic detection of SARS-COV-2 genome in patients with hematologic neoplasms admitted to our hospital to receive chemotherapy. We also analysed the impact the positive results had in the clinical course of the infected patients.

Methods

Study setting and population

We designed a retrospective, observational and descriptive study. We included the results of RT-qPCR in nasal swabs from patients older than 18 years of both genders, with diagnosis of leukaemias, lymphomas and myelomas, in any treatment phase, who were admitted to our hospital, a tertiary centre in Buenos Aires, Argentina, to receive chemotherapy from the first confirmed case of COVID-19 in Argentina, March 3rd, 2020, to February 28th, 2022.

We included patients with no symptoms compatible with COVID-19, that is, cough, fever (not even if ascribed to the hematologic disease), dyspnoea, headache, nausea, vomiting, myalgia, anosmia, or disgeusia, and without any unprotected exposure to confirmed COVID-19 patients, within the 7 days prior to admission. Also, the test had to be performed out of any clinical or radiological suspicion. As for patients with prior diagnosis of COVID-19, we only included those with a negative test between the disease and the admission.

The identity of the patients included was preserved. This study was approved by our Hospital's Ethics Committee (Comité de Ética del Hospital de Clínicas "José de San Martín" – IRB approval number: 112821). The procedures followed were in accordance with the Helsinki Declaration of 1975 (revised in 2013) of the World Medical Association.

Data sources

Data were retrospectively collected from medical charts. We registered clinical and epidemiologic information, and, for those patients with positive results, we also registered the Ct value in the respiratory sample. Our laboratory uses the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel 500 rxn (Integrated DNA Technologies, IDT® – Iowa, USA), based on N1 and N2 probes for detecting SARS-CoV-2, and the human RNaseP (RP) as an RNA extraction quality control. We obtained a mean value combining both results.

Data analysis

We determined the rate of SARS-COV-2 positivity among the asymptomatic patients. 95% confidence intervals (95% CI) were also calculated using the 2-sided Clopper-Pearson (exact) method. Statistical analysis was conducted using Stata® 13.1 (StataCorp).

We also analysed the clinical course of the SARS-COV-2 infection in the positive patients, and established what decision was made with regard to the chemotherapy treatment on each individual basis.

Results

Demographic and clinical characteristics

56 RT-qPCR tests for SARS-COV-2 were eligible. The most remarkable characteristics of the patients involved are presented in Table 1.

Table 1 – Characteristics of the origin of the respiratory samples used for SARS-COV-2 screening

		N (%)
Gender	males	26 (46.4)
	females	30 (53.6)
	total	56 (100)
Age	minimum	19
	maximum	82
	average	47
Hematologic neoplasm	lymphoma	33 (58.9)
	acute lymphoblastic leukaemia	13 (23.2)
	acute myeloid leukaemia	8 (14.3)
	myeloma	2 (3.6)

N – number

Primary outcome

Among the total of RT-qPCR tests (n=56), 2 (3.5%; 95% CI = 0.3–12.8%) resulted positive for SARS-COV-2 genome. They belonged to adult males with a diagnosis of lymphoma who denied any related symptoms and had no evidence of clinical or radiological evidence of pneumonia. One of the Ct values was 30 cycles. We were not able to retrieve the other one. Since their clinical status was favourable, we decided to withhold the chemotherapy for 7 days. After this period of time, they received their treatments with no immediate or ulterior complications.

One 49-year-old female (1.8%; 95% CI = 0.004–9.5%) with diagnosis of lymphoblastic T-cell lymphoma had been admitted to receive consolidation treatment. On that day, 48 hours after a negative RT-qPCR test was obtained, she developed fever and odynophagia. The test was repeated and, this time, the result was positive, with a Ct = 26 cycles. She had no signs of pneumonia. The patient was discharged 3 days later fully recovered and returned for chemotherapy on the 10th day after symptom onset with no complications.

Discussion

Patients with hematologic neoplasms have a higher risk of experiencing severe forms of respiratory infections, including COVID-19, than the general population, for several reasons related to the nature of their diseases and their treatments. Among them, these are the most relevant: 1) lymphopenia and neutropenia, 2) hypogammaglobulinemia, and 3) corticosteroid treatment (Assi et al., 2020). The American Society of Hematology reported a 28% mortality rate in a registry of 250 patients with hematologic neoplasms who contracted COVID-19 (Wood et al., 2020). In our country, that very same value was 20.8% (Basquiera et al., 2021).

The prevalence of asymptomatic infection by the novel coronavirus in patients with hematologic neoplasms in the population analysed (3.5%) was higher than reported by Shah et al. (2020) at a tertiary centre in New York: 0.64% among 621 PCR tests performed. In Wuhan, Yin et al. (2020) reported a 2.9% prevalence rate, but it was composed of patients with both lymphomas and solid tumours. We have not succeeded in finding local data to compare our results.

Several elements could influence these values: 1) the collection, storage and processing technique of the respiratory sample obtained by a nasal swab; 2) the homogeneity of the patient's selection criteria; 3) the exclusion of patients with fever which, it is widely known, may be a presenting symptom of acute leukaemia in up to 50–75% of cases (Gavillet et al., 2020). We bring this up because, if those patients whose disease presented with fever had been included, the prevalence would have most likely been even lower.

A standard RT-qPCR test performs a maximum of 40 cycles, after which the result is considered negative. *In vitro*, studies have shown that respiratory samples with Ct > 34 cycles do not possess the capability to infect cell cultures (La Scola et al., 2020). Both Ct values described in our manuscript were relatively high, which correspond to a low viral load.

The decision to withhold chemotherapy for 7 days was an extension of our local sanitary protocols for high-risk exposure and confirmed COVID-19 in immunocompetent patients, given the lack of compelling evidence for this particular population. Needless to say, we cannot tell what the clinical course would have been should we had administered the treatments at the time COVID-19 was diagnosed. On the other hand, the negative test of the female patient mentioned was followed by COVID-19 only 48 hours later, which is why we believe it was of no use, in terms of the purpose for which it was performed.

None of the three SARS-COV-2 positive patients received any specific treatment, as it was not indicated per our hospital's protocols. Each case was assessed on an individual basis, given the lack of universal guidelines. The Ct in the respiratory samples, albeit used with care since it has not been universally validated – as already stated – combined with a chest image, may guide clinical and therapeutic decisions such as whether to proceed with chemotherapy or not.

It is worth investigating whether remdesivir could lower the risk of disease progression in asymptomatic patients with haematological neoplasms. Although this was confirmed in general for outpatients, the cancer population was underrepresented (Gottlieb et al., 2022) and further specific studies are necessary.

These ideas should be highlighted: 1) the prevalence of asymptomatic SARS-COV-2 infection in patients with hematologic neoplasms is relatively low (studies report an even lower rate than our series, which was 3.5%) and its clinical implication is uncertain; 2) the economic cost of universal screening should be contextualised in a worldwide scenario of limited resources; 3) the interpretation of a positive result may be misleading and could derive in treatment delays with

devastating consequences in patients with, for example, an acute leukaemia; 4) a negative test only documents absence of viral excretion through the nasopharynx at the time of the testing. This being said, other screening methods could be considered.

To the best of our knowledge, there is no firm evidence that screening for SARS-COV-2 using RT-qPCR is better, in terms of cost-effectiveness, than the clinical and radiological (Meti et al., 2021). Each institution could consider confronting their own prevalence series with the economic cost of each RT-qPCR test performed.

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A Critical Analysis of the Magnetic Resonance Imaging Lesion Diameter Threshold for Adverse Pathology Features

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Abstract: To investigate the relationship between lesion size determined using multiparametric magnetic resonance imaging (mpMRI) and histopathological findings of specimens obtained after mpMRI fusion biopsy and radical prostatectomy (RP). We retrospectively analysed 290 patients with PCa who underwent an MRI fusion biopsy. We measured the diameter of suspicious tumour lesions on diffusion-weighted mpMRI and stratified the cohort into two groups. Group A included patients with a suspicious tumour lesion 10 mm and Group B included those with a suspicious tumour lesion > 10 mm. In Group B, the PI-RADS score determined in mpMRI was higher than Group A, and there was a statistically significant difference between the two groups in terms of clinical T-stage. The PCa detection rate and the number of positive cores were statistically significantly higher in Group B than in Group A. In addition, there was a statistically significant difference between the two

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groups in relation to the biopsy, the International Society of Urological Pathology (ISUP) grade values, and the presence of clinically significant PCa. In Group B, pathological T-stage and extraprostatic extension (EPE) and surgical margin (SM) positivity were found to be higher among the patients who underwent RP. In the multivariate analysis, the mpMRI lesion size being > 10 mm was found to be an independent predictive factor for SM and EPE positivity. The clinical results of this study support the modification of the lesion size threshold as 10 mm for use in the differentiation of PI-RADS scores 4 and 5.

Introduction

Prostate cancer (PCa) is the most common cancer among males and the second most common cancer worldwide. PCa is divided into clinically significant (csPCa) and clinically insignificant tumours (cisPCa), and this differentiation is directly related to the survival of the patient (Epstein et al., 2016). Although the widespread use of serum prostate specific antigen (PSA) screening has led to a decrease in cancer-related deaths, it also results in a greater rate of cisPCa diagnosis and treatment (Schröder et al., 2014). The priority in the management of patients diagnosed with PCa is to accurately evaluate the presence of csPCa, effectively demonstrate the extent of the disease at the time of diagnosis, and predict the risk of progression (Schröder et al., 2014). For this purpose, multiparametric magnetic resonance imaging (mpMRI) has been increasingly used in recent years, extending the area of use of targeted biopsies and increasing accuracy rates in the differentiation of csPCa and staging (Turkbey et al., 2011). The Prostate Imaging Reporting and Data System (PI-RADS) scoring, which is used to classify and standardize findings defined in the prostate, facilitates the clinical use of mpMRI (Weinreb et al., 2016). Since the PI-RADS scoring used during the evaluation of mpMRI includes some subjective criteria, the sensitivity of the examination varies depending on the experience of the evaluating physician (Weinreb et al., 2016). This increases the importance of using parameters that can be standardized, such as prostate lesion size in order to increase the capacity of mpMRI in determining morphological and functional results, and the lesion size is considered to be correlated with clinical parameters (Lee et al., 2013).

In this study, we aimed to investigate the relationship between lesion size determined using mpMRI and histopathological findings of specimens obtained after mpMRI/transrectal ultrasound (TRUS) fusion biopsy and radical prostatectomy (RP).

Material and Methods

Patient selection and data collection

After obtaining institutional review board approval (2021-258), we retrospectively reviewed the medical records of the patients who were admitted to the urology clinic of our hospital with the suspicion of PCa from January 2017 to June 2019. Inclusion criteria were (i) patients who underwent 3-T mpMRI, (ii) patients who had Prostate Reporting Imaging and Data System v2 (PI-RADS v2) ≥ 3 peripheral zone

lesion with PSA value > 4 ng/ml (iii) patients who had a PI-RADS 2 peripheral zone lesion with PSA value > 10 ng/ml and/or digital rectal examination (DRE) positivity. The exclusion criteria were absence of a 3-T mpMRI examination or the available mpMRI examination having non-diagnostic image quality, having any contraindication to MRI, and absence of fusion biopsy results. The patients included in the study and considered to be eligible for RP underwent robot-assisted laparoscopic RP (RALP) performed by two expert surgeons (S.Ş., A.İ.T.) using DaVinci Xi Surgical System® (Intuitive Surgical, USA). The clinical features of the patients, including age, PSA levels, PSA density (PSAD), prostate volume (PV), number of positive biopsy cores, the largest diameter of suspicious tumour lesions on diffusion-weighted MRI (DW-MRI), postoperative Gleason score, pathological stage, extraprostatic extension (EPE), surgical margin (SM) positivity, seminal vesicle invasion (SVI), and tumour volume were recorded. csPCa was defined according to the Epstein criteria (Epstein et al., 2016).

Multiparametric MRI examination and image analysis

mpMRI was performed using a 3.0-T MR unit (Verio; Siemens Medical Solutions, Germany) with a 16-channel pelvic phased array coil. Imaging sequences comprised thin-section turbo spin echo T2-weighted images (number of slices, 20; slice thickness, 3 mm with no intersection gap; TR/TE, 5800/100 ms; number of signals acquired, 2; and resolution, 0.8×0.8 mm) in the transverse, sagittal and coronal planes. DW images were obtained using multiple b-values (b-factor, 50/500/1000/1500 s/mm^2; number of slices, 20; slice thickness, 3 mm; TR/TE, 3900/75; and resolution, 1.4 mm × 1.4 mm) in the transverse plane and apparent diffusion coefficient maps were constructed from the b50, b100, b1000 and b1500 images by utilizing SyngoVia WorkStation software. Dynamic contrast-enhanced (DCE)-MRI sequences (T1 high-resolution isotropic volume with fat suppression) obtained after the administration of a gadolinium injection (slice thickness, 3 mm; intersection gap, none; TR/TE, 5.08/1.77; resolution, 1.4 mm × 1.4 mm, contrast agent injection started 24 seconds after first acquisition; temporal resolution, 8 seconds; total DCE time, 200 seconds; and number of dynamic time points). The categories determined according to the probability of the csPCa is existed. PI-RADS 2 score is defined as a csPCa unlikely to be present, PI-RADS 3 is equivocal and PI-RADS 4, 5 results were considered as a malignancy is likely to be present. We stratified the study cohort into two groups using a tumour diameter of 1 cm. Group A consisted of patients with normal MRI findings or a suspicious tumour lesion of 1 cm (Figure 1).

Biopsy protocol

Biopsies were performed with the Toshiba (Japan) Aplio-500 Platinum image fusion system. Regions suspicious for malignancy on mpMRI (targeted lesions) were sampled with two cores. This was followed by standard 10-core systemic biopsy. Each biopsy was performed by the same experienced radiologist (R.T.).

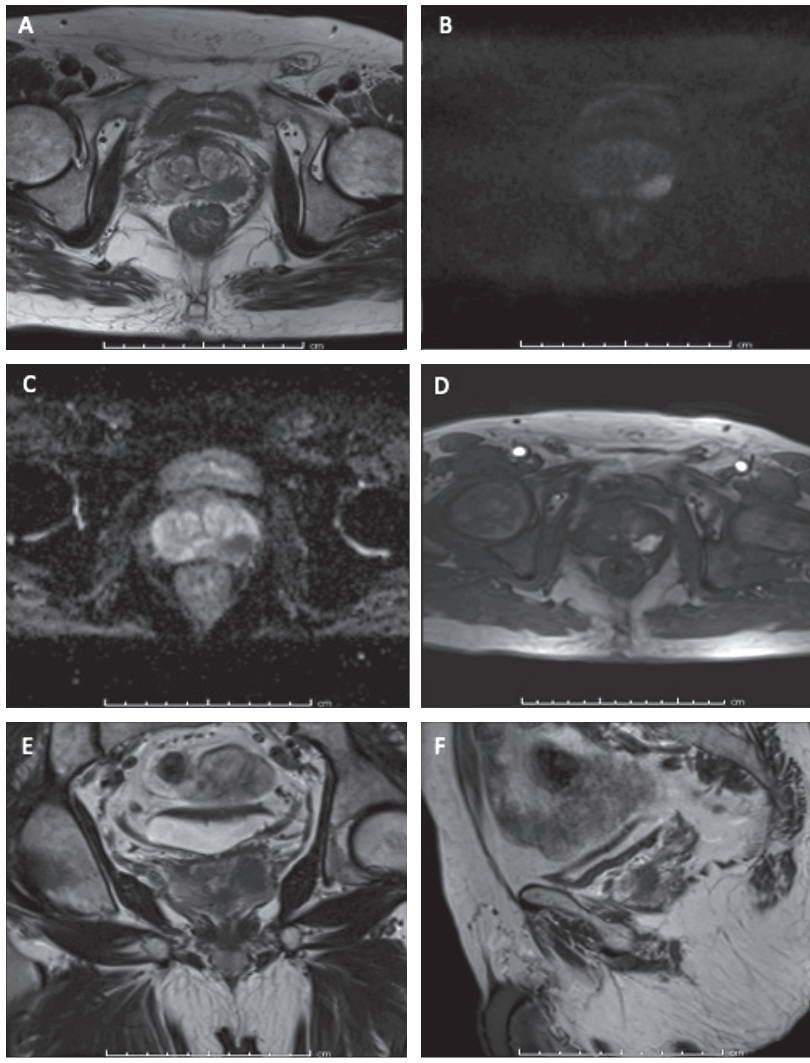

Figure 1 – T2 weighted images of a patient with history of high PSA (prostate specific antigen) values (4.8 ng/ml). There is a lesion on left peripheral zone which is hypointense on axial T2 weighted image (A) and showing diffusion restriction (B and C) and early arterial enhancement (D). The lesion was reported as PI-RADS 5 and largest dimension of lesion was delineated and measured better on coronal T2 sequence (E) than sagittal (F) and axial (A) T2 weighted images. After biopsy, the histopathological result was Gleason 4 + 5.

Histopathological analysis

The histopathological analysis of the biopsy materials was performed by an experienced uropathologist (F.T.). The reports were structured in accordance with the 2016 the International Society of Urological Pathology (ISUP) Gleason grading system (Epstein et al., 2016). The pathological long-axis diameter of the lesion on

the specimens and the biopsy core numbers for the pathologic lesions were also recorded.

The RP specimen's features were recorded. After separating the seminal vesicles from the specimen, 2 mm-thick slices were taken from the apex and bladder neck for the SMs of the apex and bladder neck. The remaining prostate tissue was sliced at 4–5 mm thickness, starting from the apex. All the slices were mapped as right, left, anterior and posterior, and each quadrant was processed with a separate block. Then, all the seminal vesicles separated into right and left were processed with cross-sections. Routine hematoxylin-eosin stained sections with a thickness of 4 micrometers were examined under a microscope after 12 hours of routine tissue processing.

In addition to the SMs of the apex and bladder neck, the anterior, anterolateral, posterior and posterolateral SMs were evaluated, and the tumour quadrants were marked and mapped. All the tumour-containing blocks were examined and graded according to the 2016 ISUP consensus (Schröder et al., 2014). The prognostic parameters of tumours included in RP reports were as follows: perineural invasion, lymphovascular invasion, SVI, EPE, tertiary pattern if present, ratio of secondary pattern to tumour, diameters of predominant tumours, ratio of tumour tissue to the whole prostate, presence/absence of prostate incision, presence/absence of prostate incision, and involvement of lymph nodes, if any. In addition, the presence of intraductal involvement in tumours was investigated and reported. Mostly, the diagnosis of acinar type adenocarcinoma and the presence and rate of ductal differentiation were also noted.

Statistical analysis

Statistical analysis was performed using SPSS v. 15.0 for Windows. Categorical variables were given as numbers and percentages. The conformance of continuous data to a normal distribution was evaluated using the Shapiro-Wilk test. The independent *t*-test was used for the comparison of groups with a normal distribution, and the Mann-Whitney U test was used for the comparison of groups that did not comply with a normal distribution. In the comparison of categorical variables, the Pearson chi-square and exact tests were used as appropriate. Parameters with a possible predictive value associated with EPE and positive SM were evaluated using univariate and multivariate logistic regression analyses. A p-value of < 0.05 was considered statistically significant.

Results

A total of 290 patients were stratified into Group A (n=144) and Group B (n=146). The mean age of the patients was 63.9 ± 7.9 years, and the median PSA value was 6.49 ng/dl (range: 4.7–9.5 ng/dl). According to the mpMRI examination, 17 (5.9%) cases were evaluated as PI-RADS 2, 77 (26.6%) as PI-RADS 3, 165 (56.9%) as PI-RADS 4, and 31 (10.7%) as PI-RADS 5. The fusion biopsy results revealed

the detection rates of ISUP Grade 1, 2, 3, 4 and 5 to be 65 (22.4%), 51 (17.6%), 34 (11.7%), 13 (4.5%), and 6 (2.1%), respectively. RALP was performed in 53 (18.3%) of the patients included in the study, who underwent a fusion biopsy. The histopathological analysis of these cases after RALP showed that 22 (41.5%) patients had EPE, 16 (30.1%) had SM positivity and four (7.5%) had SVI positivity.

Table 1 – Clinical characteristic of the study groups according to the multiparametric magnetic resonance imaging lesion size

Variables	mpMRI lesion size		p-value
	<10 mm (n=144)	≥10 mm (n=146)	
Age, years	63.3 ± 7.7	64.7 ± 8.1	0.137
PSA, ng/dl	6.0 (4.6–9.2)	6.9 (4.8–9.8)	0.078
PSAD, ng/dl/ml	0.13 (0.00–0.21)	0.16 (0.11–0.28)	**0.012**
Prostate volume, ml	48 (35–63)	45 (30–61.25)	0.195
MRI lesion size, mm	7 (6–8)	13 (11–16.25)	**<0.001**
Number of positive cores	2 (1–5)	4 (1–7)	**0.007**
Biopsy results			
Benign	74 (51.4)[a]	47 (32.2)[b]	**0.001#**
PCa	70 (48.6)[a]	99 (67.8)[b]	
PI-RADS score, n (%)			
II	8 (5.6)[a]	9 (6.2)[a]	
III	50 (34.7)[a]	27 (18.5)[b]	**<0.001#**
IV	85 (59.0)[a]	80 (54.8)[a]	
V	1 (0.7)[a]	30 (20.5)[b]	
Clinical T-stage			
T1c	132 (91.7)[a]	108 (74.0)[b]	**<0.001#**
T2	10 (6.9)[a]	30 (20.5)[b]	
T3	2 (1.4)[a]	8 (5.5)[a]	
Biopsy-ISUP grade, n (%)			
Benign	74 (51.4)[a]	47 (32.2)[b]	
I	38 (26.4)[a]	27 (18.5)[a]	
II	21 (14.6)[a]	30 (20.5)[a]	**<0.001#**
III	8 (5.6)[a]	26 (17.8)[b]	
IV	3 (2.1)[a]	10 (6.8)[b]	
V	0[a]	6 (4.1)[b]	
Disease significance, n (%)			
No PCa	74 (51.4)[a]	47 (32.2)[b]	**<0.001#**
Clinically insignificant	14 (9.7)[a]	8 (5.5)[a]	
Clinically significant	56 (38.9)[a]	91 (62.3)[b]	

#Pearson's chi-square test; PSA – prostate specific antigen; PSAD – prostate specific antigen density; MRI – magnetic resonance imaging; PI-RADS – Prostate Imaging Reporting and Data System; ISUP – International Society of Urological Pathology; same superscripts show no statistically significant difference between variables

When the patients were evaluated according to the mpMRI lesion size, it was observed that the PSAD value was statistically significantly higher in Group B than in Group A (p=0.012). The PI-RADS score was also higher in Group B compared to Group A, and the two groups statistically significantly differed in terms of clinical T-stage (p<0.001 and p<0.001, respectively). According to the fusion biopsy results, the rate of PCa detection and the number of positive cores were statistically significantly higher in Group B than in group A (p=0.001 and p=0.007, respectively). In addition, there was a statistically significant difference between the biopsy-ISUP grade values of the two groups (p<0.001). Another significant difference was detected in relation to the presence of clinically significant PCa (p<0.001). While the rate of csPCa detection among all biopsies was 62.3% in Group B, it was determined

Table 2 – Clinical data and pathological results of patients that underwent radical prostatectomy

Variables	MRI lesion size		p-value
	<10 mm (n=26)	≥10 mm (n=27)	
Preoperative clinical T-stage, n (%)			
T1c	21 (80.8)	21 (77.8)	0.728^
T2	4 (15.4)	6 (22.2)	
T3	1 (3.8)	0	
Pathological T-stage, n (%)			
T2	21 (80.8)[a]	11 (40.7)[b]	**0.003#**
T3	5 (19.2)[a]	16 (59.3)[b]	
Biopsy-ISUP grade, n (%)			
I	10 (38.5)	9 (33.3)	
II	11 (42.3)	12 (48.1)	0.919^
III	4 (15.4)	4 (11.1)	
IV	1 (3.8)	2 (7.4)	
RP-ISUP grade, n (%)			
I	3 (11.5)	5 (18.5)	
II	15 (57.7)	10 (37.0)	0.313^
III	8 (30.8)	10 (37.0)	
IV	0	2 (7.4)	
Gleason upgrade, n (%)	10 (38.5)	10 (37.0)	0.915#
csPCa			
EPE, n (%)	5 (19.2)	17 (63.0)	**0.001#**
SM, n (%)	3 (11.5)	13 (48.1)	**0.004#**
SVI, n (%)	1 (3.8)	3 (11.1)	0.610^
Lymph node metastases, n (%)	0	1 (3.7)	1.000^

#Pearson's chi-square test; ^Fisher's exact test; MRI – magnetic resonance imaging; ISUP – International Society of Urological Pathology; csPCa – clinically significant prostate cancer; EPE – extraprostatic extension; SM – surgical margin; SVI – seminal vesicle invasion; same superscripts show no statistically significant difference between variables

to be 38.9% in Group A. The rate of cisPCa detection was statistically similar in the two groups (Table 1).

It was observed that the pathological T-stage in the patients who underwent RALP was more advanced in Group B (p=0.003). In addition, the EPE and SM positivity rates were higher in Group B compared to Group A (p=0.001 and p=0.004, respectively). The two groups were statistically similar in terms of preoperative clinical stage, ISUP grade of specimen pathology, Gleason upgrade rate, and SVI and lymph node metastasis (LNM) detection rates among the patients who underwent RALP (Table 2).

Possible variables associated with EPE positivity after RALP (age, PSA, PSAD, biopsy-ISUP grade, number of positive cores, clinical T-stage, and mpMRI lesion size) were evaluated using the univariate analysis, and the mpMRI lesion size being > 10 mm was determined to be significant in predicting EPE positivity. The multivariate analysis revealed only the mpMRI lesion size being > 10 mm as an independent predictor of EPE positivity. According to the univariate analysis of the possible variables associated with SM positivity (age, PSA, PSAD, PI-RADS, biopsy-ISUP grade, number of positive cores, clinical T-stage, D'Amigo risk group, and mpMRI lesion size), the mpMRI lesion size being > 10 mm and the presence of biopsy-ISUP grade 2 significantly predicted SM positivity. In the multivariate analysis, the mpMRI lesion size being > 10 mm was found to be an independent predictive factor for SM positivity (Table 3).

Discussion

The result of the analyses undertaken in our study showed that PCa aggressiveness increased clinically and histopathologically in the patients with an index lesion size over 10 mm and the increase in lesion size was able to predict the aggressiveness

Table 3 – Results of the logistic regression analysis of parameters associated with EPE and SM positivity in patients that underwent radical prostatectomy

		Multivariate		
		OR	**95% CI**	**p-value**
*EPE**				
MRI lesion size (cat.)	<10 mm	ref		
	≥10 mm	10.023	2.008–50.036	**0.005**
*SM**				
MRI lesion size (cat.)	<10 mm	ref		
	≥10 mm	15.303	1.390–168.466	**0.026**

*age, PSA, PSAD, biopsy-ISUP grade, number of positive cores, and clinical T-stage; EPE – extraprostatic extension; cat. – categorical; SM – surgical margin; ISUP – International Society of Urological Pathology; OR – odds ratio; CI – confidence interval; ref – reference variable

of the disease. We took 10 mm as the threshold lesion size since a sphere of 0.5 cc corresponds to 1 cm, which is the standard limit for cisPCa according to the Epstein criteria (Epstein et al., 2016). Lee et al. (2013) determined that lesion size detected in mpMRI was an independent predictive factor for the presence of cisPCa.

The role of mpMRI in PCa management has been continuously increasing over the last decade. The guidelines recommend the use of mpMRI in various indications in patients who have not yet been diagnosed with PCa or before treatment in those who have been diagnosed with this cancer (Mottet et al., 2019). In addition, the use of mpMRI has become more popular in the last decade to increase the detection of csPCa and reduce the number of complications associated with biopsy procedures (Godtman et al., 2015; Caverly et al., 2016). PI-RADS scoring system, 15 mm lesion size was determined as the cut-off value in T2-weighted and DW imaging in distinguishing between category 4 and 5 lesions (Weinreb et al., 2016). Rosenkrantz et al. (2017) reported that when they reduced the 15 mm size criterion to 10 mm, resulting in increasing PI-RADS score 4 to 5, they detected PCa in 33 (79%) of 42 cases and csPCa in 26 (62%) and suggested that the size limit in score 5 should be reduced to 10 mm for PI-RADS versions. In a study by Lee et al. (2013) including 188 patients, when the index lesion size cut-off value was taken as 10 mm, no difference was found between the groups in terms of the number of positive biopsy cores and clinical T-stage. However, in our study with a higher number of patients, we determined that the rate of positive cores, clinical T-stage, biopsy-ISUP grade, and PI-RADS scores were higher among the patients in Group B. An mpMRI-targeted fusion biopsy is known to have a higher rate of detecting csPCa compared to the standard systematic TRUS biopsy, and the former also has higher upgrade rates in the Gleason score obtained from RP (Steinberg et al., 1997; Freedland et al., 2007). In our study, an mpMRI fusion biopsy was performed in all patients, and it was observed that the patients in both groups had similar rates (38.5% vs. 37%) in terms of Gleason upgrade, and these rates were consistent with the literature (Arsov et al., 2015).

According to the PCa risk classification models, the pathological stage in the RP specimen can be predicted by examining tumour size, localization and extension in mpMRI images. Studies on this subject have revealed that mpMRI not only provides anatomical tumour localization but also predicts pathological stage in the RP specimen (Lebacle et al., 2017; Morlacco et al., 2017). In our study, when we took the lesion size cut-off value as 10 mm in the patients who underwent RALP, there was no difference in the clinical T-stage of the patients, but we observed higher pathological T-stage in Group B. In contrast, Lee et al. (2013) determined no difference in pathological T-stage between the patients with a lesion size of less than or more than 10 mm.

In studies investigating the relationship between the PI-RADS index lesion size determined in mpMRI and the ISUP-Gleason grade, it has been reported that the ISUP grade was more advanced and the tumour progressed more aggressively in

larger lesions. It has also been shown that increased lesion size and other factors had prognostic value for the course of the disease (Kattan et al., 1997; Toledano and Obuchowski, 2016; Nassiri et al., 2018). Considering these factors, it has been suggested that mpMRI has a potential role in risk classification before definitive treatment in patients with PCa (Felker et al., 2016). EPE, SVI, LNM, and SM are important oncological prognostic markers in histopathological evaluation after RP (Sanda et al., 2008; Ho et al., 2016). Dvorak et al. (2005) showed that when the maximal tumour lesion size was 13 mm and above, the positivity of SM was significantly higher. Tonttila et al. (2018) investigating the relationship between lesion size in mpMRI and the pathology of the RP specimen, found higher EPE, SVI and LNM rates and higher ISUP grades in patients with lesions larger than 15 mm. In our study, we observed that the index lesion size being > 10 mm was an independent predictive factor for EPE and SM positivity.

In the PAIREDCAP study, the PCa detection rates based on PI-RADS scores determined according to the index lesion size were evaluated and the effect of lesion size on PCa detection was emphasized. That study provided guidance in determining the treatment protocol according to lesion size (Elkhoury et al., 2019). Related to this, Lee et al. (2013) stated that if the lesion size measured in mpMRI was over 10 mm, there was a much higher possibility of csPCa, and these patients were not suitable for active surveillance (AS). They found that among the patients with PCa who were suitable for AS, there was a significant rate of Gleason upgrade according to the prostatectomy pathology those with a DW-MRI lesion diameter of > 10 mm. Thus, the authors suggested that patients with a lesion larger than 10 mm were not suitable for AS (Lee et al., 2013). Similarly, in our study, we found an increased probability of having csPCa among the patients with a lesion size of over 10 mm. Özden et al. (2021) reported that the rate of csPCa detection increased in patients with an mpMRI lesion size of > 10 mm among those who underwent a cognitive-targeted biopsy. Considering these findings, our study supports the literature and can shed light on future studies to revise the 15 mm criterion used for the differentiation of PI-RADS 4 and 5 categories.

This study has several strengths, including all biopsies being in the form of fusion biopsies performed by a single experienced radiologist, RALP being performed by two specialist urologists, and histopathological evaluation being undertaken by a single uropathologist. The use of fusion biopsy combined with systematic biopsy in all patients reduced the possibility of overlooking csPCa in patients with large prostate volumes. Since the number of cisPCa was low in our study, the difference between the two groups may not have been statistically significant. The limitations of the study include the retrospective design and the low rate of RALP in our cohort.

Conclusion

The radiologists and clinicians should be aware of the possibility of presence of features that may affect local staging, such as EPE positivity, in the presence of

lesions larger than 10 mm in which prostate cancer is detected. For index lesion size, 10 mm was determined as a cut-off value for the prediction of the positivity of SM and EPE, which are prognostic factors affecting survival after RP. However, the results obtained from our study need to be supported by prospective studies with a higher number of patients.

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Red Cell Distribution Width on First Day Intensive Care Unit Admission in Paediatrics

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Abstract: Red distribution width (RDW) has recently been acclaimed as prognostic marker for mortality in critically-ill patients. However, this claim is still unclear and reports are still inadequate for the association between RDW and mortality in critically-ill paediatric patients. This research assessed the correlation between RDW within 24 hours of PICU (paediatric intensive care unit) admission and PELOD-2 score. A cross-sectional study was carried out involving 59 pediatric patients admitted to the PICU Haji Adam Malik Hospital, Medan, Indonesia, from May to July 2019. The association between RDW and PELOD-2 score was assessed by using Spearman correlation test. The RDW level of paediatric patients in the PICU on the first 24 hours was elevated (median 14.7%, range 11.4–31.2%). The median of PELOD-2 score assessment was 8 (range 2–21). There was no significant correlation between RDW and PELOD-2 in this research (r=0.187, p=0.156).

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Introduction

Red distribution width (RDW) is recently acclaimed as prognostic marker for mortality in critically-ill patients (Bazick et al., 2011). It is also reported to be significantly related to ventilator requirement, postoperative outcome, and intensive care unit care outcome (Said et al., 2017; Fernandez et al., 2018). However, most of the available reports were involving adult patients. The role of RDW as prognostic factor in paediatric patients still remains unclear. Yanni et al. (2019) conducted a study to paediatric patients with sepsis and reported no significant correlation between the increase in RDW and severity of diseases or mortality. On the contrary, Sachdev et al. (2018) reported that high RDW at admission and the persistent high levels were associated with high mortality and longer PICU (paediatric intensive care unit) stay.

This study aims to assess the correlation between RDW and PELOD-2 score, specifically on the first day of PICU admission. We compared RDW level and PELOD-2 score on the first day of PICU admission.

Methods

This research was a cross-sectional design study conducted at Haji Adam Malik Hospital, Medan, Indonesia from May to July 2019. The research subjects were paediatric patients aged between 1 month old and 18 years old admitted to the paediatric intensive care unit. RDW was measured within 24 hours of PICU admission. RDW level normal range was 11.5–14.5%, and the level above 14.5% was considered elevated. The patients who refused to do blood examination and involved in this research were excluded from the study.

Statistical analysis was perform using SPSS 20. Non-normally distributed data was presented as median and analysed by using Spearman correlation test. The Health Research Ethical Committee of Medical Faculty of Universitas Sumatera Utara approved this research under the number 518/TGL/KEPK FK USU-RSUP HAM/2019.

Results

There were a total of 59 research subjects admitted to the PICU involved in the study, where 20 (33.9%) of them were female and 39 (66.1%) were male. The patients admitted to PICU were treated for the following conditions: respiratory (13.6%), central nervous system (28.8%), cardiovascular (27.1%), nephrology (16.9%), and postoperative (13.6%). The characteristics of the research subjects are presented in Table 1.

PELOD-2 score and RDW were measured within 24 hours of PICU admission. The median value of PELOD-2 score was 8 (ranging from score 2 to 21). The median value of RDW was 14.7% (ranging from 11.4% to 31.2%). Using Spearman correlation test, the correlation between PELOD-2 score and RDW within 24 hours PICU admission in the research subjects was not significant ($r=0.187$, $p=0.156$). Figure 1 shows the correlation assessed by Spearman correlation test.

Table 1 – Characteristic of the research subjects

Variable		N=59
Age (year); median (min–max)		5.75 (0.08–17.00)
Gender; n (%)	Male	39 (66.1)
	Female	20 (33.9)
PELOD-2 score; median (min–max)		8 (2–21)
Underlying disease; n (%)	Respiratory	8 (13.6)
	Central nervous system	17 (28.8)
	Cardiovascular/circulation	16 (27.1)
	Nephrology	10 (16.9)
	Post surgical	8 (13.6)
Outcome; n (%)	Survive	33 (55.9)
	Die	26 (44.1)
Red cell distribution width (%)		14.7 (11.4–31.2)

N – number

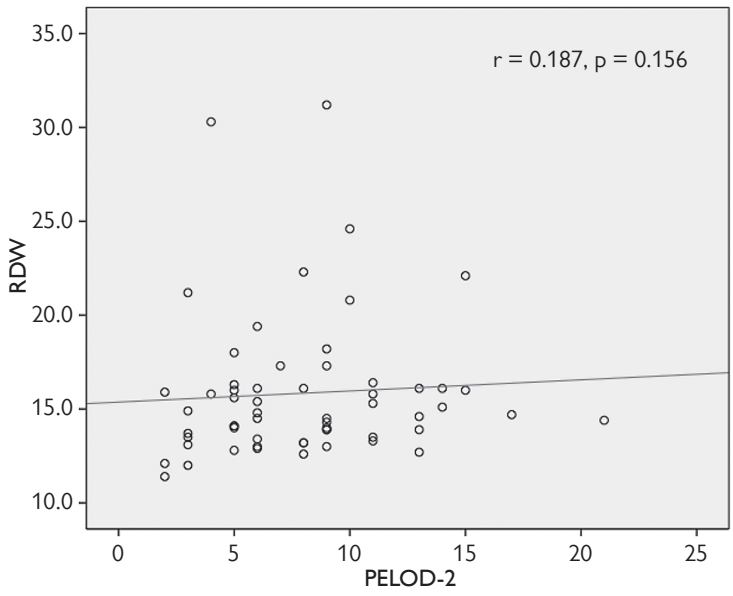

Figure 1 – Correlation between RDW (red distribution width) and PELOD-2 score.

Discussion

The research subjects in this study were admitted to PICU with underlying diseases, such as respiratory, central nervous system, cardiovascular, and nephrology diseases. Some of them were also admitted to the PICU as postoperative measures. The patients in this study showed elevated RDW, with a median of 14.7% and the highest RDW level elevation recorded was up to 31.2%. Although RDW has been

traditionally used for anaemia diagnosis (Weiss and Goodnough, 2005), patients who are critically ill usually have elevated RDW levels as a response to the systemic inflammatory processes (Bazick et al., 2011). Studies have shown that elevated RDW levels may indicated adverse outcomes and mortality among patients with heart failure and coronary heart diseases (Tonelli et al., 2008). Zhang et al. (2020) reported the association between high RDW on admission and increased risk of long-term mortality in patients suffering from respiratory failure. Furthermore, there have been many previous studies that link RDW to other various diseases and their adverse outcomes (Bazick et al., 2011; Said et al., 2017).

Despite several reports on the link between RDW and various clinical conditions, as well as mortality, the exact pathophysiologic mechanisms have not been clearly established (Weiss and Goodnough, 2005). RDW is known to be elevated in situations of inefficient red cell synthesis and excessive red cell death, which are typical in a number of viral and inflammatory diseases (Qurtom et al., 1998; Scharte and Fink, 2003; Sipahi et al., 2004). Another theory is that RDW is a surrogate for inflammation, which has been proven to promote RDW due to the reduced production of red blood cells. RDW could reflect inflammation, oxidative stress, anaemia, changes in life-span or deformability of the red blood cells, in which they are the risk factors for mortality (Weiss and Goodnough, 2005). Moreover, RDW has been linked to inflammatory indicators in blood, such as interleukin-6 and C-reactive protein (Perlstein et al., 2009). Inflammatory cytokines are known to disrupt red blood cells maturation in the bone marrow through a variety of mechanisms, including suppression of erythropoietin synthesis or responses – resulting in impaired iron metabolism and shorten red cells survival, hence increasing RDW (Fujita et al., 2013).

Ramby et al. (2015) reported the link between RDW measured within 24 hours and the length of intensive care unit stay above 48 hours in patients without sepsis, while link to mortality in all patients. This means that RDW at the time of PICU admission may provide the physicians to group the patients – whether they are the critically-ill-paediatric population with a high risk for adverse outcomes. Early identification of these group of patients allow intervention to improve outcomes and maximize the use and allocations of resources (Ramby et al., 2015). Our data showed no correlation between RDW within 24 hours PICU admission and PELOD-2 score. However, RDW recorded was elevated and PELOD-2 score was high at the median value of 8. The data set allowed us to categorise the critically-ill populations to enable immediate management to improve outcome. The markers of inflammation, however, were not examined in this research. Fernandez et al. (2018) in their study reported that high RDW level classified patients with higher mortality risk, allowing RDW to become the marker for severity of diseases and mortality. RDW that is routinely assessed during complete blood cell counts may be served as an alternative indicator to predict disease prognosis and progression (Zhang et al., 2020).

Nonetheless, the prognostic potential of RDW has obtained a special relevance because RDW is regularly included in a complete blood count analyses in hospitalized patients. Hence, it is available at no additional cost to doctors as prognostic markers and may be a predictive sign for both short-term and long-term mortality in critically-ill patients (Wang et al., 2010; Bazick et al., 2011; Braun et al., 2011). RDW can be associated with mortality and death from various critical conditions, such as cancer, cardiovascular, respiratory, and other diseases. However, it is necessary to note that RDW serves as a significant, but non-specific marker (Pan et al., 2019). The mechanism of low-grade inflammation, which is common in healthy individuals and many diseases, can even increase RDW (Cohen et al., 2008). In the conditions with stress and poor health can lead to delayed red blood cell circulation in the blood stream and elevate RDW. Therefore, RDW level elevation besides serving as prognosis marker in critically ill patients, it can also be served as a prognosis marker of physiological stress and poor health condition in general (Pan et al., 2019).

In conclusion, this study confirmed that RDW was elevated in paediatric patients admitted to PICU Haji Adam Malik Hospital. There was no significant correlation between RDW level and PELOD-2 score on the first day of PICU admission.

Conclusion

The RDW level of paediatric patients in the PICU on the first 24 hours was elevated (median 14.7%, range 11.4–31.2%). The median of PELOD-2 score assessment was 8 (range 2–21). There was no significant correlation between RDW and PELOD-2 in this research (r=0.187, p=0.156).

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Analysis of the Causes of Newborn Priapism: A Retrospective Clinical Study

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Key words: D-dimer – Neonate – Priapism

Abstract: Priapism is a rare condition in the newborn. The aim of this study was to investigate the demographic, etiologic and clinical features of neonatal priapism. We retrospectively analysed the data of 11 patients diagnosed with neonatal priapism in the neonatal intensive care unit between 2000 and 2019. Priapism was defined as an erection in the neonatal period, lasting more than 4 hours. Etiological examinations revealed polycythemia in one (9.09%) patient, D-dimer elevation in three patients, and heterozygous methyltetrahydrofolate 667 gene mutations in one patient. Other patients were considered idiopathic. Detumescence was achieved in all 11 (100%) patients during the follow-up period. The median hospitalization duration was 6 (IQR [4, 8]; range, 2–9) days. The median follow-up duration was 38 (IQR [30, 42]; range, 13–94) months for patients followed-up in our hospital after discharge. Neonatal priapism is a rare condition. Successful treatment results can be achieved with conservative methods. Data acquired from our study showed that diseases with a tendency to hypercoagulation belong to the etiology by damaging penile microcirculation and make the response to conservative treatment more challenging.

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Introduction

Priapism is a partial or complete penile erection lasting more than four hours without any sexual stimulus (Broderick et al., 2010). It can be divided into two groups: The low flow (ischemic or venous) and the high flow (non-ischemic or arterial). Ischemic priapism lasting more than four hours is a compartment syndrome requiring urgent treatment. Non-ischemic (high flow) priapism is a less common kind of priapism caused by irregular cavernous flow. Erection is painless and not completely rigid. Preservation of normal erectile function is the essential point in priapism management (Aktoz et al., 2011; Dust et al., 2011; Song and Moon, 2013). Although nearly 50% of all priapism cases are idiopathic, there are some specific known causes. Intracavernous treatment with papaverine, phentolamine, alprostadil or the combinations of these agents in adults is the most common cause of ischemic priapism (El-Bahnasawy et al., 2002).

Although the most common cause of low flow priapism is sickle cell anemia in children, in high flow priapism, trauma is the most common cause (Hekal and Meuleman, 2008). Priapism is a quite rare condition in neonates. Apart from idiopathic cases, the most frequently accused factor in the etiology of priapism, reported in the literature, is the increased blood viscosity in polycythemia (Walker and Casale, 1997; Meijer and Bakker, 2003). We thought that other factors that cause hyperviscosity in the neonatal period can be also involved in the etiology of priapism. Neonatal priapism cases are challenging for physicians due to a lack of experience and well-defined guidelines on this subject. Thus, the evaluation and management strategy remain uncertain. A limited number of case presentations are available on neonatal priapism in the literature. To the best our knowledge, we present the first original article written on this subject. The aim of our study was to extend our knowledge on the etiology of neonatal priapism and to suggest effective treatment methods.

Material and Methods

After obtaining consent from the local ethics committee (2018/229), patients who were diagnosed as having priapism in neonatal intensive care unit between 2000 and 2019 were investigated.

Patients having a duration of erection exceeding four hours in the newborn period were accepted as priapism and included in the study (Figures 1 and 2). Cases considered as transient physiological erection of the newborn that developed after the neonatal period were excluded from the study. Data on prenatal maternal history, maternal age, birth week, birth weight, delivery method, maternal medication, clinical characteristics, laboratory findings, radiological tests, treatment modalities and clinical management were evaluated. Penile blood gas analysis was performed in all cases. In addition, penile Doppler examination was performed by the radiology specialist in all cases.

Continuous data were reported as median with interquartile range (IQR). Clinical follow-up of the patients was carried out by the pediatricians and clinical management was carried out by the pediatricians and urologists together. Long-term follow-up was performed by the pediatricians.

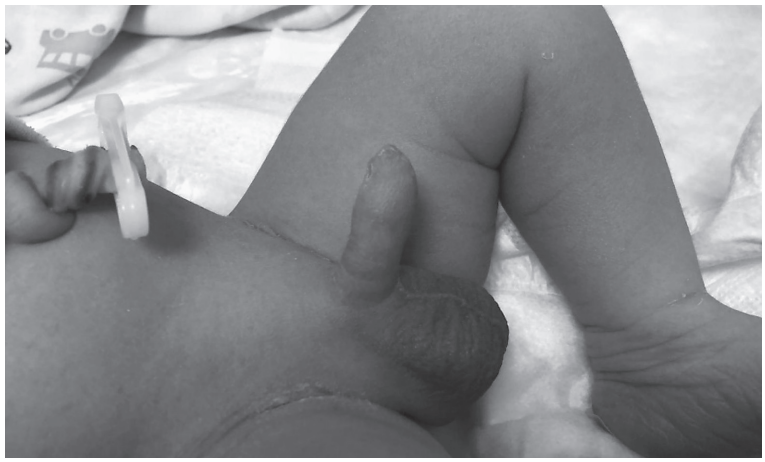

Figure 1 – A presentation of newborn priapism.

Figure 2 – A presentation of newborn priapism.

Results

In prenatal routine scannings, no evidence was found on blood group incompatibility. Two (18.1%) mothers had histories of smoking, two (18.1%) had bacterial vaginitis, one (9.1%) had folate deficiency, one (9.1%) had hypothyroidism and one (9.1%) had a history of preeclampsia. Apart from the mother who had thyroxine treatment due to hypothyroidism, none of the cases had a history of medication during pregnancy. Delivery was through spontaneous vaginal delivery in four (36.3%) and caesarean section in seven (63.6%) of the cases, and no complications occurred in any of the cases. Cesarian section was performed due to the demand of the mothers in two (18.1%)

Table 1 – Prenatal histories, characteristics of infants and mothers, etiological factors, laboratory findings and treatment methods

Case	Prenatal history	Maternal age	Birth week	Birth weight (g)	1. and 5. min APGAR	Infant's blood group	Maternal blood group	Htc	D-dimer elevation	Treatment
1	maternal smoking	26	38	3370	9-9	0 Rh+	0 Rh+	63	–	iv hydration
2	–	24	37	3390	9-10	0 Rh–	A Rh+	61	×	iv hydration
3	–	29	35	3060	9-9	A Rh+	B Rh+	58	–	iv hydration
4	preeclampsia	23	34	2890	7-8	AB Rh+	AB Rh+	74	–	iv hydration
5	–	17	38	3330	9-10	B Rh+	B Rh+	55	–	iv hydration
6	–	32	40	3230	8-9	A Rh+	A Rh+	58	×	iv hydration
7	–	28	42	3110	9-9	0 Rh–	0 Rh+	60	–	iv hydration
8	maternal smoking	29	38	3090	7-9	AB Rh+	B Rh+	61	–	iv hydration
9	MTHFR 667ct mutation	30	37	3860	8-8	AB Rh+	A Rh+	62	×	iv hydration
10	–	28	36	3040	8-9	B Rh–	AB Rh–	57	–	iv hydration
11	–	27	37	3390	9-9	B Rh–	B Rh–	56	–	iv hydration

Htc – hematocrit; iv – intravenous; MTHFR – methylenetetrahydrofolate reductase

cases, preeclampsia in one (9.0%), cephalopelvic disproportion in two (18.1%) and history of multiple caesareans in two (18.1%) cases. Median maternal age was 28 (IQR [24, 29]; range, 17–32) and median birth week was 37 (IQR [36, 38]; range, 34–42). Median birth weight was observed as 3,230 g (IQR [3060, 3390]; range, 2890–3860) (Table 1).

In the first physical examination, one of the patients had a one sided undescended testicle. The physical examinations were normal for other patients. Urine output and post-void residual volume were normal in all patients. One (9.09%) patient had polycythemia based on the etiologic examinations (Htc – hematocrit > 65). It was found that in this case preeclampsia developed during pregnancy and delivery was performed by cesarian section. D-dimer positivity was detected in three patients (27.2%), and it was observed that in one of three cases, heterozygote methylenetetrahydrofolate reductase (MTHFR) 667ct gene mutation positivity was detected in the mother before delivery. While hyperhomocysteinemia was observed in one of the patients, but the homocysteine level was normal in the others. No abnormal findings were observed in the other organ systems of these patients. Apart from these, no etiologic factors were found through imaging methods and laboratory findings in seven (63.6%) patients and these cases were regarded as idiopathic.

The median time to observe priapism was 3 days (IQR [3, 7]; range, 1–9). Median duration of episode was 3 days (IQR [3, 4]; range, 1–7). In this priapism cases, there were no stuttering priapism. No penile blood gas examination was found in favour of ischemic priapism. Normal arterial and venous flow was detected in all cases in colour Doppler ultrasonography. Detumescence was provided in all of the patients (100%) after 50 cc/kg/day fluid treatment and follow-up (Table 1). Median hospitalization duration was 6 days (IQR [4, 8]; range, 2–9). Median follow-up duration was 38 months (IQR [30, 42]; range, 13–94) for the patients in our hospital after discharge. No recurrent priapism attacks were detected in the patients during follow-up.

Discussion

Neonatal priapism is a rare case with only 18 cases reported since 1876 in the literature. The real incidence of neonatal priapism is unknown, but Merlob and Livne (1989) found the incidence as 0.15 in 1,000 live births between 1974 and 1988 in their center in a small surveillance study. In neonatal males, erection generally occurs through the slightest tactile simulation, and may often be stimulated by a completely full bladder. Typically, these physiological erections last a few minutes and quickly ends after the stimulus disappears (Burgu et al., 2007). Merlob and Livne (1989) stated that the term neonatal priapism was not suitable, and it would be more appropriate to call it as prolonged neonatal penile erection.

The etiology of priapism differs significantly among patient populations, but blood dyscrasias, pharmacotherapy, neurologic conditions, malignancy, and trauma are among common identifiable causes. Sickle cell disease constitutes nearly 70% of pediatric priapism but does not occur in the neonatal period due to the fetal

hemoglobin dominance (Mishra et al., 2020). Although neonatal priapism is most commonly idiopathic, polycythemia is the most common cause among its identifiable etiologies. The most important factor here is hyperviscosity and the resultant slowing of microcirculation. This situation results in decreased penile venous outflow and permanence of penile erection. Four of 18 neonatal priapism cases reported to date were related to polycythemia (Humbert et al., 1969; Merlob and Livne, 1989; Dust et al., 2011). Although penile blood gas examination was not performed, a non-ischemic course of priapism was seen in these cases.

In our study, the cause was observed to be polycythemia in one patient (9.09%) which is a lower ratio to the cases in the literature. In that patient, the mechanism underlying polycythemia was considered as preeclampsia during pregnancy. When etiologic factors other than polycythemia in the literature are considered, it was observed that one case had recurrent blood transfusions and hypoxia, one case was secondary to congenital syphilis, one case had central nervous system trauma secondary to forceps use and another case was secondary to bilateral spontaneous pyocavernositis (Larocque and Cosgrove, 1974; Amlie et al., 1977; Sood et al., 2006). The etiology of the cases could not be determined in the remaining nine cases, which were regarded as idiopathic. Different from the cases in literature, the cause of the priapism was considered to be secondary to microthrombosis in penile microcirculation in three patients in our study, and the thrombosis in one of these was related to heterozygote MTHFR 667ct gene mutation positivity.

MTHFR is an important enzyme in folate metabolism and is formed by 656 aminoacids (Homberger et al., 2000; Rosenblatt, 2001). A mutation occurring in MTHFR gene (C677T polymorphism is the most common) lowers enzyme activity. Levels of 5-methyltetrahydrofolate (MTHF) decreases, and the amount of 5, 10-MTHF and plasma homocysteine levels increases as the result of decreased MTHFR activity (Peng et al., 2001). Due to the hypomethylation and acylation effect of sulfhydryl group of homocysteine, homocysteine is known to cause harmful effects on vascular endothelium (Födinger et al., 2000). It was stated that homocysteine increased platelet consumption due to this resultant vascular damage and thus caused thrombosis (Donnelly and Rock, 1999).

Clinically, D-dimer is most commonly used for venous thromboemboli and disseminated intravascular coagulation diagnosis and follow-up (Righini et al., 2008; Wada et al., 2014). Apart from these, D-dimer also increases due to thromboses formed in other regions inside the body (Taylor et al., 2001). Including the one heterozygote MTHFR 667ct gene mutation positive case, plasma D-dimer levels were detected high in three cases in our study. Even though verifiable thromboses were not detected in all three cases in Doppler examinations, it was considered that D-dimer levels could have increased secondary to a thrombosis that was present in the penile venous microcirculation but could not be detected in imaging.

Although the starting time was generally reported as the first or second day of life in the priapism cases presented in literature, it was reported as the 37th day in one case

(Amlie et al., 1977). Priapism duration is quite variable, but the mean was reported as 4–5 (range 2–12) days. In our study, the median priapism starting time was reported as 3 (IQR [3, 7]; range, 1–9) days after birth, the median duration of the episodes 3 (IQR [3, 4]; range, 1–7) days and the median duration of hospitalization duration as 6 (IQR [4, 8]; range, 2–9) days.

When the management of priapism detected in the neonatal period was examined, it was reported that detumescence was provided through follow-up in 75% of the cases in literature, and two cases related to polycythemia were in this group (Humbert et al., 1969; Larocque and Cosgrove, 1974; Walker and Casale, 1977). In two other cases for which the etiological factor was polycythemia, detumescence was not provided through follow-up, and in one case it was provided through phlebotomy and through exchange transfusion in another (Humbert et al., 1969; Walker and Casale, 1977). Detumescence was provided immediately after intravenous (iv) ketamine infusion in one of the idiopathic cases (Stothers and Ritchie, 1992). Contrary to the ratio of the cases in literature, detumescence was provided after follow-up and iv fluid administration in all of 11 cases (100%) in our study. On the other hand, polycythemia is the common etiological factor in resistant cases in the literature and only one patient had polycythemia in our cohort. We think that this is the origin of our different results to those in the literature.

According to literature data, the follow-up was unfortunately limited to early infancy and the longest follow-up duration was 8 years (Merlob and Livne, 1989). The median follow-up duration of the patients was 38 (IQR [30, 42]; range, 13–94) months in our study, and the longest follow-up was 94 months; no priapism recurrence was detected in any of the patients.

Erectile dysfunction is a problem that should be considered in priapism cases occurring in childhood and adulthood. The connection of priapism with erectile dysfunction risk cannot be presented clearly because neonatal priapism cases presented in the literature are rare and there were no long-term follow-ups. In the case presentation of Sood et al. (2006), it was stated that long-term neonatal priapism could cause severe complications such as pyocavernositis.

Apart from the neonatal physiologic erection, a careful evaluation should be performed for polycythemia, neurologic conditions, and urinary tract obstruction in priapism cases. Starting from high D-dimer levels and the MTHFR mutation in some patients, our study showed that hypercoagulation was among the risk factors that should be considered in the etiology of priapism. The arterial blood flow of patients should be evaluated through penile Doppler ultrasonography and also pediatric urologists should evaluate penile blood gas analysis. As in all cases, penile blood gas and penile Doppler ultrasonography showed high flow priapism in our cases. These findings support “neonatal priapism is highly non-ischemic” theory in the literature. Rather than an aggressive intervention, observation alone is enough in these cases in the first stage (Merlob and Livne, 1989; Dust et al., 2011). Even though penile Doppler ultrasonography findings do not take us to venous return disorder in patients with priapism without regression through follow-

up, it should be kept in mind that polycythemia or other diseases creating a tendency to hypercoagulation may contribute to priapism occurrence by harming penile venous microcirculation and make the response to conservative treatments challenging.

Although the current study reported new findings, it had several limitations. First, the study was performed in a retrospective manner and had a small patient population. In this study, data related to the results and controls were collected by retrospective table review of the patients followed-up in the pediatric clinic. This could have introduced collection bias in the results.

Conclusion

Although no etiologic factor other than polycythemia could be presented in literature, data acquired from our study showed that diseases producing a tendency to hypercoagulation could be included in the etiology of priapism by damaging penile microcirculation and makeing the response to conservative treatment challenging.

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