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RISTO VAIKJÄRV

Etiopathogenetic and clinical aspects of peritonsillar abscess

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II Vaikjärv R, Mändar R, Kasenõmm P. Peritonsillar abscess is frequently accompanied by sepsis symptoms. Eur Arch Otorhinolaryngol. 2019 Jun;276(6):1721–1725. doi: 10.1007/s00405-019-05424-6. Epub 2019 Apr 16.

III Kõljalg S, Vaikjärv R, Smidt I, Rööp T, Chakrabarti A, Kasenõmm P, Mändar R. Effect of erythritol and xylitol on *Streptococcus pyogenes* causing peritonsillar abscesses. Sci Rep. 2021 Aug 4;11(1):15855. doi: 10.1038/s41598-021-95367-y.

Contribution of Risto Vaikjärv to original publications:

Paper I: Data analysis, writing the paper

Paper II: Study design, patient recruitment, clinical evaluation and treatment of patients, laboratory investigation, data analysis, writing the paper

Paper III: Study design, collecting the specimens, writing the paper

ABBREVIATIONS

BHI	brain-heart infusion
CRP	C-reactive protein
CT	computed tomography
DNA	deoxyribonucleic acid
ERY	erythritol
FAA	fastidious anaerobe agar
F. necrophorum	*Fusobacterium necrophorum*
F. nucleatum	*Fusobacterium nucleatum*
Ig	immunoglobulin
IM	infectious mononucleosis
MIC	minimum inhibitory concentration
MRI	magnetic resonance imaging
NGS	next-generation sequencing
PCR	polymerase chain reaction
PCT	procalcitonin
Ph.	Phylum
PTA	peritonsillar abscess
R. mucilaginosa	*Rothia mucilaginosa*
RNA	ribonucleic acid
SAG	*Streptococcus anginosus* group
Sepsis-2	sepsis criteria based on 2001 International Sepsis Definitions Conference
SIRS	systemic inflammatory response syndrome
SOFA	sequential organ failure assessment
S. anginosus	*Streptococcus anginosus*
S. mitis	*Streptococcus mitis*
S. parasanguinis	*Streptococcus parasanguinis*
S. pneumoniae	*Streptococcus pneumoniae*
S. pyogenes	*Streptococcus pyogenes*
TI	tonsillitis index
TSBV	tryptic soy-serum-bacitracin-vancomycin
WBC	white blood cells
XYL	xylitol

INTRODUCTION

Palatine tonsils are secondary lymphoid organs located in the lateral pharyngeal wall. Their function is to perform localised immune protection and such an anatomic location is ideal to guarantee their continuous exposure to inhaled and ingested antigens. However, in the same way they are exposed to various pathogens and therefore tonsillar infections and inflammations are common clinical entities (Brandtzaeg, 2003; Klug, 2017).

Peritonsillar abscess (PTA) is a collection of pus between the palatine tonsil capsule and the superior pharyngeal constrictor muscle (Mitchelmore *et al.*, 1995; Prior *et al.*, 1995). It is the most frequent cause of acute admission in otorhinolaryngology, accounting for around a third of all acute admissions (Rusan *et al.*, 2009). Most often, the PTA is considered to be a complication of acute tonsillitis. However, the association between PTA and chronic and recurrent tonsillitis is not well established (Klug, 2017). PTA age peak is found in young adults while the incidence peak of acute tonsillitis is found in children and adolescents of school age (Windfuhr *et al.*, 2016). In addition, acute tonsillitis incidence has seasonal trends; at the same time PTA occurrence is more even throughout the year (Kordeluk *et al.*, 2011). Therefore, causes other than acute tonsillitis should be considered. It is proposed that PTA can also develop from the small salivary glands (Passy, 1994). Smoking, poor oral hygiene and male gender have been identified as risk factors for PTA (Windfuhr *et al.*, 2016).

PTA patients usually look severely ill and untreated PTA can lead to life-threatening airway obstructions from swelling or pus. In addition, infection can spread to deeper neck spaces. PTA treatment consists of surgically opening the abscess and adequate antimicrobial therapy. From the end of 19th century to 1970s and 1980s the PTA treatment gold standard was emergency tonsillectomy whereby tonsils are removed and the abscess is completely opened. However, from 1980, needle aspiration and incision and drainage were introduced and all three methods are now accepted surgical interventions.

Most frequently the PTA pus contains mixed microbiota of aerobes and anaerobes. The exact causative agents are still unclear. It is recognised that *Streptococcus pyogenes* (*S. pyogenes*) can cause PTA. Recently, there has been a growing interest in anaerobic bacteria. However, it is still unclear which microbes can be considered to be the true causative agents and which are contamination from oral and tonsillar microbiota (Ehlers Klug *et al.*, 2009; Galioto, 2017; Slouka *et al.*, 2020). Therefore, there is no common understanding of what adequate antimicrobial therapy should be. Antimicrobial therapy and treatment recommendation vary widely and not only between countries but also among physicians (Powell and Wilson, 2012; Wikstén *et al.*, 2014).

It is still unclear what leads to the development of PTA, and the lack of consensus regarding which microbes are causing PTA has led to wide variations in antimicrobial treatment options. Also, it is not ultimately clear if the patients

should be treated as in- or outpatients and which PTA patients need closer monitoring.

The goal of the present PhD thesis is to evaluate several aspects of the clinical picture of PTA patients, to clarify etiopathogenic factors and novel therapeutic options. The collection of clinical data and all management of patients was conducted at the Institute of Clinical Medicine, Department of Otorhinolaryngology, University of Tartu. All microbiological analyses were performed at the Institute of Biomedicine and Translational Medicine, Department of Microbiology, University of Tartu.

REVIEW OF LITERATURE

1. Anatomy, histology and immunology of the palatine tonsils

1.1 Anatomy of palatine tonsils

Tonsils are secondary lymphatic organs located in the oro- and nasopharynx of most mammals except rodents. Humans have four sets of tonsils, the lingual, palatine, pharyngeal and tubal tonsils (**Figure 1**). Together they form a circumferential array known as Waldeyer's ring, which provides immunologic surveillance and produces immunoglobulins (Ig) (Cesta, 2006; Sidell and Shapiro, 2012).

The palatine tonsil represents the largest accumulation of lymphatic tissue in Waldeyer's ring and, in contrast to the lingual and pharyngeal tonsils, constitutes a compact body with a definite thin capsule on its deep surface. Palatine tonsils' size and volume correlate with age, height, weight and body mass index. The average tonsil volume is 1.5–1.8 cm^3 for the right and left sides (Flint and Cummings, 2010; Öztürk, 2017; Aydin and Uner, 2020).

Tonsillar crypts are narrow epithelial diverticula, which considerably increase the available surface area for direct antigenic stimulation. They invaginate deeply into the tonsillar tissue and are lined with stratified squamous epithelium. In an average adult's palatine tonsils the estimated epithelial surface area of the crypts is 295 cm^2, in addition to the 45 cm^2 of epithelium covering the oropharyngeal surface (Perry and Whyte, 1998; Nave *et al.*, 2001).

The tonsillar capsule is a specialised part of the pharyngobasilar fascia that covers the surface of the tonsil and extends into it to form septa that conduct the nerves and vessels. The tonsil is not, therefore, easily separated from its capsule, but the capsule is united largely by loose connective tissue to the pharyngeal muscles. It is easily possible to dissect the tonsil from its normal position by separating the capsule from the muscle through this loose connective tissue. This is the case with normal tonsils. In the case of inflammation (PTA or chronic tonsillitis), the tonsil capsule is fixed with muscle fascia and pharyngeal muscles. The easily distinguishable boundary between tonsil capsule and muscle might therefore disappear and the tonsils are no longer easily dissectible (Flint and Cummings, 2010).

The tonsillar fossa is composed of three muscles: the palatoglossus muscle, which forms the anterior pillar; the palatopharyngeal muscle, which is the posterior pillar; and the superior constrictor muscle of the pharynx, which forms the larger part of the tonsillar bed. The muscular wall is thin, and immediately against it on the outer wall of the pharynx lies the glossopharyngeal nerve. The nerve innervates the posterior third of the tongue to provide general and taste sensation.

The arterial blood supply of the palatine tonsils is provided by four different arteries. The lower pole of the tonsil is supplied by the following arteries and

their branches: the lingual artery provides the dorsal lingual branches, while the facial artery provides the ascending palatine artery and the tonsillar branch, and the ascending pharyngeal artery. The upper pole of the tonsil is supplied by the ascending pharyngeal artery, which originates from the internal maxillary artery. Venous drainage is through the peritonsillar venous plexus to the lingual and pharyngeal veins, which in turn drain into the internal jugular vein (Flint and Cummings, 2010; Ed. Pasha, 2017).

The nerve supply of the palatine tonsils is provided by the maxillary division of the trigeminal nerve, giving the descending branches of the lesser palatine nerves, and by the tonsillar branches of the glossopharyngeal nerve. The latter can easily be injured during tonsillectomy or temporarily affected by postoperative oedema, which leads to reduced or loss of general sensation and taste sensation to the posterior third of the tongue. Referring otalgia, frequently encountered following tonsillectomy, is via the tympanic branch of the glossopharyngeal nerve. Efferent lymphatic drainage courses through the upper deep cervical lymph nodes, especially the jugulodigastric or tonsillar node located behind the angle of the mandible (Flint and Cummings, 2010; Ed. Pasha, 2017).

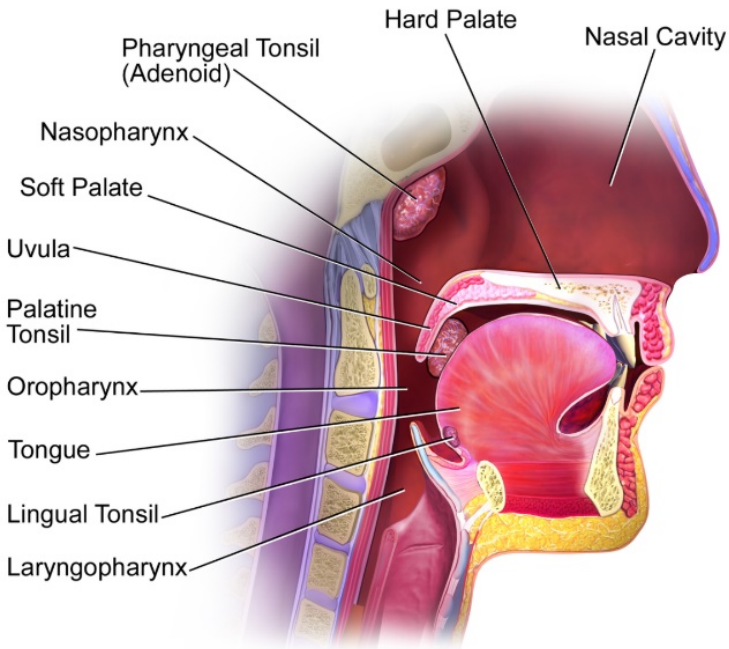

Figure 1. Anatomy of upper airway and tonsillar tissue. Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436

1.2 Histology of palatine tonsils

The oropharyngeal surface of the palatine tonsil is covered with stratified squamous non-keratinized epithelium, whose superficial cells are polygonal in shape and possess microridges on their apical membrane. These cells are connected by tight junctions providing the continuity of the epithelial surface. The superficial cell layer of the crypt epithelium consists of polygonal squamous cells with numerous microridges on the apical surface; small pores – microcrypts, situated between these cells. The crypt epithelium forms a reticular structure where epithelial cells with their slender cytoplasmic processes form a network with intercellular spaces filled with non-epithelial cells, mostly lymphocytes (Nave *et al.*, 2001; Jović *et al.*, 2015). Crypt epithelial cells are connected by numerous desmosomes. In addition, specialised epithelial cells (M cells) for the capture of luminal microparticles are present.

Fenestrated capillaries are frequently present in the subepithelial region and, in the epithelium, small blood vessels, 20 μm in diameter, might be present. Some of these vessels pass the whole thickness of the crypt epithelium. Moreover, Langerhans cells might be found in the crypt epithelium. Tonsillar lymphoid follicles consist of the lymphoid and non-lymphoid cells. Two types of non-lymphoid cells are placed in the follicular germinal centre: small reticular cells with thin reticular fibres forming the reticular framework and large antigen-presenting cells and macrophages. Lymphocytes are present inside the follicle reticulum. Interfollicular lymphoid tissue mostly consists of the reticular cells, reticular fibres and lymphocytes – but large interdigitating dendritic cells are present and endothelial venules and arterioles might also be found (Jović *et al.*, 2015).

1.3 Immunology of palatine tonsils

When antigens enter the oropharyngeal cavity the reticulated crypt epithelium is the first tonsillar compartment that is challenged immunologically. The M cells not only transport the antigens across the epithelial barrier, but also form a specific intraepithelial micro-compartment that brings together high concentrations of foreign antigens, lymphocytes, and antigen-presenting cells such as macrophages, B cells and dendritic cells (**Figure 2**) (Brandtzaeg and Halstensen, 1992; Nave *et al.*, 2001).

After passing the crypt epithelium inhaled or ingested antigens reach the extrafollicular region or the lymphoid follicles. In the extrafollicular region, interdigitating dendritic cells and macrophages process the antigens and present them to CD4+ T lymphocytes (Brandtzaeg and Halstensen, 1992; Nave *et al.*, 2001).

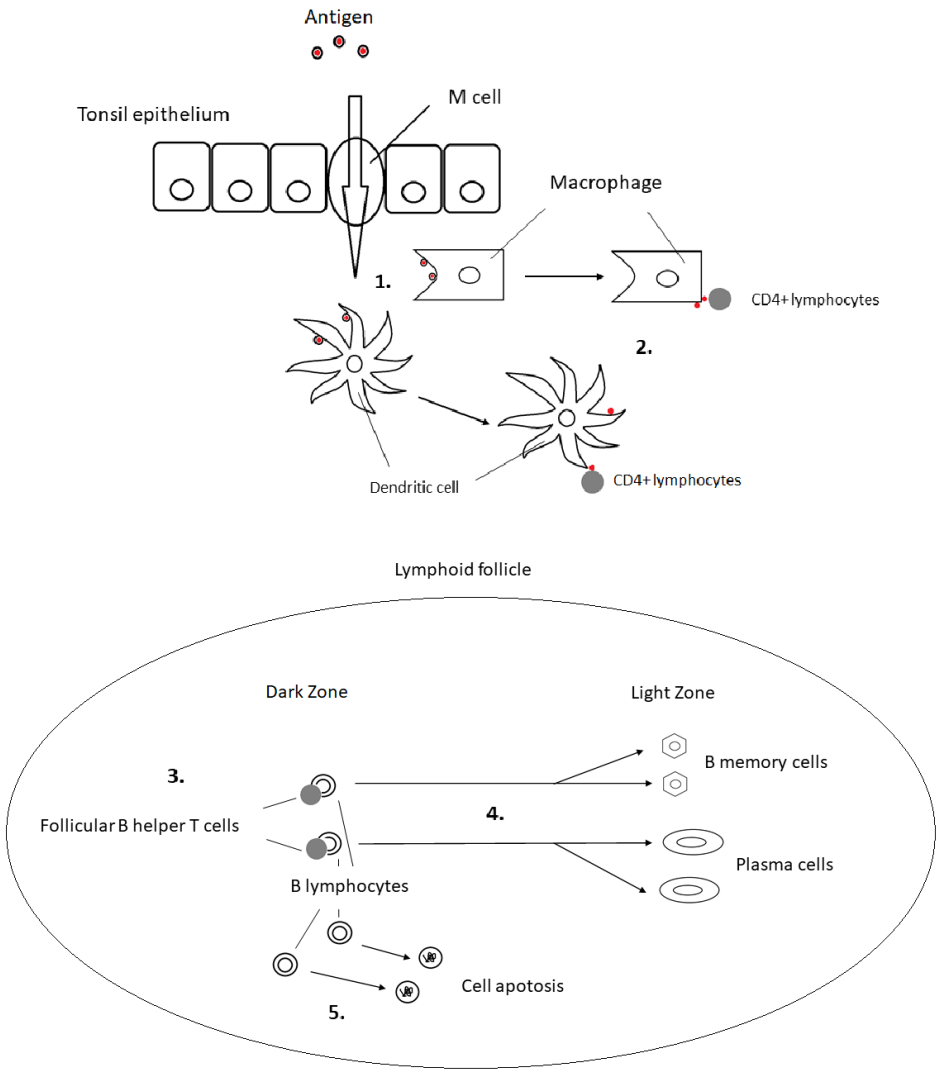

Figure 2. Schematic presentation of some important events in the immunology of palatine tonsils. 1. Antigen is transported from tonsil crypt lumen through M cells to the extrafollicular region. 2. Antigen is processed by dendritic cells and macrophages and is presented to CD4+ lymphocytes (T helper cells). 3. In lymphoid follicle B lymphocytes are stimulated by T helper cells. 4. Stimulated B lymphocytes proliferate and develop into B memory cells and plasma cells. 5. B lymphocytes without stimulation by T helper cells undergo apoptosis. Modified from (Kasenõmm, 2005).

The lymphoid cells found in the spaces of the reticulated crypt epithelium of the human palatine tonsil are mainly B lymphocytes and T helper cells (CD4+). Approximately 50 to 90% of the intraepithelial lymphocytes are B cells. The majority of B cells in the crypt epithelium are mature memory B cells with a

high antigen presenting potential, permitting an early contact between antigen presenting B cells and T cells, and leading to a rapid secondary antibody response. Various Ig isotypes are produced in the palatine tonsils. Approximately 82% of the immunocytes in the germinal centre produce IgD, 55% IgM, 36% IgG and 29% IgA. IgA is a substantial component of the tonsillar humoral immune system (Perry and Whyte, 1998; Nave *et al.*, 2001).

Follicular B helper T cells then stimulate follicular B lymphocytes so that these proliferate and, while migrating from the dark zone of the lymphoid follicle to the light zone, develop into antibody-expressing B memory cells and antibody-producing plasma cells. The presence of antigen allows B cells to survive this migration while, in the absence of stimulation, they undergo apoptosis (Brandtzaeg and Halstensen, 1992; Nave *et al.*, 2001; Brandtzaeg, 2003)

The immune response additionally requires the help of different cytokines. Cytokines are predominantly produced at sites of local antigen stimulation by intraepithelial lymphocytes, other lymphoid cells and non-lymphoid cells (Andersson *et al.*, 1994; Nave *et al.*, 2001).

2. Peritonsillar abscess

2.1. Definition and epidemiology

PTA, formerly known as quinsy, is characterised by the accumulation of a purulent secretion between the fibrous capsule of the palatine tonsil and the pharyngeal superior constrictor muscle (Mitchelmore *et al.*, 1995; Prior *et al.*, 1995; Mazur *et al.*, 2015). PTA has traditionally been regarded as a complication of acute tonsillitis (Galioto, 2017). However, some studies have shown that PTA can also develop from Weber gland infection and is not always secondary to acute tonsillitis (Passy, 1994). In acute tonsillitis, inflammation is limited to the tonsil. In the case of peritonsillitis, inflammation is within the surrounding tissue of the tonsil and this might lead to the formation of PTA. Differentiation between peritonsillitis and PTA could be clinically challenging. In some rare cases, the abscess can form inside the tonsillar tissue (intratonsillar abscess), which is clinically indistinguishable from the real PTA (Blair *et al.*, 2015).

Annual PTA incidence rate varies between different geographic regions. In the USA it has been estimated to be 30 episodes per 100 000 people a year (Herzon, 1995). In Israel, the incidence rate for PTA is lower: 9 episodes per 100 000 people a year (Marom *et al.*, 2010). At the same time, in the Nordic countries it tends to be higher: 37 episodes per 100 000 people a year in Sweden (Risberg *et al.*, 2008) and 41 episodes per 100 000 people a year in Denmark (Ehlers Klug *et al.*, 2009). PTA can affect people in every age group, but it is less common among children under 12 years of age and among elderly patients. The typical PTA patient is a teen or young adult. Among adolescents the annual PTA case rate is significantly higher – 124–167 episodes per 100 000 people a year (Risberg *et al.*, 2008; Klug, 2014).

2.2. Etiopathogenetic aspects

2.2.1. Causative agents

PTA pus contains mixed aerobic and anaerobic microbiota. The most significant pathogen in PTA is considered to be *S. pyogenes* (Group A streptococci). However, *S. pyogenes* has been only been found in roughly one third of cases (Kieff *et al.*, 1999; Lepelletier *et al.*, 2016; Slouka *et al.*, 2020). Other bacteria often associated with PTA are anaerobic Gram-negative rod *Fusobacterium necrophorum* (*F. necrophorum*) and the *Streptococcus anginosus* group (SAG, containing *S. anginosus*, *S. constellatus, S. intermedius)* (Jousimies-Somer *et al.*, 1993; Ehlers Klug *et al.*, 2009; Hidaka *et al.*, 2011; Wikstén *et al.*, 2015). SAG is part of viridans group streptococci. Other viridans group streptococci include the *Streptococcus mitis* group, *Streptococcus salivarius* group and *Streptococcus mutans* group (Maeda *et al.*, 2010).

Findings regarding PTA microbiota differ greatly between studies, especially as concerns anaerobic microbiota percentage (Jousimies-Somer *et al.*, 1993; Ehlers Klug *et al.*, 2009; Slouka *et al.*, 2020). It may be that some microbes play a bigger role in some geographic areas than in others. *F. necrophorum* has been found to be the main causative agent in Denmark and Finland (Jousimies-Somer *et al.*, 1993; Ehlers Klug *et al.*, 2009) but in other studies in different countries it is much less commonly cultivated (Gavriel *et al.*, 2009; Mazur *et al.*, 2015). A different spectrum of causative agents might also be related to methodological factors.

In many cases, the patients have received antibacterial therapy before PTA is treated and microbiological analyses are taken. This may also affect the results and it is also often a reason why the microbial growth is not found in cultures taken from PTA (Prior *et al.*, 1995; Marom *et al.*, 2010). Another problem with identifying the correct cause of PTA is that bacterial cultures are obtained from an area that is normally heavily colonised. The question then arises of what are the true causative agents and what are the contaminants of normal oral microbiota (Brook, 1981).

Moreover, seasonal and age-related trends in PTA microbiota have been shown. A study performed in Denmark showed that *S. pyogenes* is much more commonly found in PTA pus in winter and spring than in summer and autumn (Klug, 2014). Moreover, a study in the Czech Republic showed that *S. pyogenes* is less frequently found in PTA pus among patients older than 50 years than among younger patients (Slouka *et al.*, 2020). At the same time these trends differ between studies and generalisations are difficult to make (Segal *et al.*, 2009; Klug, 2014; Slouka *et al.*, 2020)

2.2.2. Risk factors and pathogenetic mechanisms

PTA patients are generally healthy young adults without chronic diseases. But it has been found that smoking is much more common among PTA patients than in the general population (Lehnerdt *et al.*, 2005; Marom *et al.*, 2010). Moreover, in a Danish population, smoking increased the risk of having PTA by approximately 150% (Klug *et al.*, 2013). Some studies have found male gender to be another risk factor – the predominance ratio being 3:1 (Matsuda *et al.*, 2002; Ong *et al.*, 2004). On the other hand, most studies showed only small male dominance or did show any gender difference at all (Templer *et al.*, 1977; Segal *et al.*, 2009; Shaul *et al.*, 2015). However, it has been found that, in women, PTA presents at an earlier age than in men and that a higher rate of PTA morbidity among men can be explained by the increased smoking rate (Risberg *et al.*, 2008; Kordeluk *et al.*, 2011; Klug, 2014).

Some studies have also found seasonal trends in PTA cases, but there is no consensus as to how seasons affect PTA and in which direction (Beeden and Evans, 1970; Spires *et al.*, 1987; Marom *et al.*, 2010). A Danish study by Klug looked at PTA-causing microbes and found no seasonal changes in incidences of PTA, although there were seasonal changes in causing microbes (Klug, 2014). Seasonal changes in microbes might explain these differences in different geographic regions and seasons.

PTA is mostly considered to be a suppurative complication of acute tonsillitis where the bacteria spread from tonsillar mucosa to the surrounding tissue. It is not described how the bacteria penetrate the tonsillar capsule and there is no scientific literature which provides direct supporting evidence for this (Powell *et al.*, 2013; Klug *et al.*, 2016). If PTA evolves from the Weber salivary gland it is found that a blockage from scarring of the gland or their duct leads to infection – peritonsillitis, and this could evolve into PTA. The blockage is thought to be caused by previous tonsillar infection or poor oral hygiene (Passy, 1994; Powell *et al.*, 2013; Klug *et al.*, 2016). A study conducted in Finland showed that most of the bacteria found from PTA pus were not opsonized by complement components or Ig (Lilja *et al.*, 1998). The same study group had previously shown that this is generally the rule under healthy conditions for bacteria on tonsillar surfaces which are coated with surface IgA (Stenfors and Räisänen, 1996). It is suggested that this blockage of Weber salivary glands may lead to decreased IgA production (Lilja *et al.*, 1998; Powell *et al.*, 2013).

2.2.3. Complications

The earliest description of surgical drainage of PTA originates from the 14th century by the French surgeon Guy de Chauliac and later by Chassaignac, who first reported “tonsillectomy a` chaud” (quinsy tonsillectomy) in 1859 (McCurdy, 1977; Battaglia *et al.*, 2018). In the 19th century, deaths due to PTA in England varied between 110 and 623 individuals a year (Klug, 2017). Nowadays, due to surgical and antimicrobial therapy, severe complications of PTA

are rare. A recent systemic review of literature of PTA complication found that the most common complications were para- and retropharyngeal abscess, necrotizing fasciitis and Lemierre`s syndrome and mediastinitis. That study found that simultaneous diagnosis of PTA and complication (59%) was more common than the development of complications after PTA treatment (36%) or recognition of complications prior to PTA (6%) (Klug *et al.*, 2020).

2.3. Clinical evaluation and diagnosis

2.3.1. Signs and symptoms

PTA patients are commonly found to be obviously ill, uncomfortable and in pain, especially children and older adults. They usually present with high fever and throat pain that is more pronounced on one side. Drooling is caused by odynophagia and dysphagia. Trismus is frequently present as a result of irritation of the pterygoid musculature by the pus collection and inflammation. As a result of trismus PTA patients often have problems swallowing and may become dehydrated. Lymphadenopathy and cervical muscle inflammation may result in limitations in neck mobility (Flint and Cummings, 2010; Bochner *et al.*, 2017; Hur *et al.*, 2018).

Diagnosis of PTA is usually made by clinical examination. A typical clinical finding in PTA is asymmetric peritonsillar swelling with displacement of the tonsil downward and medially with reflection of the uvula toward the opposite side. PTA must be distinguished from severe acute tonsillitis, peritonsillitis and infectious mononucleosis (IM). At the same time, in rare cases IM and PTA may co-exist. Retrospective studies have found that 1.5–6% of PTA patients may have co-existent IM (Arkkila *et al.*, 1998; Ryan *et al.*, 2004; Powell and Wilson, 2012).

Clinically distinguishing between peritonsillitis and PTA can, in particular, be difficult. It can be performed with needle aspiration to detect or exclude pus formation. However, false negative results may be present due to inadequate technique or atypical abscess location (Klug, 2017).

2.3.2. Radiographic examination

Computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound are the three most frequently used radiological examination methods to assist in the diagnosis of PTA, in addition to clinical examination. CT/MRI and ultrasound are used when patients are more ill than clinical findings suggest, and extension of the infection into surrounding tissues of the neck is suspected.

Ultrasound is used as both a diagnostic tool and an assistive tool for aspiration with high efficiency. Both intraoral and transcutaneous ultrasound are used. Intraoral ultrasound is more sensitive, but because of trismus its use is not always possible and it might also need specially trained personnel to use (Araujo Filho *et al.*, 2006; Powell and Wilson, 2012; Nogan *et al.*, 2015).

CT with contrast is highly sensitive for PTA and it is used in particular when the spread of the infection is suspected (most often into parapharyngeal space or mediastinum) and its use in diagnosing PTA has increased over time. A study in Taiwan found, for diagnosing PTA in children, CT use has increased from 1.4% in 2000 to 12% in 2012 (Lee *et al.*, 2019). However, CT comes with cost, waiting time and high radiation exposure, which limit its use (Smith-Bindman *et al.*, 2009; Nogan *et al.*, 2015). MRI has the advantages of improved soft tissue detail and allowing for the assessment of the carotid sheath without the associated radiation of a CT. However, MRI takes longer, tends to be more expensive and has greater problems of availability compared with CT (Powell and Wilson, 2012).

2.3.3. Other diagnostic methods

C-reactive protein (CRP) and white blood cells (WBC) are the most frequently used infection markers from blood in the monitoring of head and neck infection. These parameters are frequently used to assess the severity of infection and whether the origin of the infection is viral or bacterial (Christensen *et al.*, 2013). In the case of PTA, CRP and WBC are usually also elevated. Venous blood tests can be also used to distinguish PTA from other diseases such as tumours, which may present similar symptoms as PTA. In addition, a study conducted in Taiwan by Liu showed that decreased haemoglobin level is an independent predictor for longer hospital stay in the case of PTA (Liu *et al.*, 2017).

Some methods other than imaging and clinical examination have been proposed to help distinguish between acute tonsillitis, peritonsillitis and PTA. Biomarkers in saliva and serum have been searched (Spiekermann *et al.*, 2017). Even artificial intelligence and machine learning are used to diagnose PTA with an accuracy of 72.3% (Wilson *et al.*, 2019). Currently however, other diagnostic methods are not widely used.

2.4. Treatment options

2.4.1. Non-surgical treatment

Treatment for PTA usually consists of opening abscess with adequate antibacterial therapy. At the same time there is no clarity regarding what adequate antibacterial therapy should be. There are significant differences between studies and countries. Based on microbiological findings some authors recommend wide-spectrum antibiotics such as penicillin with metronidazole, third-generation cephalosporin with metronidazole, clindamycin and amoxicillin with clavulanate acid (Powell and Wilson, 2012; Galioto, 2017). In contrast, it has been shown that the use of penicillin alone does not lengthen the hospital stay and many authors recommend monotreatment with penicillin, especially in the Nordic countries (Kieff *et al.*, 1999; Risberg *et al.*, 2008; Ehlers Klug *et al.*, 2009; Wikstén *et al.*, 2014). Even though there are some surgical guidelines for

the treatment of PTA (Windfuhr *et al.*, 2016), there are no common clinical practice guidelines available for the medical treatment of PTA (Wikstén *et al.*, 2014).

However, in some cases medical treatment or surgery alone might be enough. A retrospective nationwide cohort study in Taiwan of 28 837 PTA patients showed that in 40.8% of cases antibiotic therapy alone was an effective treatment for PTA (Wang *et al.*, 2014). A study performed in the USA by Lamkin showed that, among patients who received antibiotics as the first line of treatment, only 4% needed further surgical treatment (Lamkin and Portt, 2006). Treatment with antibiotics as a monotreatment has been shown to cause less pain, opioid use and days off work. However, this option is only possible in PTA cases without complications (Battaglia *et al.*, 2018).

How the addition of glucocorticosteroids to PTA treatment affects clinical outcome has been analysed in several studies. Steroids have been shown to improve trismus and resolution of fever, and offer improved oral intake and decreased length of hospital stay (Ozbek *et al.*, 2004; Chau *et al.*, 2014; Battaglia *et al.*, 2018).

In 2020, a meta-analysis was performed to compare medical treatment alone and surgical intervention. There was no difference in odds of treatment failure for patients with PTA managed through medical intervention alone compared to surgical intervention. At the same time, the quality of many studies involved in that meta-analysis was low (Forner *et al.*, 2020). When choosing monotreatment with antibiotics, clinicians should be aware of potential PTA life-threatening complications. A systematic review of literature conducted by Klug showed that men and patients over 40 have a significantly increased risk of PTA complications (Klug *et al.*, 2020).

2.4.2. Surgical treatment

There are three surgical options for the draining of PTA: needle aspiration, incision and drainage, and acute tonsillectomy. Treatment options and protocols vary widely between countries and even within them (Wikstén *et al.*, 2014). Treatment with needle aspiration or incision and drainage leaves the opportunity for PTA to recur. PTA recurrence rate varies between 9 and 22% (Sørensen *et al.*, 1991; Powell and Wilson, 2012; Chung *et al.*, 2014). Acute tonsillectomy has fallen out of favour in some areas because of postoperative haemorrhage. However, acute tonsillectomy is usually preferred in cases of young children where cooperation is difficult and patients with severe trismus (Flint and Cummings, 2010; Bochner *et al.*, 2017). Moreover, acute tonsillectomy is the method of choice when the spread of the disease to other spaces is suspected.

Usually, surgical treatment is combined with antibiotics (Galioto, 2017). However, a German study by Knipping found no higher complication rate when no additional antibiotic treatment was used after acute tonsillectomy (Knipping *et al.*, 2009). Another study found that 8 patients out of 9 who were treated with ineffective antibiotics (antibiotics to which the putative causative agents were

resistant) had complete resolution of PTA after the abscess was drained (Sowerby *et al.*, 2013). These findings suggest that, in some cases, if the PTA is surgically well treated and the abscess fully drained then surgical treatment alone might be enough. Nonetheless, future studies are required to confirm this.

* * *

In summary, PTA is the most frequent cause of emergency hospitalisation in otorhinolaryngology. At the same time, it is not fully agreed how PTA develops and what the true causative agents would be. This has led to a wide variety of treatment options. PTA pus is usually polymicrobial and there seems to be at least some geographic differences. PTA is mostly studied in Western countries, especially in Northern Europe, but information is lacking about Estonia and the other Baltic States.

PTA patients are frequently severely ill when they are hospitalised. It is unclear how many PTA patients have sepsis at the time of hospitalisation and the effect of sepsis on treatment outcomes.

Moreover, since the pathomechanisms of PTA are unclear, there are few evidence-based suggestions on how to prevent PTA or reduce its prevalence.

Our research has attempted to fill these gaps.

AIMS OF THE STUDY

The main aim of the present thesis was to evaluate the etiopathogenetic factors, aspects of the clinical picture and possible novel ways to treat and prevent peritonsillar abscess.

Therefore, the specific objectives of the present thesis may be summarised as the following:

1. To clarify the risk factors and pathogenetic aspects of PTA.
2. To evaluate the frequency and clinical significance of sepsis clinical picture in the case of PTA.
3. To compare different specimens (tonsil fossa, pus, blood) and methods (culture, NGS) in identifying the causative agents of PTA.
4. To assess antibiotic susceptibility of potential pathogens isolated from PTA patients.
5. To assess the utility of some polyols for the treatment and prevention of PTA.

MATERIAL AND METHODS

3. Subjects and study design

The present research included 114 PTA patients. Patients were recruited in the framework of two studies and the results were published as three separate papers. All investigations were conducted as prospective studies. All patients were admitted to the Department of Otorhinolaryngology, Tartu University Hospital, Estonia with suspicion of PTA and were undergoing bilateral emergency tonsillectomy. PTA diagnosis was confirmed intraoperatively by pus in the peritonsillar space. The first group consisted of 22 patients enrolled from November 2011 until May 2012. The second group consisted of 92 patients enrolled from April 2016 until August 2017 (**Table 1**).

Table 1. Subjects and methods

	Enrolment	Number of subjects	Methods
Study I	November 2011–May 2012	22	Macroscopic oropharyngeal signs Tonsillitis index Bacterial cultures of pus, tonsillar fossa, blood Antibiotic susceptibility testing
Study II	April 2016–August 2017	92	Sepsis clinical signs Collection of *S. pyogenes* strains Amylase in pus Biomarkers from blood Next-generation sequencing Polyol susceptibility of *S. pyogenes* strains

3.1. Participants of bacteriological study

All patients of the first study group were hospitalised due to PTA at the Department of Otorhinolaryngology, Tartu University Hospital, between November 2011 and May 2012. This study group consisted of 22 consecutive patients (16 males and 6 females with a median age of 32, range 14–74). Patients with unilateral PTA were selected and the opposite side to PTA was used to evaluate the macroscopic oropharyngeal signs. The macroscopic oropharyngeal signs were evaluated and tonsillitis index (TI) was calculated before tonsillectomy for PTA. During operation, the microbiological samples were collected for cultures from three different locations – pus from the peritonsillar space, tonsillar fossa biopsy and peripheral blood. From all outgrown bacteria the opportunistic pathogens were selected for antibiotic susceptibility testing.

3.2. Participants of sepsis evaluation and molecular study

All PTA patients admitted with PTA from April 2016 until the end of August 2017 were enrolled into the second study group. This group consisted of 54 males and 38 females (with a median age of 31.5 years, range 13–67). Exclusion criteria included age under 12 years (previous studies have shown that PTA is rare under the age of 12), current administration of immunosuppressive medications, immunosuppressive disease, current chemotherapy or radiotherapy, and diabetes (both type I and type II).

The sepsis clinical signs were evaluated. Before emergency tonsillectomy, blood samples for the detection of biomarkers were taken. Intraoperatively, the pus and the tonsillar biopsy was collected for the evaluation of amylase and for microbiological analyses (cultures and next-generation sequencing (NGS)). All *S. pyogenes* strains outgrown from PTA pus were used for polyol susceptibility testing.

4. Methods

4.1. Clinical information and evaluations

For both groups, the following information was obtained: age, gender, type of antimicrobial therapy before admission, axillary temperature with an accuracy of 0.1 °C. Measurement of axillary temperature was used over sublingual, because of the practice in our clinic to avoid excessive inconveniences for patients with oral and pharyngeal abscesses (including PTA) and patients after surgeries in that area.

4.2. Oropharyngeal evaluations

In the first group, evaluation of the presence or absence of five macroscopic oropharyngeal signs was made on the tonsil in the opposite side of PTA and these were as follows: tonsillar sclerosis, obstruction of the tonsillar crypts, scar tissue on the tonsils, cryptic debris and lymphatic tissue aggregates. Tonsillar sclerosis was defined as increased tightness of the tonsillar and peritonsillar tissue by palpation together with fixation of the palatine tonsil in the tonsillar fossa. Obstruction of the tonsillar crypts was documented when narrowing of the crypt openings was observed, resulting in the loss of a clear cryptic pattern of the tonsillar surface. Scar tissue on the tonsils was defined as white tissue spots or streaks on the tonsillar surface. Cryptic debris was described as any white or yellow matter in the crypts or in the supratonsillar cleft. Multiple round or elongated yellow-coloured patches on the retropharyngeal mucosa were described as lymphatic tissue aggregates, which are presumably due to the enlargement of normal lymphatic structures in the throat.

We also calculated TI by multiplying the number of tonsillitis episodes per year by the morbidity period in years (Fujihara *et al.*, 2005). It approximately represents the total number of tonsillitis episodes the patient has ever

experienced. The basic clinical parameters of this group are presented in **Table 1** of **Paper I**.

4.3. Assessment of sepsis

In the second group, heart rate and respiratory frequency were measured. Before surgery, the blood samples were collected. Patients were also evaluated for sepsis criteria based on the 2001 International Sepsis Definitions Conference (Sepsis-2). Sepsis is defined as being the clinical syndrome by the presence of both infection and systemic inflammatory response syndrome (SIRS) (Levy *et al.*, 2003). Criteria for SIRS are considered to be met if at least two of the following four clinical findings are present: Temperature higher than 38 °C or lower than 36 °C; Heart rate higher than 90 beats per minute; Respiratory rate higher than 20 breaths per minute or arterial carbon dioxide tension lower than 32 mm Hg; WBC count higher than 12 $x10^9$/L or lower than 4 $x10^9$/L or with 10% immature forms. The basic clinical parameters of this group are presented in **Table 1** of **Paper II**.

4.4. Therapeutic procedures

All patients underwent emergency tonsillectomy that was performed within 24 hours after diagnosis of PTA. All operations were performed under general anaesthesia. Both tonsils were removed by blunt dissection.

During hospitalisation, the patients received intravenous antibiotics and analgesics. Intravenous administration of fluids was used if needed. Most patients were discharged from hospital the day after surgery. Oral antibiotics and analgesics were prescribed at home. The most commonly used antibiotic was penicillin.

4.5. Sample collection

For microbiological culture analyses, the samples were collected from three regions – pus from peritonsillar space, tonsillar fossa, blood. The pus aspirate was collected just before removing the tonsils when the patient was already under general anaesthesia by puncturing the peritonsillar space. The second microbiological sample was tissue biopsy from tonsillar fossa. A biopsy was taken after removing the tonsil from the side of the PTA. Within 5 minutes of removing the infected side tonsil, the peripheral blood cultures were drawn aseptically into BACTEC Plus Aerobic/F and BACTEC Plus Anaerobic/F blood culture bottles (Becton Dickinson, USA). Pus and biopsy specimens were placed in sterile containers and immediately taken to a microbiology laboratory and frozen or cultured within 2 hours.

For microbiological molecular analyses, the samples were collected from two areas – PTA pus and tonsillar biopsy. The pus sample was taken after opening the PTA with a swab. The tonsillar biopsy sample was taken after operation from the removed tonsil from the PTA-affected side.

4.6. Microbiological analyses

4.6.1. DNA extraction

In total, 182 samples (91 washouts of the pus swab materials and 91 tonsillar biopsy specimens) were analysed by next-generation Illumina sequencing. Both samples were collected from the same patient.

Bacterial DNA from washouts of the swab material was extracted with the PureLink™ Microbiome DNA Purification Kit (Invitrogen, USA), using an ELMI Sky Line instrument (ELMI Ltd, Latvia) according to manufacturer's instructions.

The frozen biopsy specimens (~25 mg) were suspended in 500 µL of lysis buffer (200 mM Tris-HCl (pH 8.0), 25 mM ethylene diamine tetraacetic acid, 300 mM NaCl, 1.2% sodium dodecyl sulfate) and 20 µL of proteinase K (400 µg/mL) for DNA extraction. The mixture was incubated at 37 ºC for 24 h. The procedure of DNA extraction was continued according to the tissue protocol of QIAamp DNA Mini Kit (Qiagen, Germany).

Extracted DNA samples were quantified with Qubit and diluted to 5 ng/µL.

4.6.2. Next-generation sequencing

DNA was amplified using the primers 16SF (5′- TCGTCGGCAGCGTCAGA TGTGTATAAGAGACAGCCTACGGGNGGCWGCAG -3′) and 16SR (5′- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGT ATCTAATCC -3′) for PCR amplification of an approximately 460 bp region within the hypervariable (V3-V4) region of prokaryotic 16S ribosomal RNA gene (Klindworth *et al.*, 2013). The first PCR mixture contained 12.5 µL of KAPA HiFi HotStart ReadyMix (2X) (Kapa Biosystems, USA), 1 µL of each primer (10 µM) and 6 µL of template DNA (5 ng/µL). The reaction volume was brought to 25 µL with milliQ water. PCR conditions were 95 °C for 3 min followed by 24 cycles of 95 ºC for 30 sec, 55 ºC for 30 sec, and 72 ºC for 30 sec with a final extension at 72 ºC for 5 min. The PCR products were purified using a 0.8x solution of AMPure XP Beads (Beckman Coulter, Inc.). The purified products were quantified with Qubit, diluted to 10 ng/µL and used as a template for indexing PCR. Indexes and sequencing adapters were attached to the PCR products in the indexing PCR, using Illumina Nextera XT dual index primers (Illumina Inc., USA). The indexing PCR contained 5 µL of each index primer, 15 µL of KAPA HiFi HotStart ReadyMix (2X) and 5 µL of template DNA (10 ng/µL).

The conditions for the second PCR cycling were 95 ºC for 3 min followed by 7 cycles of 95 ºC for 30 sec, 55 ºC for 30 sec, and 72 ºC for 30 sec with a final extension at 72 ºC for 5 min. Indexed PCR products were purified using a 1.9x solution of AMPure XP Beads, quantified and combined into a final library pool in equimolar concentrations. The library pool was quantified using Illumina-specific KAPA Library Quant Kit (Kapa Biosystems, USA). Sequencing was carried out on an Illumina MiSeq System using MiSeq Reagent Kit v3 in paired end 2 × 300 bp mode.

DNA sequence data were analysed using BION-meta, currently unpublished open source program, according to authors' instructions (McDonald *et al.*, 2016). First, sequences were cleaned at both ends using a 99.5% minimum quality threshold for at least 18 of 20 bases for the 50-end and 28 of 30 bases for the 30-end, then joined, followed by the removal of contigs shorter than 350 bp. The sequences were cleaned from chimeras and clustered by 95% oligonucleotide similarity (chimera length of 8 bp, step size 2 bp). Lastly, consensus reads were aligned to the SILVA reference 16S rRNA database (v123) using a word length of 8 and similarity cut-off of 90.

4.6.3. Culture methods

Pus and biopsies were cultured semi-quantitatively in the anaerobic box with a 10 µl sterile loop. The biopsy samples were ground in a mortar under sterile conditions before cultivation. Blood agar plates (Oxoid; UK) were employed for aerobic bacteria, chocolate agar plates for *Haemophilus sp*, Tryptic Soy-Serum-Bacitracin-Vancomycin agar (TSBV; Oxoid, UK) for *Aggregatibacter sp,* and Fastidious Anaerobe Agar (FAA; LabM, UK) for anaerobic bacteria. The plates were incubated at 36.6 ºC either aerobically for 2 days (blood agar), in a CO_2-enriched atmosphere for 5 days (chocolate and TSBV agar) or anaerobically for 7 days using the anaerobic glove box (FAA). The count of bacteria was evaluated semi-quantitatively: the growth in the first sector (+), in the second sector (++), in the third sector (+++), and in the fourth sector (++++). Identification of isolated microorganisms was performed using MALDI Biotyper (Bruker Daltonics).

4.6.4. Antibiotic susceptibility testing

For antibiotic susceptibility testing, only the opportunistic pathogens were selected. The selected microorganisms were *S. pneumoniae*, *S. pyogenes*, *S. parasanguinis*, SAG, *R. mucilaginosa*, and *F. necrophorum*. Minimum inhibitory concentrations (MIC) were assessed using the E-test method and the guideline "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0, 2015. http://www.eucast.org." Mueller-Hinton agar plates were used, and the cultures were incubated for 24 hours at 35 ºC. Streptococci were incubated in a microaerophilic atmosphere (10% CO_2) and anaerobes were incubated in an anaerobic glove box (Sheldon Manufacturing Inc, with a gas mixture: 5% H_2, 5% CO_2, 90% N_2).

Exact MIC values, which were used to determine sensitivity for each specimen, were: *S. parasanguinis* – penicillin, ampicillin/sulbactam (MIC susceptible ≤ 0.25; resistant > 2); clindamycin (MIC susceptible ≤ 0.5; resistant > 0.5); SAG – penicillin, ampicillin/sulbactam (MIC susceptible ≤ 0.25; resistant > 2); clindamycin (MIC susceptible ≤ 0.5; resistant > 0.5); *S. pneumoniae* – penicillin (MIC susceptible ≤ 0.06; resistant > 2); ampicillin/sulbactam (MIC susceptible

≤ 0.5; resistant > 2); cefuroxime, erythromycin (MIC susceptible ≤ 0.25; resistant > 0.5); clindamycin (MIC susceptible ≤ 0.5; resistant > 0.5); ciprofloxacin (MIC susceptible ≤ 0.125; resistant > 2); *S. pyogenes* – penicillin, ampicillin/sulbactam (MIC susceptible ≤ 0.25; resistant > 0.25); clindamycin (MIC susceptible ≤ 0.5; resistant > 0.5); *F necrophorum* – penicillin (MIC susceptible ≤ 0.25; resistant > 0.5); ampicillin/sulbactam (MIC susceptible ≤ 4; resistant > 8); clindamycin (MIC susceptible ≤ 4; resistant > 4); metronidazole (MIC susceptible ≤ 4; resistant > 4).

4.6.5. Polyol susceptibility testing

A total of 36 *S. pyogenes* strains, 31 from pus of PTA patients from Study II and 5 throat strains from culture collections (CCUG 23117, CCUG 53553, DSM 25932, DSM 25943 and DSM 11728) were included. Erythritol (ERY), (Cargill R&D Centre Europe, Belgium) and xylitol (XYL), (≥99%, Sigma-Aldrich Co, USA) were tested. Tested substances were sterilised by filtration at the desired concentration and added to the brain-heart infusion (BHI), (Oxoid Limited, UK) medium (sterilisation by autoclaving at 121 °C for 15 min). The bacteria were incubated at 37 °C in 10% CO_2 for 24 hours on blood agar (Oxoid Limited, UK).

The microtiter plate wells (Cellstar® 96 well polystyrene suspension culture microplates, F-bottom, Greiner Bio-One GmbH, Austria) were inoculated with equal amounts (200 µl) of bacterial (the final test-concentration of 10^5 CFU/ml) and polyol solution. The bacterial cells were grown at 37 °C in 10% CO_2 on the microtiter plate. The density of bacterial growth was detected spectrophotometrically in an absorbance microplate reader (Labsystems Multiskan® MCC/340, Fisher Scientific, USA) at 620 nm. The measurement time points were 0 and 24 hours.

The tested polyol concentrations (weight/volume) were 2.5%, 5% and 10%. The polyol effect was calculated as the difference between average BHI and polyol test values. Polyol effectivity scores were calculated as a summary effect of three polyol concentrations (2.5%, 5% and 10%) where each polyol concentration gave -1, 0 or +1 points depending on whether the *S. pyogenes* growth increased, remained unchanged or decreased in the presence of polyols compared to the growth in BHI, respectively. All experiments were performed in triplicate and repeated twice. Each value represents the average of two median results from triplicate experiments.

Four *S. pyogenes* strains were selected for the growth curve study, two with high polyol effectivity scores (ERY score 3, XYL score 3) and two with low polyol effectivity scores (ERY score -3, XYL score -1). One of the high score and one of the low score strains were from high amylase pus and one of the high score and one of the low score strains were from low amylase pus. For the growth curve study, the microtiter plates were prepared similarly to the growth inhibition study. The following incubation at 37 °C took place in a spectrophotometer with an integrated incubator (Multiscan Go 1510-00361, Thermo

Fisher Scientific Oy, Finland. Skanit Software 3.2) in aerobic conditions. The density of bacterial growth in microwells was detected spectrophotometrically at 620 nm in 1-hour intervals from 0 to 24 hours with preceding shaking of 5 seconds.

4.7. Other laboratory analyses

Blood samples were transported to the United Laboratory of Tartu University Hospital where white blood cell count, CRP, procalcitonin (PCT), anti-streptolysin O and amylase were determined using standard methods. Amylase levels in the 5-fold diluted pus were measured by kinetic colorimetric method in United Laboratory of Tartu University Hospital.

We also measured amylase in PTA pus. For that we diluted pus 5-fold and transported the diluted pus samples to the United Laboratory of Tartu University Hospital. Amylase was measured by a kinetic colorimetric method.

4.8. Statistical analysis

For statistical analyses, SigmaStat (Systat Software, USA) and Excel (Microsoft, USA) software programs were used. The differences between the groups were calculated with t-test (in the case of normal distribution) and Mann-Whitney rank sum test (in the case of nonparametric distribution) as well as Fisher's exact test and Chi-squared test. Spearman correlation was used to determine correlations between the parameters. Statistical significance was assumed at $p < 0.05$ for all parameters.

The NGS data were analysed using t-test, Mann-Whitney U test and Wilcoxon rank sum test. The diversity of microbiota was described by richness, Shannon 'H' diversity and Simpson's indices. SILVA reference 16S rRNA database (release 138) with a word length of 8, similarity cut-off of 90 and renewed taxonomic paths was used (**Figure 4**). All names of validly described species in the SSU and LSU databases have been checked for changes (basonyms, synonyms and orthographical corrections) against the DSMZ "Bacterial Nomenclature up to date (PNU)" service as of June 2019 (https://www.arb-silva.de/documentation/release-138/). Therefore, the taxonomic bacterial classification names (e.g. for some phyla) may differ slightly from the traditional nomenclature (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi).

4.9. Ethical considerations

Participation in the studies was voluntary. All subjects were informed about the studies' nature and, after the signing an ethics-committee-approved informed consent form, they were entered into the studies. The studies were conducted in compliance with the "Ethical principles for medical research involving human subjects" of the Helsinki Declaration and approved by the Ethics Review Committee on Human Research of the University of Tartu (protocols no: 202T-2 and 255/T-1).

RESULTS AND DISCUSSION

5. Clinical picture in PTA patients

5.1. Oropharyngeal and other characteristics

Study I consisted of 22 patients and Study II consisted of 92 patients with PTA (**Table 2**). In both studies there was a male dominance: in the first study 72.7% and in the second study 58.9% of the PTA patients were male. Some other authors have found that PTA is seen with equal representation among both sexes while the others have noted male dominance (Kronenberg *et al.*, 1987; Sørensen *et al.*, 1991; Ehlers Klug *et al.*, 2009). In our first study male dominance was at a ratio 3 to 1 – this is among the greatest gender differences in the literature, similarly to the findings of Matsuda (Matsuda *et al.*, 2002).

Table 2. Clinical data of the PTA patients

	Study I		**Study II**	
Gender	16 M (72.7%), 6 F (27.3%)		54 M (58.7%), 38 F (41.3%)	
Antimicrobial therapy before admission (%)	72.7%		48.9%	
	Median	Range	Median	Range
Age (years)	32	14–74	31.5	13–67
Temperature (ºC)	38.4	37.1–39.1	37.6	36.0–39.4
Duration of symptoms before hospitalisation (days)			5.5	1–30

In both studies, the median age, age range and duration of symptoms were very similar. Our data are similar to some other Scandinavian data where the average duration of symptoms before diagnosis of PTA was 5 days, but with a greater range of 1–60 days. At the same time the median age in Denmark was 2 days and patients affected with PTA were accordingly younger, 21 years in Denmark and 25.6 years in Sweden (Risberg *et al.*, 2008; Ehlers Klug *et al.*, 2009).

In the first study 68.2% and in the second study 73.9% of patients had a fever of more than 37.1 ºC. Antibiotic consumption before admittance was higher in Study I. Antibiotic therapy was mostly prescribed by family doctors and it ranged widely. Antibiotics that were prescribed in the first study were penicillin V, amoxicillin, cefadroxil, and clindamycin. Three patients out of 16 who had taken antibiotics before hospitalisation had taken two courses, the second choice being always clindamycin. In the second study, antibiotics that patients had taken before hospitalisation were penicillin V, amoxicillin, amoxicillin with clavulanic acid, azithromycin, clarithromycin, clindamycin, cefadroxil, cefuroxime, cefprozil and ciprofloxacin.

In Denmark and Sweden, 38% and 21% of the patients had antibiotic treatment before hospitalisation, respectively (Risberg *et al.*, 2008; Ehlers Klug *et al.*, 2009). In addition, in Sweden the majority of patients were treated with penicillin V and 94% patients in Denmark with penicillin-type antibiotics. In both our studies less consistency was seen in the choice of antibiotics and significantly wider spectrum antibiotics were used. This is probably due to the fact that Estonia does not have nationwide clinical guidelines for the treatment and management of PTA.

5.2. Comparison of patients with and without sepsis symptoms

The patients of Study II were divided into two groups based on fulfilling sepsis criteria (we used criteria referred to as Sepsis-2) (Levy *et al.*, 2003). Forty-seven patients out of 92 (51.1%) had sepsis symptoms during hospitalisation. There were no differences between patients' age or duration of symptoms before hospitalisation between those groups.

Patients with sepsis symptoms had statistically significantly higher values in terms of body temperature, heart rate, respiratory rate and WBC count in comparison with the non-sepsis group. This is understandable because those are the four clinical findings which sepsis diagnosis is based upon. WBC had a strong positive correlation with CRP ($r=0.943$, $p=0.0167$) and PTA patients with sepsis seemed to have higher CRP levels, but this finding was not statistically significant ($p=0.071$). Patients who had not received any kind of antibiotic treatment before hospitalisation had more sepsis symptoms (accordingly 31 vs 16). The positive effect of antibiotics has previously been shown: a systematic review showed that antibiotic prescription significantly reduces PTA incidents compared to a placebo (Spinks *et al.*, 2013). Moreover, we found that patients who smoked had significantly more sepsis symptoms: 36% of the patients in sepsis group were smokers compared to 11% of patients in the group without sepsis symptoms.

There was no difference between pus amylase levels in the groups with and without sepsis symptoms. Out of 9 patients with very high levels of pus amylase 5 patients had sepsis symptoms (1304–7004 U/L) and 4 patients were without sepsis symptoms (860–10019 U/L).

We also evaluated two inflammation biomarkers – CRP and PCT. Two patients had very high levels of PCT – 38.05 and 49.94 ng/mL (one with symptoms of sepsis and the other without). If we excluded these two values, then there was no difference in PCT levels between study groups (median values 0.08 and 0.07) and PCT overall increase in blood samples was very small.

Table 3. Clinical and laboratory data of the patients of Study II

	Mean ± SD [median (range)]			*P* values
	Total (n=92)	Without clinical picture of sepsis (n=45)	With clinical picture of sepsis (n=47)	
Age (years)	33.5 ± 13.8 [31.5 (13–67)]	35.5 ± 15.7 [33 (13–67)]	31.6 ± 11.6 [31 (15–59)]	NS
Temperature (°C)	37.6 ± 0.6 [37.5 (36.0–39.4)]	37.2 ± 0.5 [37.2 (36.0–39.0)]	37.9 ± 0.6 [38.0 (36.5–39.4)]	<0.001
Duration of symptoms before hospitalisation (days)	5.5 ± 4.1 [5 [1–30)]	5.5 ± 2.7 [5 (2–14)]	5.6 ± 5.1 [4 (1–30)]	NS
Antibiotic treatment before hospitalisation (n,%)	45 (49%)	29 (64%)	16 (34%)	0.003
Smokers (n,%)	20 (22%)	5 (11%)	15 (32%)	0.016
Smoking years	11.0 ± 9.2 [9.5 (0.25–40)]	11.3 ± 16.3 [5 (0.25–40)]	10.9 ± 6.2 [10 (2–20)]	NS
Smokes per day	10.9 ± 6.6 [10 (0.5–20)]	8.4 ± 7.2 [6 (1–20)]	11.7 ± 6.5 [10 (0.5–20)]	NS
Smoking in pack-years	6.3 ± 6.0 [4.3 (0.1–20)]	4.6 ± 5.3 [4 (0.1–13.3)]	6.9 ± 6.3 [4.5 (0.5–20)]	NS
Pulse (x/min)	90.1 ± 16.1 [90 (52–140)]	80.2 ± 12.2 [83.5 (52–102)]	100.1 ± 13.2 [99 (72–140)]	<0.0001
Respiratory rate (x/min)	19.1 ± 5.8 [18 (12–59)]	17.9 ± 3.6 [17 (12–35)]	20.5 ± 7.3 [19 (12–59)]	<0.0001
O_2 saturation (%)	96.6 ± 2.2 [97 ± (88–100)]	96.8 ± 2.2 [98 ± (90–100)]	96.4 ± 2.1 [97 ± (88–99)]	NS
Procalcitonin (ng/mL)	1.2 ± 6.9 [0.07 ± (0.05–49.94)]	1.3 ± 7.7 [0.07 ± (0.05–49.94)]	1.1 ± 6.1 [0.08 ± (0.05–38.05)]	NS

	Mean ± SD [median (range)]			*P* values
	Total (n=92)	Without clinical picture of sepsis (n=45)	With clinical picture of sepsis (n=47)	
C-reactive protein (mg/L)	100.1 ± 67.4 [86.9 (6.9–334.4)]	88.6 ± 65.9 [74.0 (6.9–274.0)]	111.1 ± 67.7 [106.4 (13.7–334.4)]	0.071
White blood cells (x10^9/L)	13.6 ± 3.7 [13.8 (6.8–22.7)]	11.6 ± 3.4 [10.7 (6.8–18.9)]	15.4 ± 2.9 [15.4 (10.2–22.7)]	<0.001
Anti-streptolysin O (IU/mL)	259.9 ± 405.3 [147.0 (20.0–3079.0)]	270.2 ± 487.8 [115.5 (20.0–3097.0)]	250.0 ± 311.8 [162.5 (20.0–1541.0)]	NS
Amylase in serum (U/L)	45.3 ± 17.5 [43.0 (20.0–108.0)]	48.0 ± 18.7 [49.0 (20.0–108.0)]	42.6 ± 16.0 [42.0 (20.0–83.0)]	NS
Amylase in pus (U/L)	765.3 ± 2064.5 [<3 (<3–10019)]	589.2 ± 1968.9 [5 (<3–10019)]	984 ± 2205.7 [<3 (<3–7004)]	NS

P values indicate difference between the patients with and without sepsis clinical picture
NS – not significant

5.3. Association with Weber salivary gland infection

Twelve patients out of 92 had amylase levels in their pus at least twice as high (94–10019 U/L) than in their serum. Average pus amylase was 765.3 U/L and median pus amylase was less than 3 U/L (**Table 3**). Average serum amylase was 45.3 U/L with range (20–108 U/L) and median 43.0. There was no difference in other clinical symptoms between patients who had high amylase levels in their pus versus patients who did not. This result is similar to the finding of El-Saied, who did not see any clinical differences between the high levels of amylase group and low levels of amylase group (El-Saied *et al.*, 2012). The only difference between the groups in the study was that the patients with recurrent PTA always had lower levels of amylase (lower than 65 U/L). We were not able to confirm that, because our study groups did not include patients with recurrent PTA. Also, in two studies conducted by El-Saied, almost half of the patients had amylase of more than 65 U/L (El-Saied *et al.*, 2012, 2014). In our study only 13% of patients had high elevation of amylase in their pus. High amylase levels in PTA pus are associated with Weber salivary glands. Therefore, only a small number of our patients can be associated with PTA originated from Weber salivary glands.

6. Anamnesis of PTA patients

6.1. Former tonsillitis episodes

In Study I, we collected data about previous tonsillitis episodes and macroscopic oropharyngeal signs which refer to previous tonsillar infection and chronic tonsillitis. Most of the patients (63.6%) did not report any previous problems with their tonsils, 31.8% patients had one to three episodes of tonsillitis a year and only one patient had tonsillitis episodes more often than three times a year. Calculated TI score median was 0 and mean 4.04 (with range 0–36). After excluding the one person with a TI score of 36, the mean value of TI dropped to 2.16. Other studies have reported recurrent tonsillitis percentage from 7.9% to 85% (Templer *et al.*, 1977; Stringer *et al.*, 1988; Wolf *et al.*, 1994; Chung *et al.*, 2014; Tachibana *et al.*, 2014) This may be related to the fact that recurrent tonsillitis is very differently defined in different studies and thus, comparison is difficult. None of the patients in our study had previously experienced PTA, but it might be due to fact that the vast majority of the patients in our region are treated with acute tonsillectomy which prevents future episodes.

We evaluated 5 oropharyngeal signs that were previously shown to indicate long-term recurrent inflammatory process in tonsils (Kasenõmm *et al.*, 2004). In Study I the median value was 4 out of 5 (range 0–5), 83.3% of patients had cryptic debris; 72.2% of patients had scar tissue on tonsil and tonsillar sclerosis; 61.1% of patients had obstruction of the tonsillar crypts; 55.6% of patients had lymphatic tissue aggregates on the retropharyngeal mucosa. This indicates that

most of the patients had an underlying chronic or recurrent inflammatory process in their tonsils although their own complaints were small. Long-term chronic or recurrent inflammation leads to a sclerotic process in the tonsil and in the surrounding tissue (Kasenõmm *et al.*, 2004). When Weber's salivary glands theory was put forward by Passy he argued recurrent tonsillitis probably plays a role in the development of PTA. Repeated tonsillar infections leads to scarring in the tonsils and surrounding tissues. This scarring can cause ductal obstruction to Weber's salivary mucous gland secretions, resulting in infection and eventually PTA formation (Passy, 1994).

6.2. Other possible risk factors

In Study II, 22% of PTA patients were smokers. This is similar to the overall smoking rate in the Estonian population of 21% (Tekkel and Veideman, 2017). This is in contrast to many other studies, where smoking is associated with higher risk of PTA (Schwarz *et al.*, 2018; Kim *et al.*, 2020). In a study performed in the UK researchers found that smoking increased PTA risk by 70% and in Denmark researchers found that smoking increased the PTA risk by approximately 150% (Dilkes *et al.*, 1992; Klug *et al.*, 2013). However, in Canada Sowerby *et al.* reported an even lower smoking rate among PTA patients than in their region overall (Sowerby *et al.*, 2013). On this basis we are not able to confirm that smoking increases PTA, at least in the Estonian population.

7. Oral microbiota in PTA patients

7.1. Microbiota of pus and tonsillar fossa – bacteriological study

A total of 62 different bacteria were found from pus, tonsillar fossa biopsy and blood samples (**Table 4**). All 22 tonsillar fossa biopsies were positive while only 16 (72.7%) of pus samples and 2 blood samples were positive. A total of 49 different bacteria were discovered from tonsillar fossa, 32 different bacteria from pus and 3 from blood samples. The median number of different bacteria found in tonsillar biopsies was 6 (range 1–10, average 5.7) and in pus samples 3 (range 0–9, average 2.7). Our findings are similar to other studies where average species counts found from pus samples are between 1 and 3 but the highest average species count found from pus is 7.7 (Brook, 1981). However, not all studies have evaluated anaerobes in PTA (Matsuda *et al.*, 2002; Megalamani *et al.*, 2008). Also, in Study I, the preceding antibiotic treatment rate was very high, and may affect positive culture results and the number of microbes detected in PTA pus, tonsillar fossa and blood samples.

Pus is the most common microbiological specimen for PTA patients. To the best of our knowledge our study was the first to evaluate bacterial growth from tonsillar fossa biopsy, in addition to pus. A slightly similar study was conducted in Denmark where they evaluated growth from PTA pus, on PTA tonsil surface

and from tonsil core (Klug *et al.*, 2011). In this study the researchers also found that pus aspiration gave the smallest average bacterial growth – 3.7 isolates while from surface swabs 5.1 isolates and from tonsillar core 5.8 isolates were detected. This study is in good agreement with ours, indicating that isolates found from PTA pus are less numerous than from other areas, and thus tonsillar fossa biopsy or tonsillar core biopsy should be considered as preferred specimen.

In tonsillar fossa, the most frequent genera of bacteria were *Streptococcus* spp., followed by *Neisseria* spp. and *Actinomyces* spp. The most common species were *S. parasanguinis* and *S. pneumoni*ae, both with 55%. *R. mucilaginosa* was present in 31.8% of tonsillar fossa biopsies but was never found in pus samples and blood samples. Also, in tonsillar fossa we found 6 *Staphylococcus* spp., but none of them were found from pus cultures or blood samples.

In 54.5% of **pus samples** multiple organisms were present, in 18.2% of pus samples only one organism was present and 27.3% of pus sample gave no growth. The most frequently found bacteria in pus cultures were different streptococci. Out of 16 positive pus cultures SAG was present in 9 cases. At the same time SAG was much less frequently found in tonsillar fossa biopsies where only 4 samples were positive for SAG.

Most of the studies relating to PTA have looked at SAG as a member of the viridans streptococci group. Viridans group streptococci are very frequently found in PTA pus (Brook, 1981; Matsuda *et al.*, 2002; Sakae *et al.*, 2006). But there is also great variability among different studies (Sunnergren *et al.*, 2008; Ehlers Klug *et al.*, 2009; Gavriel *et al.*, 2009; Hsiao *et al.*, 2012). The difference in percentage arises if all viridans streptococci are looked at together or SAG is looked at separately. In addition, viridans streptococci can also be found as commensals in the oral and nasopharyngeal microbiota of healthy individuals (Salavert *et al.*, 1996). Therefore, in some cases their occurrence in PTA pus samples might also reflect contamination from oral microbiota (Sunnergren *et al.*, 2008; Ehlers Klug *et al.*, 2009; Gavriel *et al.*, 2009; Segal *et al.*, 2009; Mazur *et al.*, 2015).

S. pyogenes is the only bacterium which is recognised as a true pathogen in PTA (Klug, 2017). At the same time, we only found *S. pyogenes* in 4 tonsillar fossa biopsies (18.2%) and from 6 pus samples out of 22 (27.2%). Our study is well aligned with other authors and their studies, where *S. pyogenes* is usually found in a quarter to a third of cases. In addition, *S. pneumoniae* has been associated with PTA in some former studies and this bacterium was also frequently found in our specimens (Haeggström *et al.*, 1987; Sunnergren *et al.*, 2008).

We used special culture media and growth conditions to reveal anaerobic bacteria. The most common anaerobes that are associated with PTA are *F. necrophorum* and *Prevotella* spp. (Mitchelmore *et al.*, 1995; Ehlers Klug *et al.*, 2009). In our study *F. necrophorum* was only once found from pus samples and twice from tonsillar fossa biopsy. *Prevotella* spp. was also only once found from pus samples and three times from tonsillar fossa. Another fusobacterium, *F. nucleatum* that may be also associated with PTA, was isolated from 3 pus samples (Jousimies-Somer *et al.*, 1993).

Table 4. Microorganisms isolated from pus, tonsillar fossa and blood of PTA patients.

Phylum	Family	Bacteria	Pus	Tonsillar fossa	Blood
Proteobacteria	*Comamonadaceae*	*Acidovorax temperans*	1		
	Moraxellaceae	*Moraxella/Branhamella catarrhalis*		1	
	Pasteurellaceae	*Aggregatibacter aphrophilus*	1		
		Haemophilus influenzae		1	
		Haemophilus parainfluenzae		2	
	Neisseriaceae	*Neisseria flavescens*	1	7	
		Neisseria macacae	4		
		Neisseria mucosa/macacae		2	
		Neisseria perflava	2	5	
		Neisseria perflava/flavescens		3	
		Neisseria subflava	1	1	
		Neisseria spp.		1	
Firmicutes	*Bacillaceae*	*Bacillus licheniformis*		1	
	Carnobacteriaceae	*Granulicatella adiacens*	1	1	
	*Clostridiaceae**	*Clostridium halophilum*	2	3	
	*Peptoniphilaceae**	*Parvimonas micra*	2	1	
	*Peptostreptococca-ceae**	*Peptostreptococcusm anaerobius*			1
	Lactobacillaceae	*Lactobacillus catenaformis*	2		
		Lactobacillus fermentum	1		
		Lactobacillus plantarum		1	
	Staphylococcaceae	*Gemella haemolysans*		1	
		Gemella haemolysans/morbillorum		1	
		Gemella sanguinis		1	
		Staphylococcus aureus		1	
		Staphylococcus epidermidis		3	
		Staphylococcus hominis		1	
		Staphylococcus warneri		1	

Phylum	Family	Bacteria	Pus	Tonsillar fossa	Blood
Actinobacteria	*Streptococcaceae*	*Streptococcus anginosus*	2	2	
		Streptococcus constellatus	5	1	
		Streptococcus dysgalactiae		1	
		Streptococcus gordonii		2	
		Streptococcus intermedius	3	1	
		Streptococcus intermedius/anginosus	2		
		Streptococcus mitis			1
		Streptococcus mutans		2	
		Streptococcus oralis		5	1
		Streptococcus parasanguinis	4	12	
		Streptococcus peroris	1	4	
		Streptococcus pneumoniae	4	12	
		Streptococcus pyogenes	6	4	
		Streptococcus salivarius	1	6	
		Streptococcus sanguinis	1	2	
		Streptococcus sobrinus		1	
	*Veillonellaceae**	*Dialister pneumosintes*	1		
		Veillonella atypica	1		
	Actinomycetaceae	*Actinomyces radicidentis*	1		
		Actinomyces naeslundii		1	
		Actinomyces odontolyticus	1	5	
		Actinomyces oris		3	
	Corynebacteriaceae	*Corynebacterium argentoratense*	1	2	
	*Bifidobacteriaceae**	*Alloscardovia omnicolens*		2	
		Bifidobacterium dentium	1	1	
	Micrococcaceae	*Rothia dentocariosa*	2	1	
		Rothia mucilaginosa		7	

Phylum	Family	Bacteria	Pus	Tonsillar fossa	Blood
		Kocuria marina		1	
		Micrococcus luteus		1	
Bacteroidetes	*Bacteroidaceae**	*Bacteroides pyogenes*		1	
	*Prevotellaceae**	*Prevotella intermedia*		1	
		Prevotella melaninogenica	1	2	
Fusobacteria	*Fusobacteriaceae**	*Fusobacterium necrophorum*	1	2	
		Fusobacterium nucleatum	3		

*anaerobic bacteria

In 9 patients out of 16 with positive pus samples (56.2%), one or more organisms that were found in the pus were also present in the tonsillar fossa biopsy samples (altogether 20 specimens). At the same time, all except one pus sample had one or more specimens in the pus that were not found in the tonsillar fossa biopsy.

Only in two patients were the **blood samples** culture-positive (9.1%). The bacteria found from blood cultures were *Streptococcus mitis* (*S. mitis*) and *Streptococcus oralis* in one patient and *Peptostreptococcus anaerobius* in another.

7.2. Microbiota of pus and tonsillar fossa – molecular study

The tonsil biopsies and pus samples of 91 patients were analysed by applying NGS. More than 200 genera of bacteria from 35 different phyla were found in these samples. Both sample types had quite similar species Shannon 'H' diversity (proportion of the species) and Simpson's indices (number of the species/relative abundance of the species) (**Figure 3**). The difference between the two materials was found in species richness (number of species in a community) – this marker was significantly higher in pus samples than in tonsil biopsies (p=0.014).

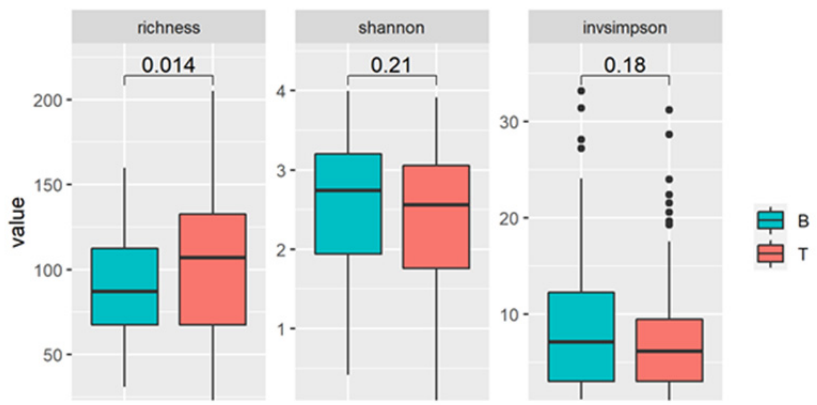

Figure 3. Species richness, Shannon 'H' diversity and Simpson indices in the tonsillar biopsy (B) and pus (T) specimens.

The majority of the bacteria found in tonsil biopsies and pus swabs belonged to **four phyla** (Ph.) – *Firmicutes*, *Fusobacteria*, *Bacteroidetes* and *Proteobacteria* (**Figure 4**). In pus swabs, the phyla *Firmicutes* ($p<0.001$) and *Actinobacteria* ($p=0.006$) were more numerous than in tonsil biopsies. In tonsil biopsies, *Proteobacteria* ($p<0.001$), *Bacteroidetes* ($p=0.009$) and *Synergistetes* ($p=0.042$) were more abundant than in pus swabs. Bacteria of phylum *Fusobacteria* were found equally from both locations and accounted for nearly a quarter of all revealed microbes.

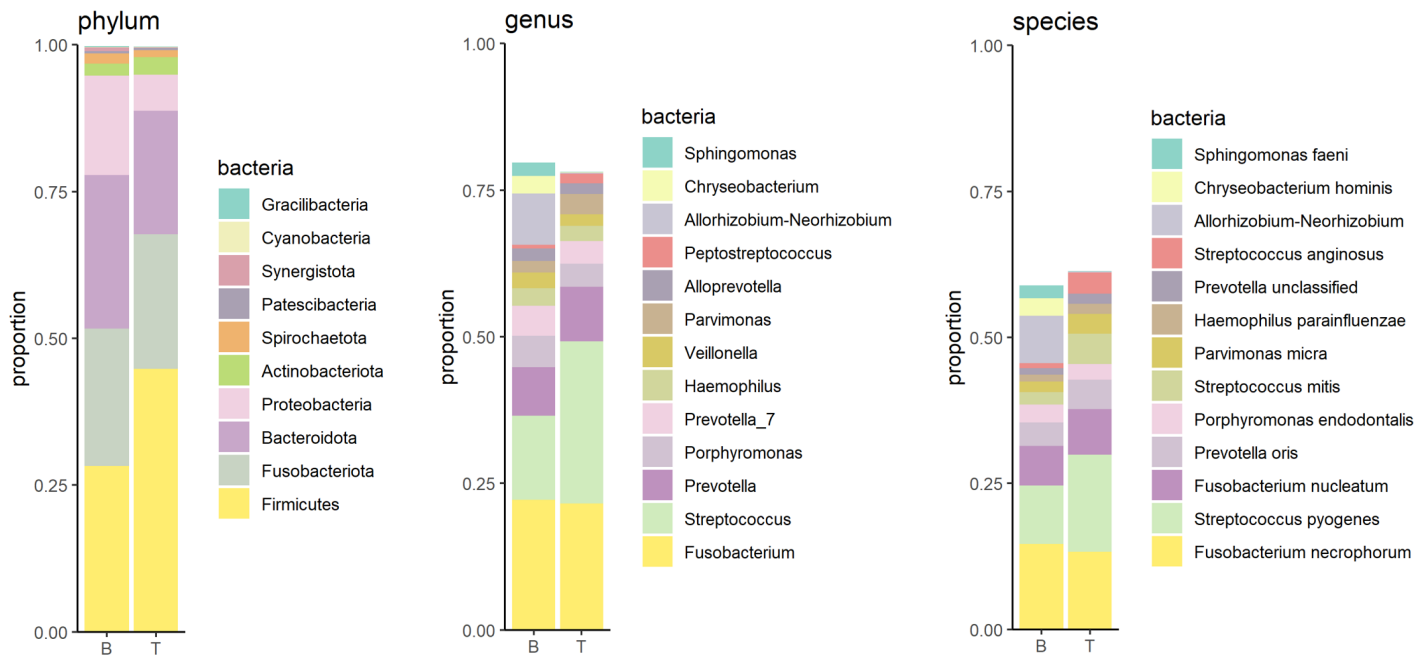

Figure 4. Microbial distribution in the tonsillar biopsy (B) and pus (T) specimens on phylum, genus and species level. The phylum names in the figure *Fusobacteriota*, *Bacteroidota, Actinobacteriota* and *Synergistota* are synonyms for *Fusobacteria*, *Bacteroidetes, Actinobacteria* and *Synergistetes* that are used in the text.

On a **genus** level, *Fusobacterium* (Ph. *Fusobacteria*) and *Streptococcus* (Ph. *Firmicutes*) were the most predominant genera, accounting for nearly half of bacterial genes found in these specimens. *Fusobacterium* displayed a similar abundance in both specimens while *Streptococcus* was more abundant in pus ($p<0.001$). *Prevotella* and *Porphyromonas* (both Ph. *Bacteroidetes*) held the 3rd and 4th place.

On a **species** level, the analysis revealed that phylum *Fusobacteria* mostly consisted of two species – *F. nucleatum* and *F. necrophorum*. Phylum *Firmicutes* primarily consisted of streptococci and the most common species were *S. pyogenes, S. mitis* and *S. anginosus*. The most common species in the phylum *Bacteroidetes* were *Prevotella oris* and *Porphyromonas endodontalis,* and in the phylum *Proteobacteria Haemophilus parainfluenzae* and *Sphingomonas faeni*.

While **comparing two sampling sites**, the analysis revealed that *F. necrophorum* was more abundant in pus than tonsillar biopsy samples ($p=0.02$) while no significant difference was found in the case of *F. nucleatum*. In addition, tonsillar biopsy displayed significantly more *Sphingomonas faeni* ($p<0.001$), *Chryseobacterium hominis* ($p<0.001$) and some environmental bacteria of the group *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* spp. ($p<0.001$) than pus. At the same time, the pus swabs revealed more *S. mitis* ($p=0.003$), *S. anginosus* ($p<0.001$), *Parvimonas micra* ($p=0.003$) and *Prevotella oris* ($p=0.024$) than biopsies while the abundance of *S. pyogenes* was not statistically different.

While **comparing the results obtained by two different methods** (cultures, NGS) we found that both methods revealed *Streptococcus* as the most common genus. Some differences were found on a species level – according to the cultures, the most common streptococci were SAG while, according to NGS, the most common was *S. pyogenes.* Some previous studies have also revealed that *S. pyogenes* may be difficult to culture from tonsil tissues while molecular methods reveal this species more frequently (Kasenõmm *et al.*, 2005). In addition to *S. pyogenes*, fusobacteria were also more frequently found by NGS.

According to NGS, ***S. pyogenes*** was present in 70% of pus samples and 66% of biopsy samples, ***F. necrophorum*** in 75% of pus samples and 82% of biopsy samples, and ***F. nucleatum*** in 86% of pus samples and 89% of biopsy samples. All pus samples contained at least one of these bacteria or their combination while four tonsillar biopsy samples were negative for all these three bacteria. We additionally compared the samples with a low proportion (<5%) of *S. pyogenes* and fusobacteria (10 pus and 13 tonsillar biopsy samples), to search for other possible causative agents. In these selected pus samples, *S. mitis*, *S. anginosus* and *Prevotella oris* were more abundant, while in tonsillar biopsy samples *S. mitis*, *Haemophilus parainfluenzae, Prevotella nigrescens* and *Mycoplasma hominis* prevailed. Further studies should reveal their actual role in PTA. The biopsy samples contained some environmental bacteria (*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* spp, *Chryseobacterium hominis*, *Sphingomonas faeni*), too, but their role in the disease should be evaluated with caution since contamination cannot be ruled out.

A culture study revealed that tonsillar fossa biopsy is more suitable for microbiological analysis than pus since more pathogens were found from tonsillar fossa. A similar result was seen in a Danish study by Klug where less microbes were cultured from pus samples than tonsil surface and core samples (Klug *et al.*, 2011). At the same time, our study revealed that both pus and biopsy samples are suitable for microbiological analysis when NGS method is applied.

Summarising the results of both microbiological studies, the most common bacteria in tonsillar biopsy and pus were *S. pyogenes* and two species of fusobacteria, *F. necrophorum* and *F. nucleatum* that can be considered as the true causative agents of PTA. Our findings are in close alignment with other authors who have considered *S. pyogenes* and *F. necrophorum* as the main causative agents of PTA (Sunnergren *et al.*, 2008; Ehlers Klug *et al.*, 2009; Wikstén *et al.*, 2015; Slouka *et al.*, 2020). According to our study, *F. nucleatum* should be added to this list. Most of the previous studies that have investigated the microbiology of PTA have used culture methods. To our knowledge this is first time that NGS was used to evaluate PTA microbiota, including comparison of different locations.

7.3. Antibiotic susceptibility of putative causative agents of PTA

We determined antibiotic susceptibility for 14 *S. parasanguinis*, 11 *S. pneumoniae*, 10 *S. pyogenes*, 10 SAG, 2 *F. necrophorum* and 7 *R. mucilaginosa* strains. We revealed high resistance only among *S. pneumoniae* for erythromycin (64%) and ciprofloxacin (82%) (**Figure 5**).

Antibiotic sensitivity has been shown to vary greatly among different geographic areas and depends a great deal on how widely the antibiotics are used and how easily they are prescribed. According to our study, in our population penicillin and other beta-lactam antibiotics are suitable as empirical treatment.

Penicillin alone is also the most commonly used antibiotic for PTA in four Nordic countries (Wikstén *et al.*, 2014). This is in contrast to some older studies where researchers have found much greater resistance to penicillin and recommend the use of penicillin with metronidazole or broad spectrum antibiotics (Hall, 1990; Brook *et al.*, 1991; Prior *et al.*, 1995; Shaul *et al.*, 2015).

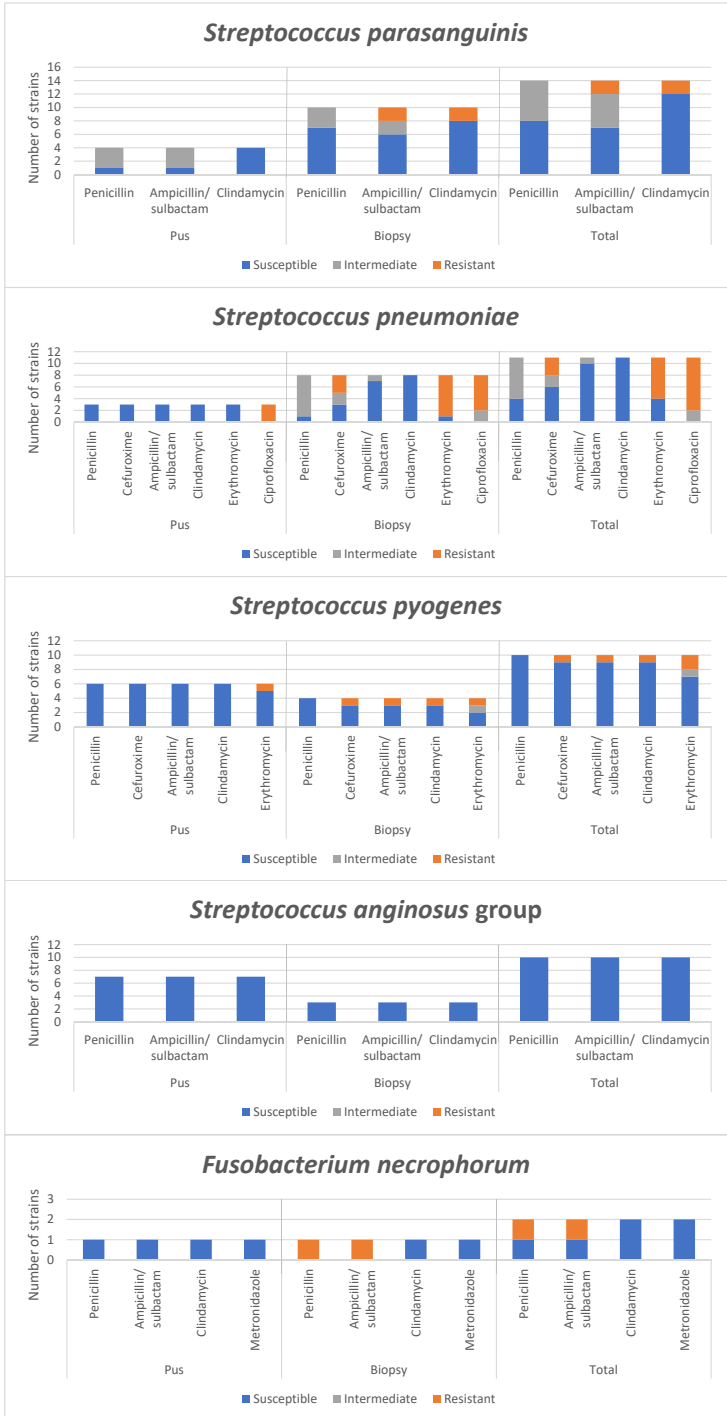

Figure 5. Antibiotic susceptibility of opportunistic bacteria isolated from tonsillar fossa and PTA pus.

We also determined the MIC values for *R. mucilaginosa*, which were 0.016 and 0.125 for penicillin; 0.047, 0.094, 0.5 and 0.38 for cefuroxime; and 0.75, 2 and 6 for ciprofloxacin. These data were not included to the table since no MIC cut-off values for *R. mucilaginosa* susceptibility and resistance exist.

8. Activity of polyols against PTA-associated *S. pyogenes*

All tested polyol concentrations, except ERY 2.5%, showed a statistically relevant inhibitory effect against PTA strains compared to BHI (**Figure 6**). Here, 10% XYL was more effective than 10% ERY. The growth of all throat-derived type strains was inhibited by both polyols in all studied concentrations.

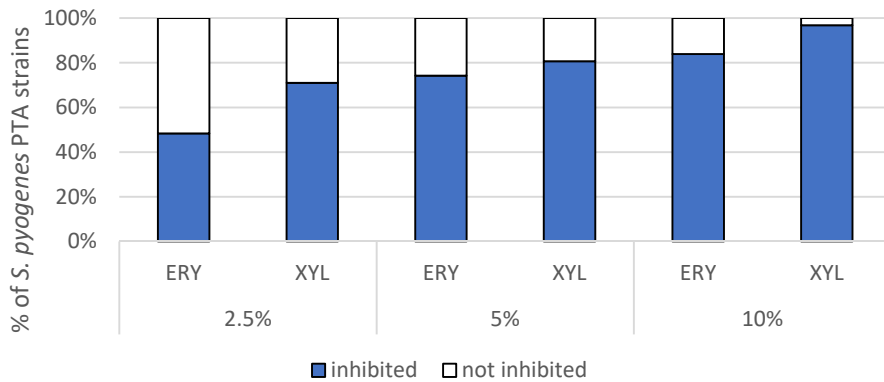

Figure 6. Effect of different concentrations (2.5%, 5% and 10%) of ERY and XYL on the growth of *S. pyogenes* PTA strains (n=31)

We also looked separately at the strains from the patients with high and low levels of amylase in their pus. ERY effectivity score of isolated *S. pyogenes* strains was negative or zero in two thirds (4/6) of high pus amylase cases compared to only 2 of 12 of low pus amylase cases. XYL effectivity score was negative in half of the high pus amylase cases (3/6) compared to 2 of 12 low pus amylase cases.

Strains with high polyol effectivity scores and low polyol effectivity scores in a 24-hour study showed similarly good effectivity of ERY and XYL in the exponential growth phase compared to BHI in growth curve study. Both polyols consistently inhibited the growth of all studied strains in all studied concentrations in the exponential growth phase. To date, the effect of polyols to *S. pyogenes* has not been studied to any great extent. A study conducted in Finland by Kontiokari looked at the XYL effect on microbiota found from pharynx and middle ear fluid. In this study XYL in concentrations of 5% and 10% showed a slight but statistically significant inhibition effect to *S. pyogenes* strains found from asymptomatic children (Kontiokari *et al.*, 1995).

GENERAL DISCUSSION

Pathogenetic aspects

Most commonly PTA is considered to be a complication of acute tonsillitis (Mitchelmore *et al.*, 1995; Prior *et al.*, 1995). However, it has been shown that PTA can also arise from small salivary gland infection (Weber salivary glands) (Passy, 1994). Other pathogenesis routes have also been speculated such as association with His duct (Iemma *et al.*, 1992), but these theories are not widely accepted. El-Saied *et al.* found that almost half of patients with PTA have high levels of amylase in the pus (more than 65 U/L). In the same study they also evaluated amylase levels in neck abscess and dental abscess pus and found that amylase is not increased in these locations (El-Saied *et al.*, 2014). They proposed that their data show the origin of PTA from Weber salivary gland infection. We conducted a similar study and observed that 13% of PTA patients had amylase levels at least twice as high as in blood, which is lower than El-Saied *et al.* found. El-Saied also found that all patients with increased amylase levels in PTA pus had their first episode of PTA (El-Saied *et al.*, 2014). We were not able to repeat that part of the study, because our first line of treatment for PTA is acute tonsillectomy, which prevents subsequent episodes of PTA. This is most likely the reason why we rarely see recurrent episodes in our population. The treatment option might also be one of the causes that affects the overall morbidity of PTA, because using tonsillectomy as the first line of treatment will reduce overall morbidity by preventing recurrent episodes.

When Passy proposed his Weber salivary glands theory, he argued that recurrent tonsillitis causes fibrosis and Weber salivary gland ducts' obstruction leads to inflammation and formation of PTA (Passy, 1994). A histological comparison study of tonsils removed due to PTA, acute tonsillitis and chronic tonsillitis revealed that the tonsils of PTA patients had more intense periductal inflammation than the two other groups, thus supporting Weber's salivary glands theory (Kaltiainen *et al.*, 2017). In Study I we found that most of patients did not report previous tonsillitis episodes before PTA. At the same time, when we evaluated macroscopic oropharyngeal signs for recurrent tonsil infection and sclerosis, we found that most PTA patients had these signs (median 4 out of 5). This is an indication of underlying sub-clinical chronic or recurrent tonsillitis. We argue that this tonsil scarring and fibrosis might be the etiopathogenetic factor for PTA. Studies have shown that the recurrence of PTA is greater among patients with prior tonsillar infection (Chung *et al.*, 2014; Wang *et al.*, 2014). At the same time acute tonsillitis is more frequent in the winter months, but peritonsillitis and PTA occurrence does not change with the seasons (Kordeluk *et al.*, 2011; Klug, 2014). This indicates that PTA may not be limited to the complications of acute tonsillitis. However, PTA pathogenesis from acute tonsillitis or Weber salivary glands might not be the complete opposite of each other. In 2016 a unified hypothesis was presented by Klug proposing that bacteria

initially infect the tonsillar mucosa and spread via the salivary duct system to the peritonsillar space, where an abscess is formed (Klug *et al.*, 2016).

Microbiological aspects

Most studies have revealed mixed oral microbiota of aerobic and anaerobic bacteria from PTA pus. At the same time the percentage of anaerobic bacteria largely differs between studies, ranging from 10 to 100 (Brook, 1981; Klug, 2017; Segal et al., 2009) and aerobes 16 to 98 (Brook, 1981; Prior *et al.*, 1995; Segal *et al.*, 2009; Mazur *et al.*, 2015; Klug, 2017). It is still unclear what the true causative agents of PTA are. The only consensus is that *S. pyogenes* can cause PTA. However, *S. pyogenes* is found from PTA pus in the case of only around a fifth to a third of cases (Ehlers Klug *et al.*, 2009; Gavriel *et al.*, 2009; Mazur *et al.*, 2015). It is proposed that other bacteria might also play a significant role in the development of PTA. In our culture study, *S. pyogenes* was grown in 6 out of 22 pus samples and from those six in 2 cases *S. pyogenes* was grown as a monoculture. Our findings confirm that *S. pyogenes* might cause PTA, but it is difficult to connect PTA with *S. pyogenes* alone. In Study I, the most frequently found bacteria were different species of genus *Streptococcus spp.* Many streptococci have been associated with tonsillar diseases. Large colony-forming beta-haemolytic streptococci from groups C and G have been recovered from tonsillar surface swabs from acute tonsillitis patients with higher frequency than from healthy controls (Meier *et al.*, 1990; Cimolai *et al.*, 1991; Turner *et al.*, 1997; Klug, 2017). SAG have been associated with abscesses in the head and neck region (Han and Kerschner, 2001; Hirai *et al.*, 2005; Foxton *et al.*, 2012). *S. pneumoniae* has been associated with rhinosinusitis and otitis media (Loughran *et al.*, 2019; Molloy *et al.*, 2020). However, there is currently a lack of consistency in the literature that SAG and other streptococci then *S. pyogenes* can be considered as the true causative agent of PTA.

We performed another microbiological study comparing the specimens from tonsillar biopsy and pus, applying NGS. This study also revealed *S. pyogenes* as a major causative agent of PTA, being present in two thirds of specimens. This study also revealed that both fusobacteria (*F. necrophorum* and *F. nucleatum*) are frequently found. These three bacteria accounted for nearly a third of the mean microbial mass in PTA pus, and at least one of these three bacteria was present in each pus specimen. Some previous studies, especially those performed in Finland and Denmark, have similarly found that *F. necrophorum* is often in PTA pus and noted that it might be causative agent of PTA (Jousimies-Somer et al., 1993; Savolainen et al., 1993; Klug et al., 2011). In our cultural study the fusobacteria were not very common and many other studies have also rarely reported *F. necrophorum* (Sakae et al., 2006; Marom et al., 2010; Hsiao et al., 2012), but this might be because culture methods are mostly used and molecular methods might give more reliable results, especially for anaerobic bacteria. Our NGS study revealed fusobacteria in the majority of pus and biopsy

specimens. The intriguing finding in our study was that *F. nucleatum* can be also very frequently found in PTA pus. Here, future studies are needed to clarify if *F. nucleatum* can also be considered as a potential causative agent of PTA.

Because there is so much inconsistency between studies, it has made it difficult to reach an agreement on other possible causative agents. At the same time, polymicrobial infection that is common in the case of many other diseases is also highly likely in the case of PTA (Galioto 2017). The infectious community may involve several streptococci and anaerobic bacteria that have a synergistic lifestyle. Inflammation is caused by this community rather than by any of these bacteria separately.

In 2004, Kasenõmm *et al*. (2004) conducted a study in our department where they collected blood cultures from patients to whom tonsillectomy was performed due to recurrent tonsillitis. In his study the rate of positive blood cultures during tonsillectomy was as high as 44% (Kasenõmm *et al.*, 2004). Tonsillectomy was performed on patients in a relatively healthy state, without preoperative antibiotics. In Study I we used the same culture media and the same protocol for collecting blood cultures during tonsillectomy for PTA. In our current study only 2 blood cultures out of 22 were positive. At the same time most of our PTA patients (72.7%) had received antibiotic therapy before hospitalisation. This could be a reason why in Study I the percentage of positive blood cultures was so low. The same argument that prior antibiotic therapy might alter microbiological sample results has also been proposed by many other authors (Hoffmann *et al.*, 1987; Jokipii *et al.*, 1988; Mitchelmore *et al.*, 1995; Klug, 2017). In addition, the patients had different diagnoses (tonsillitis versus PTA). With this in mind it has been difficult to identify other true causative agents of PTA. At the same time this shows that preoperative antibiotic therapy is effective for preventing intraoperative bacteraemia.

Choice of antibiotics for treatment of PTA depends on local antibiotic sensitivity, overall countries' antibiotic policy and physicians' preferences. Penicillin is the most commonly used antibiotic in the case of PTA in the Nordic countries (Risberg *et al.*, 2008; Wikstén *et al.*, 2014; Klug, 2017). However, in many areas authors prefer and recommend broad-spectrum antibiotics (penicillin with metronidazole, clindamycin, clarithromycin), because culture findings have shown high resistance to penicillin (Hall, 1990; Brook *et al.*, 1991; Prior *et al.*, 1995; Powell and Wilson, 2012). Although culture findings might show the resistance to penicillin of putative causative agents, the studies on clinical outcomes have not shown that broad-spectrum antibiotics give better results than penicillin alone (Hallgren *et al.*, 2021). In addition, a study performed in Finland by Wikstèn revealed that penicillin combined with metronidazole may lead to adverse effects, but does not prevent recurrence nor enhance recovery compared to the use of penicillin alone (Wikstén *et al.*, 2016). In Study I we had higher antibiotic resistance only among *S. pneumoniae* for erythromycin and ciprofloxacin. Therefore, Study I's findings correlate with Nordic studies that penicillin alone is suitable for the treatment of PTA. If we consider the possibility of the polymicrobial infection including both streptococci and anaerobic

bacteria then if we target the streptococci with penicillin then probably the anaerobic bacteria alone cannot continue the infectious process. This may explain the good clinical experience with penicillin treatment.

Risk factors

Although we excluded patients under the age of 12, which increases the median age of PTA patients in our studies, it still seems that in the Estonian population the typical PTA patient is older compared to Scandinavian studies (Risberg *et al.*, 2008; Ehlers Klug *et al.*, 2009). In part, this might be explained by the male dominance in our study population. In the studies carried out in Denmark and in Sweden the female and male morbidity in PTA was equal. However, both authors showed that women are affected at a younger age than men (Risberg *et al.*, 2008; Ehlers Klug *et al.*, 2009; Klug, 2014). Therefore, a higher proportion of men affected with PTA in Estonian population also raises the median age.
Female dominance has been shown in primary health care visits due to acute pharyngitis and acute tonsillitis (Fine *et al.*, 2012). In contrast, in both of our studies we had male dominance. In Study I we had male dominance with a ratio of 3:1. This finding might be due to the small sample size. Most of the other studies relating to PTA have also found male dominance or equal distribution between the sexes (Spires *et al.*, 1987; Brook *et al.*, 1991; Matsuda *et al.*, 2002; Ehlers Klug *et al.*, 2009). This has partially been explained by smoking.

Smoking and passive smoking have been associated with many respiratory tract infections and other diseases such as otitis media (Arcavi and Benowitz, 2004; Salvi, 2014; Toskala and Kennedy, 2015; Christensen *et al.*, 2018). Smoking is also the most well-recognized risk factor for the development of PTA (Hidaka *et al.*, 2011; Klug *et al.*, 2013; Schwarz *et al.*, 2018). In Denmark researchers calculated that 16% of PTA cases could potentially be avoided if everybody stopped smoking (Klug, 2017). At the same time, in Study II we did not find a difference between overall smoking in our population and among PTA patients. In Study II the overall smoking rate was 22% and in 2016 the overall smoking rate in Estonia among 16-year-olds and over was 21.3% (29.9% in men and 15.5% in women) (Tekkel and Veideman, 2017). In Study II we only asked about active smoking. Schwarz *et al.* reported that PTA risk is only smaller in patients who have never smoked, and even previous smoking increases risk for PTA (Schwarz *et al.*, 2018). It has been shown that smokers have different microbiota in their upper airways compared to non-smokers, containing more potential pathogens (Brook and Gober, 2005). A study performed by Hidaka found that SAG was significantly more commonly found among smokers (Hidaka *et al.*, 2011). In Study I SAG was the most commonly found bacteria in PTA pus. Moreover, it has been shown that smokers have alterations in both lymphoid and non-lymphoid compartments in their palatine tonsils (Torre *et al.*, 2005). Many studies have shown that smoking causes impairment in cell-mediated immunity (Moszczyński *et al.*, 2001; Sopori, 2002; Robbins *et al.*, 2004; Mehta *et al.*, 2008). In addition, smokers have lower levels of serum

lysozyme and smoking causes a decrease in serum Ig concentrations together with a reduction of the absolute number of nature killer cells (Moszczyński *et al.*, 2001). We argue that the negative effect of smoking on immunity is probably the reason why smokers have a more severe clinical picture of PTA and fulfilled more Sepsis-2 criteria in Study II.

Clinical aspects

Half of the patients in Study II fulfilled the Sepsis-2 clinical criteria. We found that smokers had significantly more sepsis symptoms than non-smokers. Smoking itself tended to be a risk factor since there was no relation between the number of cigarettes smoked per day or duration of smoking in years. Also, patients who had not received antibiotic treatment before hospitalisation had significantly more sepsis symptoms. However, there was no difference in complication or hospital stay between the sepsis group and the group without sepsis symptoms. Most of the patients were discharged home a day after tonsillectomy and nobody needed re-hospitalisation. We therefore did not see that assessment of Sepsis-2 clinical criteria provides additional clinical information. It has been noted before that Sepsis-2 criteria and definition might be not ideal for sepsis diagnostics (Kaukonen *et al.*, 2015; Marik and Taeb, 2017). This has led to the development of new methods to better assess sepsis and in 2016 a new definition and assessment was put forth (Shankar-Hari *et al.*, 2016). For clinical evaluation the Sequential Organ Failure Assessment (SOFA) score is recommended and it has shown superior predictive value for in-hospital mortality than the Sepsis-2 score. However, the complexity of the method, the lack of requisite data for many patients, and concerns that it may result in late identification relative to other methods have raised questions about its practicality in clinical practice (Marik and Taeb, 2017). In view of the above, new simplified version from SOFA known as “quick SOFA” or qSOFA has been created (Shankar-Hari *et al.*, 2016). Future studies could show whether qSOFA more precisely evaluates which PTA patients are at a higher risk of having complications and need closer monitoring and are not suitable for medical treatment alone and need surgical intervention.

Laboratory markers

In Study II we evaluated WBC and some other inflammatory biomarkers – CRP and PCT – to assess their usefulness in PTA. CRP is a major acute phase protein whose concentration may increase more than 1,000-fold in severe inflammatory states (Black *et al.*, 2004). PCT levels have been found to be very high in patients with severe invasive bacterial infections compared to patients with mild local bacterial infections or viral infections (Hamade and Huang, 2020). In Study II all patients had increased CRP values and WBC and CRP were in strong positive correlation. At the same time, most patient had very small increase in PCT values. Only two patients had very high levels of PCT

(accordingly 49.94 and 38.05 ng/mL). However, the course of disease of those two patients did not differ in any way from other PTA patients. Therefore, we can suggest that PCT is not a good diagnostic marker in the case of PTA. At the same time CRP and WBC can both used as a diagnostic tool for inflammation due to PTA.

Polyols' efficiency

Effect of polyols has primarily been studied on salivary microbiota. ERY and XYL have shown a reduction in dental plaques and reduction in the levels of *Streptococcus mutans* in dental plaque and saliva (Mäkinen *et al.*, 2005; de Cock *et al.*, 2016). However, during the last decade polyols have also received increased attention in otorhinolaryngology, especially XYL in treatment of chronic rhinosinusitis. This is due to its antimicrobial and anti-biofilm formation effect. In chronic rhinosinusitis, nasal irrigation with XYL added to salt water have shown an additional beneficial effect compared to salt water alone (Weissman *et al.*, 2011; Lin *et al.*, 2017). Moreover, use of XYL in nasal irrigation has been included in the latest European guideline – European Position Paper on Rhinosinusitis and Nasal Polyps (Fokkens *et al.*, 2020). As far as we are aware, our study was the first to evaluate the ERY and XYL effect on *S. pyogenes* strains from PTA patients.

In our study, we found XYL to be more effective against *S. pyogenes* strains than ERY. Both polyols showed a high inhibitory effect against most of the strains, especially among throat strains. In 2013, the Cochrane systematic review concluded that using antibiotics for the treatment of sore throat reduces PTA within two months compared to those taking placebo. However, this study also found that the overall benefits are small. Protecting sore throat sufferers against suppurative and non-suppurative complications in high-income countries requires treating many with antibiotics for one to benefit (Spinks *et al.*, 2013). The wide use of antibiotics leads to antibiotic resistance and causes adverse effects. Polyols are safe, well tolerated and they do not increase antibiotic resistance. Therefore, polyols such as ERY and XYL may have potential in preventing PTA and may have a beneficial effect in case of *S. pyogenes* throat infection and can be used in the patients with sore throats.

CONCLUSIONS

1. Risk factors for the development of PTA are scarring and fibrosis of within tonsils and peritonsillar tissue due to previous recurrent inflammations. Smoking and not receiving antibacterial treatment before hospitalisation are associated with more acute clinical picture of PTA.
2. Although half of PTA patients fulfil clinical criteria for sepsis, it does not worsen or extend clinical course of PTA. Sepsis-2 criteria seem to be too sensitive and have low clinical value for PTA diagnosis.
3. Tonsillar fossa sample is the best specimen for microbiological analysis using the culture method since it has a significantly higher yield of pathogenic bacteria than culture of free pus. In case of molecular diagnostics, both pus samples and biopsy samples are suitable. Most common causative agents of PTA tend to be *S. pyogenes,* fusobacteria and SAG.
4. Penicillin alone proves to be the most optimal initial choice for PTA treatment since the main causative agents – streptococci – are susceptible to this antibiotic.
5. The polyols ERY and XYL have inhibitory effect against most *S. pyogenes* strains and may therefore have potential in preventing PTA and beneficial effect in relieving *S. pyogenes* throat infection.

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SUMMARY IN ESTONIAN

Peritonsillaarse abstsessi etiopatogeneetilised ja kliinilised aspektid

Sissejuhatus

Peritonsillaarne abstsess (PTA) on mäda kogunemine kurgumandli kapsli ja ülemise neeluahendaja lihase (*musculus constrictor pharyngis superior*-i) vahele. PTA puhul on tegu kõige sagedasema erakorralist hospitaliseerimist vajava haigusega kõrva-nina-kurguhaiguste valdkonnas (Rusan *et al.*, 2009). Haiguse ravi seisneb abstsessi kirurgilises avamises ja adekvaatses antibakteriaalses ravis. Alates 19. sajandi lõpust on kuldstandardiks olnud erakorraline abstsess-tonsillektoomia, kus eemaldatakse kurgumandlid ja sellega avatakse täielikult ka abstsess. 20. sajandi lõpust on hakatud kasutama lisaks abstsessi aspireerimist süstlaga või abstsessi avamist lõikega ja selle dreneerimist. Kõik kolm metoodikat on aktsepteeritud ning meetodi valik sõltub väga palju piirkonna traditsioonidest ja arsti eelistustest (Powell and Wilson, 2012).

Abstsessi mädas leitakse enamasti polümikroobseid kooslusi, mis hõlmavad nii aeroobseid kui ka anaeroobseid baktereid. Kuna proovid võetakse mikroobide poolt tugevasti koloniseeritud piirkonnast, siis on raske eristada võimalikke tõelisi PTA tekitajaid suumikrobioota kontaminantidest. Üldiselt peetakse PTA kindlaks tekitajaks *Streptococcus pyogenes*-t, samas leitakse *S. pyogenes*-t ainult viiendikus kuni kolmandikus abstsessimäda proovidest. Sellepärast oletatakse, et ka teised bakterid võivad PTA tekitada, kuid puudub üldine konsensus, millised need bakterid võiksid olla. Seepärast on soovitatud antibakteriaalset ravi laiatoimeliste antibiootikumidega, mis kataks kõikvõimalikud tekitajad. Varasemad uuringud on leidnud, et monoteraapia penitsilliiniga on sama efektiivne kui ravi laia toimespektriga antibiootikumidega, samas vähemate kõrvaltoimetega. Võimalik, et abtsess-tonsillektoomia korral, kus kogu abstsess avatakse täielikult, piisab ka ainult kirurgilisest ravist ja tüsistumata juhud täiendavat antibakteriaalset ravi ei vajagi (Wikstén *et al.*, 2016).

Traditsiooniliselt on PTA peetud ägeda tonsilliidi tüsistuseks. Samas on äge tonsilliit enamasti hooajaline haigus, mis on sagedasem talvel ning harvem suvel. Sellist hooajalist erinevust enamasti PTA puhul näha ei ole. Lisaks on äge tonsilliit kõige sagedasem lastel ja teismelisel, samas kui PTA haigestumine on suurim noortel täiskasvanutel. PTA on enamasti ühepoolne haigus, samas kui äge tonsilliit on alati kahepoolne. Lisaks võib PTA esineda isegi siis, kui kurgumandlitel pole tonsilliidi nähte ja eelnevat iseloomulikku haigestumist. Sellepärast arvatakse, et PTA võib alguse saada ka väikeste mandli ümber olevate süljenäärmete põletikust, mida kutsutakse Weberi süljenäärmeteks (Passy, 1994).

Seega on mitmed olulised PTA aspektid seni teadmata: kuidas PTA täpselt kujuneb ja millised on riskifaktorid, millised mikroobid mängivad abstsessi

tekkes rolli, kui paljudel patsientidel esineb sepsis ja mis on selle tähtsus ravitulemuste osas, ning kas on võimalik PTA teket ennetada.

Töö eesmärgid

Antud doktoritöö põhiline eesmärk oli hinnata PTA etiopatogeneetilisi faktoreid, haiguse kliinilist pilti ja võimalike uusi viise haiguse ennetamiseks ja raviks.

Täpsemad ülesanded olid seega:

1. Selgitata PTA riskifaktoreid ja patogeneesi;
2. Hinnata sepsise sümptomite olemasolu ja tähtsust PTA korral;
3. Hinnata erinevatest piirkondadest (mandliloož, mäda, veri) võetud mikrobioloogiliste proovide ja erinevate mikrobioloogiliste meetodite (külv, sekveneerimine) efektiivsust PTA tekitajate leidmiseks;
4. Hinnata PTA patsientidelt isoleeritud patogeensete mikroobide antibiootikumtundlikust;
5. Hinnata polüoolide võimalikku potentsiaali PTA ennetamises ja ravis.

Uuritavad ja meetodid

Kogu uurimisrühm koosnes 114 patsiendist, kes olid haaratud uuringusse kahes erinevas etapis, millest esimene viidi läbi novembrist 2011 kuni mai 2012 (hõlmates 22 patsienti) ning teine aprillist 2016 kuni august 2017 (hõlmates 92 patsienti). Kokku oli uuringutes 70 meest ja 44 naist vanuses 13–74 aastat. Kõik patsiendid hospitaliseeriti Tartu Ülikooli Kliinikumi Kõrvakliinikusse PTA kahtlusega ja neil teostati erakorraline abstsess-tonsillektoomia. Abstsessi diagnoos kinnitus mäda leiuga operatsioonil.

Esimese grupi patsientidel hinnati enne operatsiooni makroskoopilisi orofarüngeaalsed tunnuseid, mis viitavad kurgumandlite sidekoestumisele ja varasematele põletikkudele: kurgumandlite tihkenemine, kurgumandlite krüptide ahenemine, armkude kurgumandlite peal, mandlikorkide olemasolu ja lümfaatiliste koeväätide tekkimine neelu tagaseinale. Esimese grupi patsientidel arvutati välja ka tonsilliidi indeks, mis näitab elu jooksul läbipõetud ägedate tonsilliitide üldarvu. Lisaks võeti külvid kolmest erinevast kohast – abstsessi mädast, mandlilooži st ja verest. Mikroobid isoleeriti ja samastati rutiinmeetoditega. Patogeensetel bakteritel määrati ka antibiootikumtundlikus.

Teise grupi patsientidel määrati peale hospitaliseerimist sepsise kliinilised sümptomid (Sepsis-2 alusel), mille jaoks mõõdeti kehatemperatuur, pulsi- ja hingamissagedus ning leukotsüütide arv veres. Lisaks määrati neil patsientidel verest põletikumarkerid (C-reaktiivne valk ja prokaltsitoniin) Abstsessi mädast ja verest määrati ka amülaas. Operatsiooni ajal võeti proovid (abstsessi mädast ja kurgumandli biopsia) mikrobioloogiliseks analüüsideks (mikrobioloogilised külvid ja uue põlvkonna sekveneerimine). Kõikidel isoleeritud *S. pyogenes*-e tüvedel määrati tundlikkus polüoolide (ksülitooli ja erütritooli) suhtes.

Uuringus osalemine oli kõikidele patsientidele vabatahtlik ja enne andmete kogumist täitsid kõik patsiendid kirjalikult informeeritud nõusoleku vormi. Mõlemal uuringul oli eetikakomitee nõusolek (protokollid: 202T-2 ja 255/T-1). Statistiliseks analüüsiks kasutati SigmaStat (Systat Software, Chicago, IL) ja Excel (Microsoft, Redmond, WA) arvutiprogramme. Kasutati t-testi, Mann–Whitney Rank Sum, χ^2 ja Fisheri täpset testi. Statistiliselt oluliseks loeti p väärtust alla 0.05.

Tulemused

Mõlemas uuringugrupis oli mehi rohkem kui naisi. Keskmine vanus oli esimeses grupis 32 ja teises 31.5 aastat. Meie PTA patsiendid olid veidi vanemad kui Rootsis ja Taanis, kus keskmine haigestumise vanus oli vastavalt 25.6 ja 21 eluaastat (Risberg *et al.*, 2008; Ehlers Klug *et al.*, 2009). Keskmiselt tekkisid sümptomid 5 päeva enne kui diagnoositi PTA. 72.7% esimeses ja 48.9 % teises grupis olnud patsientidest olid saanud enne hospitaliseerimist antibiootikumravi. Ravi oli määratud enamasti perearsti poolt ja selleks oli kasutatud esimese grupi puhul penitsilliini, amoksitsilliini, tsefadroksiili ja klindamütsiini, teise grupi puhul lisaks neile ka amoksitsilliini koos klavulaanhappega, asitromütsiini, klaritromütsiini, tsefuroksiimi, tsefprosiili ja tsiprofloksatsiini. Võrreldes Taani ja Rootsiga oli antibiootikumi saajate protsent meie uuringus suurem ja kasutati ka erinevamaid antibiootikume. Taanis oli 38 % ja Rootsis ainult 21 % patsiente saanud enne hospitaliseerimist antibiootikumravi ja enamikel juhtudel oli kasutatud selleks penitsilliini (Risberg *et al.*, 2008; Ehlers Klug *et al.*, 2009).

Meie uuringus oli pooltel patsientidel (51.2%) hospitaliseerimisel täidetud sepsise kriteeriumid (Sepsis-2) (Levy *et al.*, 2003). Sepsise kriteeriumid täitnud ja mittetäitnud patsientidel polnud erinevust vanuses, sümptomite kestvuses, amülaasi tasemes süljes ega prokaltsitoniini tasemes veres. Samas esines sepsise sümptome rohkem neil patsientidel, kes polnud saanud enne hospitaliseerimist antibiootikumravi ja kes suitsetasid.

13 % patsienditel oli PTA mädas kõrge amülaasi tase. Seda seostatakse Weberi süljenäärmetest lähtunud põletikuga. Võrreldes El-Saied uuringuga, kus pooltel patsientidel oli märgatavalt tõusnud amülaasi tase mädas, oli meie uuringu puhul vastav protsent väiksem (El-Saied *et al.*, 2012).

Enamikul patsientidest ei olnud varasemaid kaebusi kurgumandlite osas või olid kaebused vähesed. Samas olid enamikel patsientidel olemas makroskoopilised orofarüngeaalsed tunnused, mis viitavad kurgumandlite sidekoestumisele ja varasemale põletikule. Sellepärast on kahtlus, et PTA haigetel on vaatamata ägedatele kurgumandlite põletike puudumisele olemas krooniline põletikuline protsess kurgumandlite koes.

Mädast, kurgumandlilooži biopsiast ja vereproovidest kasvas külviuuringus kokku välja 62 erinevat mikroobi. Vereproovid olid positiivsed ainult 2 patsiendil, mis võib tõenäoliselt olla tingitud sellest, et kolmveerand patsientidest oli saanud enne proovide võtmist antibiootikumravi. Mandlilooži proovidest kasvas rohkem mikroobe välja kui mädast (vastavalt 5.7 ja 2.7 erinevat

mikroobi ühe patsiendi kohta) ja seda peaks arvestama materjali kogumisel mikrobioloogilisteks külvideks. Kõige sagedamini leitavad bakterid olid streptokokid, sh *anginosus* grupi streptokokid ja tunnustatud PTA patogeen *S. pyogenes,* mis oli leitav 4 biopsia- ja 6 mädaproovist. See tulemus on sarnane teiste uuringutega, kus *S. pyogenes*-t leitakse ligi kaudu veerandil juhtudest.

Ligikaudu sarnaseid tulemusi näitas ka meie teine uuring, kus kasutasime uue põlvkonna sekveneerimist. *S. pyogenes* oli selle meetodiga leitav isegi kahes kolmandikus proovidest. Väga sageli leiti selles uuringus ka fusobaktereid, nii *F. necrophorum'*it, mille olulisust PTA tekitajana on varasemad uuringud näidanud kui ka *F. nucleatum*'it, mille seos PTA-ga vajab edasisi uuringuid. Kui külviuuringu puhul sõltus uuringu tulemus uuritavast materjalist, siis uue põlvkonna sekveneerimiseks olid nii mäda kui ka biopsia võrdselt sobilikud. Patogeensetel mikroobidel määrasime ka antibiootikumtundlikkuse. Streptokokid olid penitsilliini osas hea tundlikkusega, kõrgem resistentsus esines üksnes *S. pneumoniae*-l erütromütsiini (64%) ja tsiprofloksatsiini (82%) osas. Kuna PTA korral tuleb arvestada segainfektsiooni võimalusega, kus üheks komponendiks on streptokokid ja teiseks anaeroobsed bakterid, siis streptokokkide elimineerimisel ei pruugi anaeroobid üksinda olla suutelised infektsiooni jätkama, mis seletab head kliinilist kogemust penitsilliinraviga.

Uuritud polüoolid (ksülitool ja erütritool) näitasid kontsentratsioonist sõltuvat inhibeerivat efekti enamiku *S. pyogenes*'e tüvede suhtes, XYL inhibeeriv efekt oli mõnevõrra suurem kui ERY-l. Seega on polüoolidel potentsiaali kasutamiseks PTA ennetuses.

Uurimustöö järeldused

1. Riskifaktorid PTA tekkeks on kurgumandlite ja peritonsillaarse koe armistumine korduvate põletike tõttu. Suitsetamine ja antibakteriaalse ravi puudumine enne hospitaliseerimist on seotud PTA ägedama kliinilise pildiga.
2. Kuigi pooltel PTA patsientidel on täidetud sepsise kliinilised kriteeriumid, ei ole nende patsientide haiguse kulg kokkuvõttes pikem ega raskem. Sepsis-2 kriteeriumid on liiga sensitiivsed ja madala kliinilise tähtsusega PTA korral.
3. Mandlilooži biopsia on parim koht mikrobioloogilise analüüsi võtmiseks, kui kasutada külvimeetodit. Molekulaarsete meetodite korral sobivad mäda ja biopsia võrdselt hästi. Kõige sagedasemad PTA tekitajad on *S. pyogenes,* fusobakterid ja SAG grupi streptokokid.
4. Esmaseks PTA raviks sobib penitsilliin, kuna põhilised PTA tekitajad – streptokokid – on tundlikud penitsilliini suhtes.
5. Polüoolid ERY ja XYL omavad inhibeerivat efekti enamike *S. pyogenes*-e tüvede osas ja seega võivad omada ennetavat efekti *S. pyogenes*-e tekitatud kurgupõletike ja PTA korral.

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