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AETIOLOGY OF PERITONSILLAR ABSCESS - ROLE OF MINOR SALIVARY GLANDS

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*"On tärkeää, että on määränpää, jota kohti kulkea.
Kuitenkin, loppujen lopuksi, itse matka on tärkein."*

Kiitos aviomiehelleni, perheelleni, ystävilleni ja
ohjaajilleni tästä matkasta kohti hienoa määränpäätä.

CONTENTS

ABSTRACT

Peritonsillar abscess (PTA) is the most common deep head and neck infection. PTA is considered to be a purulent complication of acute tonsillitis (AT), although the correlation between these two has not been proven. In recent decades, infection of minor peritonsillar salivary glands has been proposed to be behind the development of PTA. Smoking has been known to predispose patients to PTA, and poor dental hygiene has been hypothesised to control the course of the disease.

Clarifying the aetiology of PTA would help physicians differentiate better the subtypes of PTA and plan the best treatment for the patient. Diagnostic tools to achieve this are lacking. Diagnosis of PTA is clinical and is based on a patient's symptoms and clinical examination. No blood tests or imaging has been used routinely. Bacterial culture is a slow method and is not used in clinical decision-making.

In the first study of this thesis, we compared bacterial findings of PTA between patients who had had a renewal and those who had not. *Streptococcus anginosus* group (SAG) was detected more often in the patients with renewal, compared to those without; however, no group A *streptococcus* was reported in patients with renewal. In addition, male patients over 40 years old had renewal episodes after a shorter period than other patients.

In the second study, we histologically analysed the presence of peritonsillar minor salivary glands and inflammation patterns from the removed tonsils. In most (67.5%) of the tonsil specimens, minor salivary glands were identified, and we observed a greater periductal inflammation in patients with PTA compared to AT and chronic tonsillitis groups.

In the third study, we collected AT and PTA patients and found a subgroup of PTA patients without any clinical signs of AT. PTA patients without tonsillar findings had significantly lower C-reactive protein (CRP) levels than PTA patients with tonsillar findings. They were also older than other patients suffering from PTA.

In the fourth study, we compared salivary samples of the patients with AT, peritonsillitis, and PTA between themselves, and with healthy volunteers. No differences between groups were observed which could imply that the composition of oral bacterial microbiota may not have a significant effect on the development of PTA. Alcohol consumption, oral hygiene and smoking had no significant effect either on numbers or distribution of saliva bacteria.

Our studies suggest that tonsillar infection is not always a pre-stage of PTA and support the hypothesis that infection of minor salivary glands, in some cases, causes PTA. We also showed that SAG predicts renewal of PTA, and it is found in older male patients with rapidly proceeding disease. By understanding the aetiology of PTA better, we could also identify patients with different aetiological factors and target treatment more accurately.

TIIVISTELMÄ

Kurkkupaise on yleisin pään ja kaulan alueen syvä infektio. Perinteisesti kurkkupaisetta on pidetty akuutin nielurisatulehduksen märkäisenä komplikaationa, mutta tieteellistä näyttöä näiden kahden taudin yhteydestä ei ole. Viimeisten kahden vuosikymmenen aikana on tutkittu pienten peritonsillaarisesti sijaitsevien sylkirauhasten roolia kurkkupaiseen taustalla. Tupakan tiedetään altistavan kurkkupaiseen kehittymiselle, ja myös huonolla hammashygienialla saattaa olla vaikutusta taudinkuvan.

Taudin etiologian tunteminen auttaisi kliinikkoa erottamaan taudin erilaiset alatyypit ja valitsemaan parhaan mahdollisen hoidon potilaalle. Kliinikot tarvitsisivat myös diagnostisia menetelmiä kurkkupaiseen aiheuttajan selvittämiseen. Kurkkupaiseen diagnoosi on kliininen ja perustuu potilaan oireisiin sekä lääkärin suorittamaan tutkimukseen. Verikokeita tai kuvantamistutkimuksia ei rutiininomaisesti tarvita. Bakteeriviljelynäytteistä ei hoitoa suunniteltaessa ole hyötyä menetelmän hitauden vuoksi.

Väitöskirjan ensimmäisessä osatyössä vertasimme kurkkupaiseiden märkäeritteen bakteerilöydöstä "uusijoiden ja ei-uusijoiden" välillä. *Streptococcus anginous* –ryhmän (SAG) bakteereita tavattiin enemmän "uusijoilla", kun taas A-ryhmän *streptokokkia* (GAS) ei "uusijoilla" todettu lainkaan. Lisäksi havaitsimme, että yli 40 vuotiailla miehillä kurkkupaise uusii muita potilaita nopeammin.

Toisessa osatyössä mikroskopoimme histologisesti nielurisaleikkauksessa poistettua peritonsillaari- ja nielurisakudosta, ja osoitimme, että valtaosalla potilaista (67.5%) on pieniä sylkirauhasia peritonsillaaritilassa. Lisäksi havaitsimme, että kurkkupaisepotilailla on pienen sylkirauhasen tiehyen ympärillä huomattavasti enemmän tulehdusta kuin niillä potilailla, joiden nielurisat on poistettu äkillisen tai pitkäaikaisen nielurisatulehduksen vuoksi.

Kolmannessa osatyössä osoitimme ryhmän kurkkupaisepotilaita, joilla ei ollut merkkejä akuutista nielurisatulehduksesta. Havaitsimme, että kurkkupaisepotilailla, joilla ei havaittu nielurisoissa infektion merkkejä oli selvästi matalampi CRP-arvo kuin potilailla, joiden nielurisoissa oli infektion merkkejä. Potilaat, joilla ei nielurisoissa havaittu tulehduksen merkkejä olivat myös selvästi vanhempia kuin muut kurkkupaisepotilaat.

Neljännessä osatyössä vertasimme terveiden vapaaehtoisten, äkillisestä nielurisatulehduksesta, peritonsilliitista ja kurkkupaiseesta kärsivien potilaiden sylkinäytteitä. Ryhmien välillä ei havaittu eroja, joka voisi viitata siihen, ettei suun mikrobiston muutoksilla ole vaikutusta kurkkupaiseen kehittymiseen. Myöskään alkoholinkäytöllä, hammashygienialla tai tupakoinnilla ei havaittu olevan merkittävää vaikutusta syljen bakteerien määrään.

Tutkimuksessamme osoitimme, ettei nielurisatulehdus aina edellä kurkkupaisetta. Tutkimuksemme tukee vahvasti hypoteesia, jonka mukaan osa kurkkupaiseista aiheutuu pienten sylkirauhasten tulehduksesta. Lisäksi osoitimme, että SAG ennustaa kurkkupaiseen uusiutumista ja on yleensä vanhempien miesten taudinaiheuttajana. Ymmärtämällä kurkkupaiseen etiologiaa voimme jatkossa paremmin tunnistaa eri etiologiset tekijät ja valita hoidon tarkemmin.

SAMMANFATTNING

Peritonsillär abscess (PTA), också kallad halsböld, är den vanligaste djupa huvud- och halsinfektionen. PTA har traditionellt antagits vara en komplikation av akut tonsillit (AT) men en korrelation mellan AT och PTA har inte påvisats. I 20 års tid har man spekulerat att en del av halsbölderna kunde bero på en infektion i de små peritonsillara spottkörtlarna. Rökning ökar incidensen av PTA och dålig tandhygien kan också vara relaterad till sjukdomen.

Identifiering av etiologin för PTA hjälper läkaren att känna igen olika typer av PTA och välja den bästa behandlingen för patienten. Dessutom strävar vi efter att hitta diagnostiska verktyg för att åtskilja kausitiva faktorer av PTA. Diagnosen av PTA är klinisk och baserad på patientens symptom och klinisk undersökning. Rutinmässiga blodprov eller radiologiska undersökningar behövs inte. Varprov är inte nödvändigt för planering av vården eftersom metoden är långsam.

I första studien av avhandlingen jämförde vi varprovens bakterier hos de patienter som återinsjuknat och de som icke återinsjuknat. Ingen av de återinsjuknade patienterna hade grupp A *Streptococcus* i varprovet. Däremot observerades *Streptococcus anginosus* -gruppens bakterier oftare i den återinsjuknade gruppen. Över 40-åriga män återinsjuknar i PTA mycket snabbare och kraftigare än andra PTA-patienter.

I andra studien analyserade vi tonsillavävnadens histologi. Vi fann små spottkörtlar i majoriteten (67,5%) av histologiska proven. Vi observerade också att periduktala inflammationen var betydligt starkare hos PTA-patienterna jämfört med AT- och CT-patienter.

I tredje studien upptäckte vi en grupp av PTA-patienter utan tecken av infektion i tonsillerna. Dessa patienter hade lägre CRP och var äldre än PTA-patienterna med tecken av infektion i tonsillerna.

I fjärde studien jämförde vi salivprov av patienter med AT, PT och PTA patienter sinsemellan och med friska referenspatienter. Vi observerade inga skillnader mellan grupperna, vilket kan tyda på att skillnaderna i salivbakterierna inte har en relevant inverkan på utvecklingen av PTA. Alkoholförbrukning, tandhygien eller rökning har ingen relevant inverkan på salivbakteriernas mängd eller kvalitet.

Vi visade att PTA inte alltid utvecklas som en komplikation av AT. Vår undersökning stöder starkt hypotesen att en del av PTA utvecklas från en infektion i mindre spottkörtlarna. Ytterligare påvisade vi att SAG förutspår återinsjuknande I PTA och förorsakar vanligen äldre mäns PTA. Genom att känna igen PTA: s etiologi, kan vi i fortsättningen känna igen etiologiska faktorerna och välja vården noggrannare.

LIST OF ORIGINAL PUBLICATIONS

1. Wikstén J., Kaltiainen E., Laakso S., Pitkäranta A., Blomgren K. (2017). Renewal of Peritonsillar Abscess; Impact of the Bacteria and Clinical Features of the Patient. Clinical Otolaryngology. Impact Factor: 2.627

2. Kaltiainen E, Wikstén J, Aaltonen L-M, Ilmarinen T, Hagström J, Blomgren K. (2017). Presence of Minor Salivary Glands in the Peritonsillar Space. European Archives of Oto-Rhino-Laryngology. Impact Factor: 1.546

3. Sanmark E, Wikstén J, Aaltonen L-M, Ilmarinen T, Välimaa H-M, Blomgren K. (2019). Peritonsillar abscess may not always be a complication of acute tonsillitis: a prospective cohort study. Submitted

4. Sanmark E, Wikstén J, Välimaa H-M, Blomgren K. (2019). Smoking or poor oral hygiene do not predispose to peritonsillar abscesses via changes in oral flora. Acta Oto-Laryngologica. Impact Factor: 0.86

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ABBREVIATIONS

AT	acute tonsillitis
CRP	C-reactive protein
CCT	chronic caseous tonsillitis
CT	chronic tonsillitis
EBV	Epstein Barr virus
FCM	flow cytometry
FN	*Fusobacterium necrophorum*
GAS	group A *Streptococcus*
HUH	Helsinki University Hospital
HUSLAB	Helsinki University Hospital Laboratory Services
ID	incision and drainage
ICU	intensive care unit
ITA	intratonsillar abscess
IV	intravenous
LGG	*Lactobacillus rhamnosus* GG
NA	needle aspiration
ORL	otorhinolaryngological
PAD	pathologic-anatomic diagnosis
POGI	*Porphyromonas gingivalis*
PTA	peritonsillar abscess
PT	peritonsillitis
RADT	rapid antigen detection test
RT	recurrent tonsillitis
SAG	*Streptococcus anginosus* group
S. mutans	*Streptococcus mutans*
SVG	*Streptococcus viridans* group
TE	tonsillectomy

INTRODUCTION

Peritonsillar abscess (PTA) is a frequent deep head and neck infection and the most common otorhinolaryngological (ORL) infection requiring tertiary medical care [1, 2]. PTA is defined as a collection of pus usually located between the tonsillar capsule and pharyngeal constrictor muscles near the tonsil´s superior pole [3, 4]. Almost 800 patients suffering from PTA are treated in Helsinki University Hospital (HUH), Department of ORL, annually. PTA is seen in all ages from infants to very old people. Depending on age, duration of symptoms, cooperation, and general overall health, treatment varies from an incision on an outpatient visit to a tonsillectomy (TE) and hospitalisation. The complications of PTA can be severe and require treatment in the intensive care unit (ICU) which consumes expensive resources. All patients also need sick leave, which further burdens the society. [2]. Therefore, it is important to treat the patients with PTA in the most accurate and efficient way from the baseline.

Despite its high incidence, the aetiology of PTA is controversial. Traditionally PTA is classified as a complication of acute tonsillitis (AT). No scientific evidence, however, supports this theory [3]. A presumption was raised over two decades ago that the development of PTA could be associated with an infection of minor salivary glands, more accurately Weber's glands [3, 4]. Few small, uncontrolled studies have shown that the minor salivary glands are infected in patients with PTA [4-7]. An Egyptian study group has observed that amylase levels in pus and serum are elevated in PTA patients compared to patients with other neck or oral abscesses [8, 9]. Salivary amylase levels can be used as a marker about salivary function [10]. Recently a large cohort study showed that the incidence of AT increases during the winter, while the incidence of PTA is steady throughout the year. This finding does not support that PTA is only a complication of AT.[11].

Though PTA is traditionally regarded as a complication of tonsillitis, it is possible that poor oral hygiene, patients´ characteristics like age and gender, smoking, and infection of minor salivary glands play a role in aetiology of PTA [3, 12, 13]. These patients with different subtypes of PTA should be recognised and treatment should be targeted based on the aetiology in the future. In this doctoral thesis, we aim to specify the diverse aetiology of PTA and find diagnostic tools to differentiate the subgroups of PTA patients with different causative agents.

1   REVIEW OF LITERATURE

1.1   Anatomy and histology of tonsils and salivary glands

1.1.1   Tonsils

The palatine tonsils are located in the lateral part of the oropharynx surrounded by the palatoglossal muscle anteriorly and the palatopharyngeal muscle posteriorly. Laterally the tonsil is covered by a fibrous connective tissue capsule. Tonsillar crypts on the medial surface are formed of non-keratinised stratified squamous epithelium. The number of tonsillar crypts varies from 8 to 20 in one tonsil. Palatine tonsils are a major component of lymphoid tissue which forms Waldeyer's ring. Other components are adenoid, lingual and tubal tonsils. The main function of Waldeyer's ring is to act as a first line of defence against pathogens. [4, 14]. Interaction between pathogens and tonsillar lymphocytes happens via apertures of tonsillar crypts [4, 15, 16].
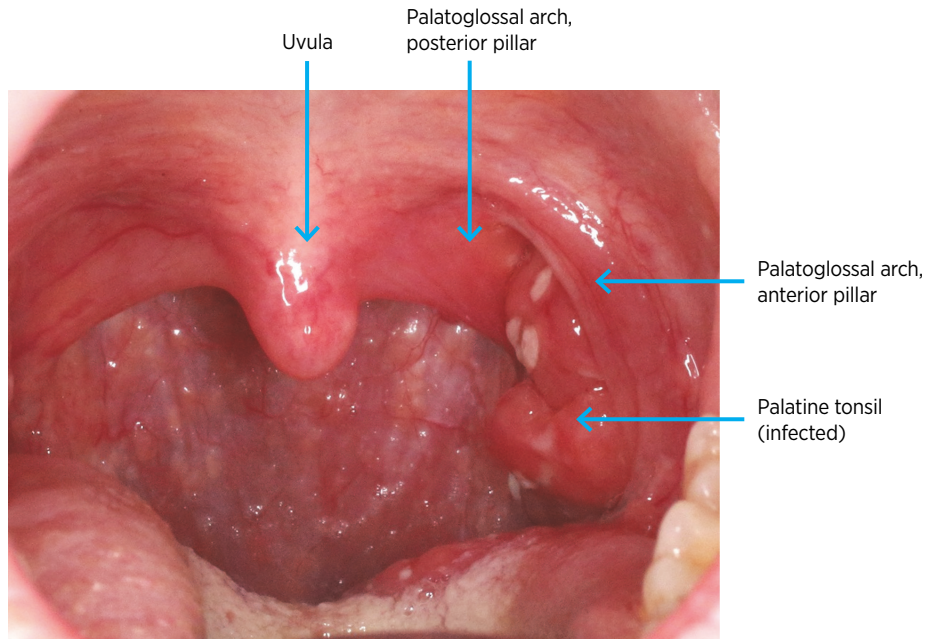
Figure 1. Anatomy of oropharynx.

1.1.2   Minor salivary glands

There is a group of 20 to 25 mucous salivary glands located in the superior peritonsillar space [5]. In this thesis minor salivary glands means minor peritonsillar salivary glands. The precise location of peritonsillar-located minor salivary glands is reported in few uncontrolled studies. These studies have shown that minor salivary glands appear at the upper, middle, and lower portions of the peritonsillar space outside the tonsillar capsule. However, only glands located in the superior pole are called Weber´s glands, described by German anatomist Moritz Ignaz Weber in 1927. [4, 5, 7]. Minor salivary glands are organised in small lobules and send the common ducts from the glands to the tonsillar crypts penetrating the tonsillar capsule on the way [4]. In histological analysis of PTA patients' tonsillar tissue, the inflammation and fibrosis of minor salivary glands has been observed [6, 7].

1.1.3   Major salivary glands

The paired parotid, submandibular, and sublingual glands comprise the major salivary glands. The parotid gland becomes inflamed most commonly. Acute and chronic sialadenitis, viral infections, and various neoplasms are the most common diseases of major salivary glands. [17, 18].

1.2   Acute tonsillitis (AT)

Acute tonsillitis (AT) is one of the most common upper respiratory infections in primary health care [19]. It affects both children and adults and the highest incidence is in those from 5 to 15 years of age [3, 11, 20, 21].

The diagnosis is clinical and the typical symptoms include sore throat, fever, and difficulties swallowing. In clinical examination a physician can detect pharyngeal erythema with or without tonsillar exudates and painful cervical adenopathy. The raised inflammation markers as C-reactive protein (CRP) and leukocytes in laboratory tests may be linked to the higher likelihood of streptococcal infection; however, routinely laboratory tests (CRP, total blood count, and electrolytes) are recommended only to those patients who require hospital admission. [20]. Based on signs and symptoms, it is impossible to differentiate causative agents. Accuracy in differentiating clinically virus from bacteria or group A *Streptococcus* (GAS) versus non-GAS is weak [20, 22]. Finnish Current Care Guidelines recommend trying to find GAS as a causative agent. [23]. Because culturing the bacteria is too slow for decision-making, rapid antigen detection tests (RADT) with high specificity (95%) and quite high sensitivity (70 to 90%) are nowadays widely used

in emergency departments for detecting GAS [24]. There are also point-of-care tests for detecting mononucleosis in clinical use [25].

50–80% of ATs are caused by viruses. The most common viruses causing AT are rhinoviruses, coronaviruses and parainfluenza viruses. GAS, *Fusobacterium necrophorum* (FN), and other betahaemolytic streptococcus are the most common bacteria causing AT and they are associated with 5–36% of tonsillar infections. Other bacteria found from bacterial culture of AT patients are *Staphylococcus aureus*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Candida spp.*, *Neisseria meningitidis*, *Yersinia enterocolitica*, *Arcanobacterium haemolyticum*, and *Neisseria gonorrhoeae.* [20, 23]. The possibility of *Corynebacterium diphtheriae* as a causative agent of AT should remembered in patients with severe course of disease [26].

Antibiotic use for tonsillitis is controversial. The treatment of viral tonsillitis is mainly conservative; however, severe cases of bacteria-caused tonsillitis are treated with aggressive antibiotic administration and hospital admission. The level of evidence for antibiotic treatment of AT is highest in GAS tonsillitis. Antibiotic treatment can modestly relieve the symptoms and reduce the duration of symptoms. Penicillin is regarded as the first-line antibiotic for GAS tonsillitis. A single-dose corticosteroid has also been shown to relieve the pain. [20, 23].

The most common complications of AT are other bacterial infections, such as PTA, invasive GAS disease, and retro- and parapharyngeal abscesses. The incidence of bacterial complications of AT does not correlate with the antibiotic use targeted to AT. [27]. Lemierre´s syndrome is a rare complication of FN tonsillitis [28].

Figure 2. Acute tonsillitis.

1.3   Chronic tonsillitis (CT)

Chronic tonsillitis (CT), more specifically chronic caseous tonsillitis (CCT), is the common cause of sore throat and halitosis (clinically detectable or non-detectable odour). CT occurs in both genders and at any age. In CT, tonsillar stones, also called tonsilloliths, are formed when the mineralised debris of food, leucocytes, and bacteria are trapped in the tonsillar crypts. About 77% of CT patients suffer from halitosis. Symptomatic CT is treated with topical antiseptics, per oral antibiotics, and in the end with TE if clinical treatment does not bring the relief. [29, 30].

1.4   Recurrent tonsillitis (RT)

In literature, recurrent tonsillitis (RT) is usually defined as recurrent acute episodes of tonsillitis, but the number of AT episodes is not specified. Chung et al. used a minimum of five episodes in the past year as a limit to the definition of RT [31]. To Kronenberg et al. two episodes confirmed by physician was enough to be categorized RT [32]. In Finnish Current Care Guidelines RT is defined as a minimum of 4 episodes in a year or three episodes in half a year [23]. In some studies, the number of episodes has not even been defined [33, 34] .

The percentage of PTA patients with a history of recurrent episodes of AT varies from 8% to 60% [31, 33-36]. Changes can be explained by the different definitions of RT. Tachibana et al. showed that patients with RT before PTA recover faster. They speculated that previous throat infections would cause adhesion around the tonsil which in turn could prevent the abscess from spreading. [36]. Opposite results have also been reported [35].

1.5   Sialadenitis of major salivary glands

The development of acute sialadenitis has been described as a process in which stasis of the salivary duct permits retrograde bacterial flow from the oral cavity into the gland. The most common bacteria causing acute bacterial sialadenitis are some of the same bacteria met in AT and PTA: GAS, *Staphylococcus aureus* and *Haemophilus influenzae*. Poor dental hygiene has also been connected to development of acute sialadenitis. [18, 37, 38]. In the majority of patients suffering from parotitis, elevated serum amylase (S-Amyl) levels have been registered but only a few patients have demonstrated elevation of CRP [37, 39].

1.6   Epidemiology of PTA

PTA is the most common deep neck infection in ORL. Usually its incidence is highest in teenagers and young adults equally in both genders. PTA develops more often in the left side and its recurrence rate is between 9% and 22%.

1.6.1   Incidence

PTA´s incidence is 10–41/100,000 [2, 3, 21, 27, 40-42]. The large variation in reported incidences has been thought to be connected to the number of tonsillectomies (TE) in the country. Low frequency of TEs, aspiration and incision and drainage (ID) of the abscess is linked to higher incidence of PTA. [2, 43, 44].

1.6.2   Age

Median age of PTA patients is 25–26 years [21, 34, 45]. The incidence of PTA is higher in teenagers and young adults, gradually decreasing after age 40 until old age [21, 34, 41]. In children the reported incidence is 18/100,000 [44]. Patients older than 40 years have a lower incidence of throat infections and also a lower probability of recurrence of PTA [32]. One possible explanation for PTA's higher incidence in young adults is their higher smoking rate [46, 47]. According to Marom et al. the incidence among elder patients is, however, increasing [48].

PTA in elder patients differs in many ways from PTA in young adults. Older PTA patients have a longer hospital stay. The rate of complications and comorbidities is higher among the older patients. Older patients have also worse oral hygiene. [35, 48].

1.6.3   Gender

Between the ages of 10 and 14, girls suffer from PTA more often than boys. After that until at least the age of 70, men predominate significantly over women [21, 41, 43]. These observation are probably due to changes in the immune system during puberty [21]. In adults the male to female ratio is reported to be between 1:1 and 2:1[21, 33, 40, 49-51]. Male predominance may be partly a result of the higher incidence of smoking in males. When the effect of smoking is observed and excluded, the risk of PTA is still 9.5% higher among males [46].

1.6.4 Side

Left predominance in PTA formation has been reported in several studies and it is found in both genders [33, 48, 50]. The cause of this slight asymmetry is unclear. It has also been proposed that it could be a coincidence [52]. Bilateral abscesses are rare, with only 0.2–1.8% of PTA patients [35, 36, 48].

1.7 Clinical characteristics of PTA

1.7.1 Location of peritonsillar abscess (PTA)

PTAs are nearly always (64–99%) located at the superior part of peritonsillar space. However, inferior and middle abscesses have also been described [5, 13, 28, 31, 53]. PTA is usually, with very few exceptions, unilateral [13, 35]. Even though rare, PTA has also been diagnosed in patients with no tonsil tissue left after TE [54]. Intratonsillar abscess (ITA) is a different disease and, compared to PTA, is extremely rare [28].

Peritonsillar abscess
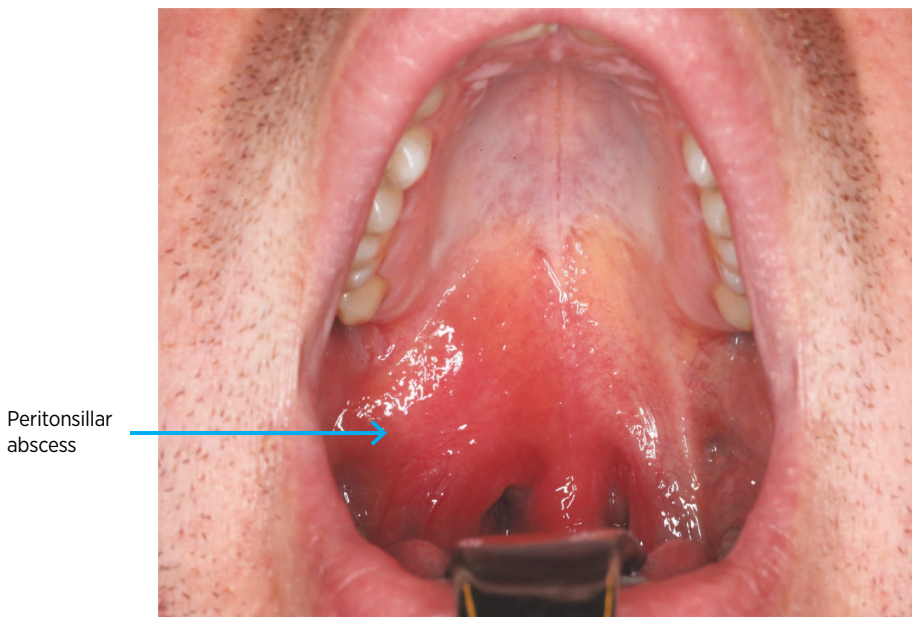
Figure 3. Peritonsillar abscess.

1.7.2 Recurrence of PTA

Recurrent PTA is defined as the recurrence of abscess formation at the same site after the initial treatment. No conclusion about the required time period after the first PTA exists. Several studies have used 0 to 2 months as an interval after the primary PTA period [31, 32, 49, 55]. Bovo et al. showed that most relapses of PTA happen during the first three months after the first PTA episode [41]. When treated with ID, recurrence rate after a single PTA episode is between 9–22% [2, 32, 41, 49, 50, 55, 56]. After the second and third PTA episodes, the risk of further recurrences increases [31, 41]. Increased recurrence rate has also been observed in patients with RT and patients with FN in bacterial culture. Jousimies-Somer observed also SAG predicted renewal, while in contrast GAS was rare in PTA patients with a renewal [31, 32, 57]. Spreading to extraperitonsillar space also predicts recurrence [31].

In a Finnish study, most (65%) recurrences occurred after 3 days, with a range of 1–42 days. No difference emerged between penicillin + metronidazole and penicillin + placebo groups or in subgroups observed by age, gender, pre-admission antibiotics, smoking, success of the primary opening, or the time point of recurrence. [55]. In another study, a significant male predominance in patients with RT was observed, but smoking had no effect on recurrence [48]. Gavriel et al. have, on the other hand, shown that anaerobic bacterial growth correlates with the higher recurrence rate [58]. Asthma and diabetes mellitus type 2 have also been associated with a readmission to hospital, but the reason for the readmission was not analysed more precisely [49].

1.7.3 Complications of PTA

Complications after PTA are uncommon, with an incidence of 3%. Complications are, however, potentially life-threatening [48]. Complications include the infection spreading into the parapharyngeal space and other deep neck spaces. These patients require inpatient treatment, intravenous (IV) antimicrobial therapy, and sometimes also monitoring in the ICU. [48]. Aspiration, airway obstruction, erosion into major blood vessels, Lemierre´s syndrome, or extension to the mediastinum are the known complications of PTA and are potentially fatal [2, 28, 49]. Possible fatal complications have been seen in 0.41% of cases [41].

1.8 Aetiology of PTA

Aetiology of PTA is controversial. The condition is usually believed to be the continuum of AT via peritonsillitis to the formation of an abscess. However, an abscess can develop without any preceding history of tonsillitis. [28]. That has raised the other main theory to explain the formation of PTA: infection of peritonsillar minor salivary glands [4]. In recent decades, the aetiology of PTA has been speculated on, but no consensus exists.

1.8.1 Theory of minor salivary glands causing PTA

Minor salivary glands are localised at the upper, middle, and lower portions of the peritonsillar space [5]. Inflammation and fibrosis of minor salivary glands has been observed in PTA patients [6, 7]. The scarring and obstruction of the draining ducts of minor salivary glands could promote the formation of PTA [28]. El Saied et al. showed that both serum and pus amylase levels are highly elevated in patients with PTA, compared to levels seen in those with deep neck abscesses and dental abscesses [8, 9]. PTA recurrence is more likely in patients with RT. This could be due to scarring or other anatomic changes in the minor salivary gland. [13, 31]. PTA has also been diagnosed in patients who have undergone TE, but these patients are a small minority [54].

AT is usually bilateral, while PTA is nearly always unilateral [28]. In the vast majority of cases (64-99%) PTA is located in the supratonsillar space of the oropharynx. This supports the theory of the infection of small salivary glands because most of the glands are found in the same location [4, 13, 54]. Kordeluk et al. also showed that although the incidence of AT increases during the winter, the incidence of PTA remains the same the whole year, so there is no seasonal correlation between the incidence of AT and PTA [11]. Other studies in different countries have produced the same results [59, 60].

1.8.2 Theory of AT causing PTA

AT has traditionally been regarded as the only cause of PTA. The disease process has been explained as pathogens spreading from the tonsils to peritonsillar space. Development of PTA starts in the intratonsillar fossa, which is situated between the upper pole and the body of the tonsil, and eventually extends around the tonsil. [6, 13, 28]. The theory does not explain, however, how the causative agents penetrate the tonsillar capsule [13].

Same major pathogens of AT and PTA are found from the pus aspirates and bilaterally tonsillar tissue [13, 47, 61]. In some studies, antimicrobial treatment had a minor preventative effect on the developing of PTA in certain subgroups. There

are, however, also studies where the difference has not been shown. [3, 5, 13, 47, 62, 63]. Recently, a large Swedish study demonstrated an even lower rate of PTA after AT in case where the patients were not treated with antibiotics during an AT episode [27]. Marom et al. showed that 66.2% of patients developed PTA in spite of prior antimicrobial therapy [48]. They also found that as many as 79% of patients reported a sore throat episode preceding PTA [34, 48]. In a recent review, Little et al. found that some PTA patients had had a sore throat episode one month before developing PTA and the researchers have interpreted this as support to the AT hypothesis. In addition, they also found that tonsillar inflammation during a sore throat episode predicts complications. Complications were, however, analysed in large groups of diseases, and subgroup analysis of PTA was not introduced in the primary study. [13, 64].

1.8.3 Smoking

Smoking is associated with increased risk to PTA in both genders. As many as 30% to 60% of patients with PTA have reported daily smoking which is significantly higher than the smoking rate in the general population. In Finland the number of daily smokers in the general population is 13%. [36, 46, 48, 49, 65, 66]. Also, the number of ex-smokers among PTA patients is high: 18% [49]. The specific mechanism of how smoking promotes these diseases is not clear. Klug et al. found no differences in PTA pathogens between smokers and nonsmokers [46]. In another study, smokers with PTA were observed to have a higher incidence of *Streptococcus viridans* (includes SAG) isolates in microbiological cultures of pus samples [48]. Torre et al. showed histological changes in both lymphoid and non-lymphoid compartments of tonsils in smokers. Changes in immune defence mechanisms via changed cell-to-cell or cell-to-matrix interactions in tonsillar tissue could be one possible mechanism explaining how smoking induces PTA. [67]. Among invasive pneumococcal diseases, Nuorti et al. have observed that smoking seems to be the most important risk factor among immunocompetent, non-elderly adults. They speculated that the higher rate of bacteria carriers among smokers, poor mucociliary clearance, or enhanced bacterial adherence could be the possible biological mechanisms enabling smoking to promote the development of pneumococcal disease. These same mechanisms could expose smokers to tonsillar diseases. [68]. Smoking alters the composition of subgingival bacterial microbiota in the development of periodontal disease and increases the depth of periodontal pockets [69, 70]. Although smokers have significantly higher CRP levels during PTA episodes, no difference in leukocyte or neutrophil levels has been shown. Klug et al. did not notice any difference in the duration of symptoms between smokers and nonsmokers. [46]. Correlation between smoking and postoperative haemorrhage has also been studied but no correlation was found [66].

1.8.4   Periodontal disease

Periodontal diseases, the group of chronic inflammatory diseases of gingiva, supporting connective tissue, and alveolar bone, can be regarded as a public health concern when it affects as much as 20–50% of the global population in both developed and developing countries. Periodontal diseases are speculated to be an aetiological factor of PTA. [12, 71]. Georgalas et al. showed that patients with PTA have considerably more periodontal diseases than patients with CT [12]. Like PTAs, oral infections are also typically polymicrobial. Same bacterial species, such as *Prevotella spp.,* and *Fusobacterium spp.*, have been identified in not only PTA but also periodontitis and in the tonsillar area [3, 5, 12]. The causality between these two diseases has, however, not been shown although the changes in oral microbiota have been speculated to participate in this process. It is known that low socioeconomic status, smoking, and diabetes are associated with upper respiratory infections. Poor oral hygiene has also been thought to be linked to these other factors and not be an individual causative factor. [3, 12, 72].

1.8.5   Other possible factors

Several host factors may also promote PTA, such as compromised immunity and previous use of antibiotics. There are, however, no studies about these topics in the scientific literature. [12]. Recently a Korean research group published a study suggesting that air pollutants (with high concentration of NO_2 and PM_{10}) could be the risk factor for PTA [73].

1.9   Diagnosis and differential diagnosis of PTA

Diagnosis of PTA is usually clinical and is based on patient's symptoms, careful clinical examination performed by a physician, as well as aspiration or ID of PTA. Routinely no laboratory tests or imaging are needed. [2].

1.9.1   Signs and symptoms of PTA

The common symptoms of PTA are sore throat, difficulties and pain in swallowing, trismus, and fever [1, 51, 74, 75]. Duration of symptoms before diagnosis is usually 2–6 days. [1, 21, 33, 40, 45, 48, 55]. The duration of symptoms in older patients is twice as long as in younger patients and they seldom suffer from fever or trismus [76]. In an ORL examination a physician may observe tonsillar exudates, asymmetric peritonsillar swelling, uvular deviation, and enlarged cervical lymph nodes [6, 28, 33, 51, 74, 77]. Patients over age 40 with no history of RT and high

CRP can be regarded as a risk group, with a longer recovery time than other PTA patients [36].

1.9.2   Diagnostic tools in PTA

Traditionally diagnosis of uncomplicated PTA has been clinical, and neither laboratory tests nor imaging has been considered necessary [2, 28]. Diagnosis is based on finding the abscess from peritonsillar space with aspiration or ID [31]. A recent review, however, recommended that CRP and full blood count should be taken from all PTA patients, but the benefits of laboratory tests in diagnosis or treatment of PTA are poorly evidenced. Because dehydration is a common symptom of PTA, electrolytes were also recommended to be analysed. [2]. Tachibana et al. have shown that serum leukocytes are higher in patients with PTA than in patients with AT. Differences in CRP levels were not observed between groups. The researchers, however, noted that high CRP is associated with longer recovery time and predicts a slower healing process after PTA. [36, 78]. In a study by Johnston et al., median serum CRP in all PTA patients was 85mg/l (range: 41–143 mg/l) [49]. PTA caused by FN is noted to increase CRP and neutrophil levels significantly more than PTA caused by other pathogens [40]. Salivary amylase levels can be used as a marker of salivary function [10]. El Saied et al. showed that both serum and pus amylase levels are highly elevated in patients with PTA, compared to levels in patients with deep neck abscesses and dental abscesses (PTA patients: mean S-Amyl 50 U/l, mean pus amylase 3045 U/l, range 20–11,000 U/l) [8, 9].

Computed tomography imaging can be used when extraperitonsillar spread is suspected. Another indication for computed tomography imaging is trismus, when a patient is unable to open his or her mouth for clinical examination. [28, 31, 49]. The use of CT in PTA diagnostics has increased in recent years, due to decreased costs and radiation doses as well as better availability of the imaging [44]. Intraoral ultrasound can be used to confirm the presence of an abscess as well as its volume, location, and relationship to the carotid artery. Ultrasound has an 89–95% sensitivity and a 79–100% specificity for diagnosing PTA [2, 28, 79].

Bacteriologic cultures are not useful in non-complicated cases of PTAs, as the bacteriological results often delay, and the timing of the results does not match the clinical need. They should be used, however, in patients with a high likelihood of infection by atypical or antimicrobial-resistant organisms—for example, in patients with a poor response to treatment, in diabetics, in immunocompromised patients, and in patients with a recurrent PTA [51].

1.9.3   Peritonsillitis (PT)

Making a differential diagnostic between peritonsillitis (PT), also called peritonsillar cellulitis, and PTA is not always easy. The differential diagnosis is mainly based on pus aspiration. [21, 41, 77, 80]. The aetiology of PT is as unclear as PTA's. PT could be the same disease as PTA with the same causative agents, only at an earlier stage, but the association of these two diseases has not been shown in the literature. The signs and symptoms of PT are like those of PTA, but without a confirmed abscess formation. Patients' characteristics and symptoms between PT and PTA patients have been compared in a study by Risberg at al. The median age of patients, days of sore throat, amount of prior antibiotics, mean duration of prior treatment, and percentage of tested GAS were all equal. [45].

1.9.4   Mononucleosis

Mononucleosis is a possible differential or co-diagnosis of both AT and PTA [2]. Approximately 1 to 10% of AT cases are caused by mononucleosis, more specifically an infection caused by Epstein-Barr virus (EBV) [20]. Retrospectively diagnosed simultaneous mononucleosis is found in 1.5%–6% of patients with PTA. Incidence of mononucleosis is higher in teenagers and young adults, and a mononucleosis test is therefore recommended in unclear cases, especially for teenagers and young adults. [2, 49, 81]. PTA patients with simultaneous mononucleosis have lower leukocyte levels than PTA patients without mononucleosis [40].

1.9.5   Retro- and parapharyngeal abscess

Retro- and parapharyngeal abscesses share similar clinical characteristics with PTA and are sometimes difficult to distinguish from PTA. Retropharyngeal abscesses occur in children while parapharyngeal abscesses affect primarily adults [17]. The annual incidence of parapharyngeal abscesses is 0.9/100,000. As many as 50% of patients with parapharyngeal abscess have a concomitant episode of PTA. [28, 82]. Parapharyngeal abscess is treated with abscess tonsillectomy or external drainage and IV antibiotics. Difficulties in breathing and limited range of motion of neck are associated with retropharyngeal and parapharyngeal abscesses, usually not PTA. The trismus is a common symptom of both PTA and retropharyngeal and parapharyngeal abscesses. [28, 83].
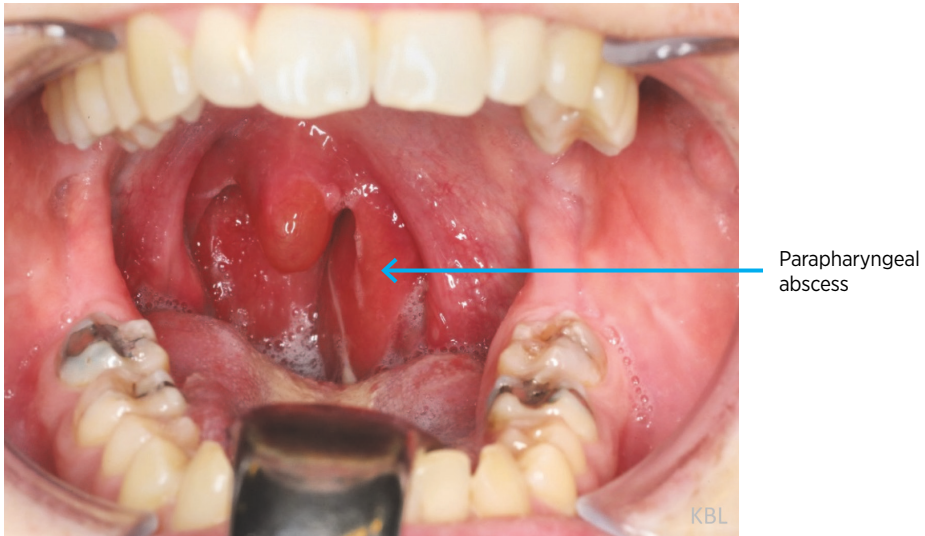
Figure 4. Parapharyngeal abscess.

1.9.6 Intratonsillar abscess (ITA)

ITAs are rare [17, 28]. They are defined as a collection of pus in the parenchyma of the tonsil. As in PTA, the pathogenesis of ITA is also uncertain. Two mechanisms of the formation of ITA have been proposed. Firstly, direct invasion of pathogens into the tonsillar crypts is followed by enlargement of the inflamed tonsil, which causes occlusion of the crypt. Secondly, bacterial seeding via the bloodstream or lymphatic system leads to abscess formation inside the tonsil. [6, 16].

1.10 Microbiology and the most important pathogens in tonsillar infections

In the literature, microbiological findings show large variability. This could result from the existing variations in handling, culturing methods, or differences in advanced technology. Because of this, the studies are only partly comparable.

The microbiology of abscesses is generally related to microbiota of the originating focus. In this thesis microbiota means mainly bacterial microbiota. That's why microbiology of PTA and other deep neck abscesses is similar, reflecting the host's oropharyngeal microbiota. [17, 28]. There is no consensus in literature whether a PTA is monobacterial or polybacterial infection. Variation in the results can be explained with different collection methods, culture techniques, and incubation times in different studies. [3, 28, 50, 84, 85]. The amount of positive bacteria culture samples in PTA patients is between 48% and 100% depending on culture methods

and incubation time [34, 40, 48, 51, 84, 85]. Microbiota may also differ because of a patient´s diet and lifestyle [3, 28, 84, 85]. There is no consensus in literature about the impact of prior antibiotic treatment of AT or PTA on microbiota. Sakae et al. found no difference in bacterial species between patients with and without prior antibiotics. Those patients with prior antibiotics (all had penicillin derivates) had, however, a lower number of different bacterial species in the bacterial culture of pus samples, which could mean that antibiotics destroyed a some bacteria. [85]. On the other hand, in some studies, differences especially in aerobes have been noticed after prior antimicrobial treatment [84].

It is still unclear which bacteria are real pathogens causing PTA, but the most frequently isolated aerobes from PTA patients´ pus samples are GAS, FN, group C and G *Streptococcus*, *Staphylococcus aureus,* and *Haemophilus influenzae.* Predominant anaerobic bacteria, on the other hand, are *Fusobacterium spp.*, *Prevotella spp.*, *Porphyromonas spp.,* and *Peptostreptococcus spp.* [28, 55, 86, 87] (Table 1). All these bacteria are regarded as real pathogens causing PTA. Growth of GAS, FN and SAG in bacterial culture is usually frequent and prominent, so they are regarded as major pathogens causing PTA. [3, 88].

According to current review, when examining from a microbiological perspective, there seem to be pathogenically two different subtypes of PTA. Type 1 contains a pure culture of a single organism, most often GAS. In type 2 extensive polybacterial growth can been observed, which often contains a variety of anaerobes. Clinically, type 2 PTA patients often have more severe disease. [3]. Recently, however, more specific subtypes of PTA have been presented. Patients with FN and GAS are younger, and high neutrophil and CRP levels are associated with FN as a pathogen [40, 87].

Table 1. Number of potential pathogens from pus specimens in studies of peritonsillar abscess microbiology published 2000-2019.

Study		Diagnosis	(n)	GAS (number of isolates)	GCS/GGS	Fusobacterium spp. (FN)	Viridans streptococci (SAG)	Staphylococcus aureus	Haemophilus spp.
Matsuda et al. [89]	2002	PTA	724	48			298		
Sakae et al. [85]	2006	PTA	30	7	2		11	3	1
Gavriel [58, 86]	2008, 2009	PTA	469	58	5	3		4	2
Sunnergren [42]	2008	PTA	89	20	5		5(2)	2	
Rusan [1]	2008	PTA	623	105	20	(154)			
Segal [90]	2009	PTA	126	29	6		2	2	1
Klug [40]	2009	PTA	847	141	39	191 (191)		15	9
Hidaka [91]	2011	PTA	117	9		3	20 (20)		
Klug [47]	2011	PTA	116	7	3	25 (19)	38	2	
Hsiao [92]	2012	PTA	56	9		15 (2)	22	2	
Takenaka [93]	2012	PTA	71	10		13	5	2	2
Albertz&Nazar [94]	2012	PTA	112	16		12			
Mazur [35]	2015	PTA	111	13	1	(1)	19 (4)	1	
Plum [95]	2015	PTA	69	19	1		9		
Vaikjärv [96]	2016	PTA	22	6		4 (1)	4 (2)		
Lepelletier [75]	2016	PTA	412	67	11	102		20	20
Tsai [97]	2018	PTA	415			17	48 (24)		
Ali [98]	2019	PTA	990	51	5	203 (176)	140 (140)	20	20

PTA, peritonsillar abscess; FN, *Fusobacterium necrophorum*; GAS, group A *Streptococcus*; SAG, *Streptococcus anginosus* group

Table 2. Number of potential pathogens from throat swabs in studies of AT microbiology published 2000–2019.

Study		Diagnosis	(n)	GAS (number of isolates)	GCS/GGS	Fusobacterium spp. (FN)	Viridans streptococci (SAG)	Staphylococcus aureus	Haemophilus spp.
Aliyu [99]	2004	AT	100			(10)			
Loganathan [100]	2006	AT	233	25	16			99	22
Rusan [1]	2008	AT	109	25	2				
Ludlam [101]	2009	AT				(14)			
Hedin [102]	2014	AT	220	66	15	(33)			
Eaton [103]	2014	AT	502	59	17	(28)			
Kjaerulff [74]	2015	AT	100	26	10	(16)			
Jensen [104]	2015	AT	179	7	55	(43)			
Centor [105]	2015	AT	312	22	28	(43)			
Furuncuoglu [106]	2016	AT	899	56					

AT, acute tonsillitis; FN, *Fusobacterium necrophorum*; GAS, group A *Streptococcus*; SAG, *Streptococcus anginosus* group

1.10.1 Group A Streptococcus (GAS)

GAS, also known as *Streptococcus pyogenes*, is a betahaemolytic, Gram-positive coccus and regarded as the most prevalent bacteria causing AT and PTA [1, 48-50, 84, 85, 107, 108] (Table 1 and Table 2). It is found in 10–75% of PTA cases [28, 84]. GAS causes PTA, as isolated bacteria, more often than other pathogens and is the most common pathogen causing PTA between ages 14 and 25 [3, 109, 110]. In AT, GAS is prevalent in younger patients, mostly in the 5- to 15-year-old age range [20]. GAS is also met in asymptomatic carriers, and the number of culture-positive individuals has been reported as between 0.4 and 11.2%, depending on age [111]. In school-aged patients with the anamnesis of recurrent tonsillitis and tonsillar hypertrophy, the number of culture-positive individuals is reported to be even higher, at 18.2% [112]. Smoking has no effect on the prevalence of GAS in PTA [46]. GAS's sensitivity to penicillin is 100% and in Finland 2–3% are resistant to macrolides [50, 113].

1.10.2 Fusobacterium necrophorum (FN)

FN is a species of rod-shaped Gram-negative anaerobic bacteria and especially associated with Lemierre's disease [28, 107]. FN is considered a pathogenic bacteria in AT and PTA (Table 1 and Table 2). Several findings support this view of FN as a significant pathogen in tonsillar infections. Klug et al. showed that FN was isolated more frequently from the tonsil core samples of PTA patients than controls [47]. In another Danish study Klug et al. showed that the majority of FN grew in pure cultures which also strengthened the theory of FN being a real pathogen in tonsillar infections [40]. In some studies, FN has not been observed in the normal microbiota of human tonsils, but in other studies FN has been detected also in the throat swabs of asymptomatic patients [61, 99, 114]. Recently several studies have reported FN also as the major pathogen in tonsillar infections and even the most prevalent pathogen in PTA, especially among patients aged 15–35 years [40, 46, 88, 91, 104, 115]. There is no difference in the prevalence of FN in PTA patients between smokers and nonsmokers [46]**.** FN is shown to predispose patients to a higher probability of renewal and it has even been speculated whether the detection of FN should be an indication of TE [40, 61].

1.10.3 Streptococcus Anginosus group (SAG)

SAG, previously called *Streptococcus Milleri* group, is a group of commensal, aerobic, Gram-positive cocci and consists of three species: *Streptococcus intermedius*, *Streptococcus anginosus* and *Streptococcus constellatus* [3, 107]. SAG is associated with head and neck infections and abscess formation [34]. Hidaka

et al. have shown an association between smoking and growth of SAG in bacterial culture [91]. SAG is also commonly observed with anaerobes in abscess pus samples [34, 91]. In older patients, especially smokers, SAG is observed more frequently than in younger, non-smoking patients. In older PTA patients, the signs of PTA are milder but the duration of symptoms as well as recovery time are longer, and comorbidities are more common. [35, 76, 87, 91].

1.10.4 Other bacteria

Streptococci groups C and G are also noted as throat pathogens in addition to GAS, FN and SAG. *Streptococci* belonging to groups C and G are repeatedly presented as some of the most numerous bacteria in bacterial cultures of AT and PTA patients (Table 1 and Table 2). In a Norwegian study, it was also shown that patients with C or G group *Streptococci* had the same clinical picture of AT as the patients with GAS. This strengthens the view that *Streptococci* groups C and G are independent pathogens in throat infections [116].

Staphylococcus aureus, *Nocardia asteroides*, *Haemophilus influenzae*, *Arcanobacterium haemolyticum* and *Streptococcus pneumoniae* have also been found monobacterial in bacterial cultures of PTA patients (Table 1 and Table 2). Which ones of these are true pathogens and which provide only a beneficial environment for pathogenic bacteria is not known. However, at least *Staphylococcus aureus* is likely the real pathogen, based on findings from abscess pus samples [3]. Besides pathogenic bacteria, tonsils are also highly colonised by normal microbiota. In bacterial cultures, as many as 67% of PTA patients' pus samples are reported to carry no pathogen growth. [58].

1.11 Periodontitis

Periodontitis is a common infection-induced inflammatory disease of the gingiva and the connective tissue surrounding the tooth. Left untreated, it leads to alveolar bone destruction. PTA patients have considerably more periodontal diseases than patients with RT, and certain bacterial species, such as *Fusobacterium spp.* and SAG, have been identified in both of these disease [3, 12, 117]. Both PTA and periodontal diseases have been speculated to have a multifactorial nature and instead of a certain pathogen, there may be a synergy of certain bacteria or other factors influencing these diseases, although the causality between these two diseases is unclear [72, 118].

1.12 Microbiology of other infections in the oral cavity

The human oral cavity, the mouth, contains more than 600 different bacteria species [28, 119, 120]. The number of anaerobic bacteria are manifold compared to aerobic bacteria in oral microbiota [28]**.** During the acute stage of pharynx inflammation in children, the number of certain bacteria (*Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis, Peptostreptococcus spp., Fusobacterium nucleatum, Prevotella spp.,* and *Porphyromonas spp.)* in saliva samples has been shown to rise from 10- to 1000-fold [121]. As PTA, oral infections are also typically polymicrobial [72, 118, 122].

1.12.1 Porphyromonas gingivalis (POGI)

Porphyromonas gingivalis (POGI)*,* a rod-shaped, Gram-negative anaerobe, is a keystone pathogen in periodontal diseases, especially in severe forms of periodontitis. It is a prominent component of oral microbiota and also colonises successfully the oral epithelium. The virulence of POGI is based on its ability to manipulate and decrease the host response to pathogens rather than induce inflammation. [72, 107, 123]. The role of POGI in aerodigestive cancers is also speculated about [123].

1.12.2 Fucobacterium nucleatum

Fusobacterium nucleatum, a rod-shaped, Gram-negative anaerobe, is an oral commensal bacterium and periodontal pathogen. It is also associated with other infections such AT, PT and PTA. [107, 124]. Smoking increases the amount of *Fusobacterium nucleatum* in the mouth, especially in patients with periodontal disease [124-126]. Brook et al. have also noticed a significant increase of *Fusobacterium nucleatum*-specific antibodies after pharyngitis [121].

1.12.3 Streptococcus mutans

Streptococcus mutans (*S. mutans*), a Gram-positive coccus*,* is a major pathogen in the formation of dental caries and is frequently isolated in caries lesions. The formation of caries is, however, a polybacterial process involving many pathogenic and non-pathogenic bacteria. [107, 127-129].

1.12.4 Streptococcus salivarius

Streptococcus salivarius is commonly isolated from the oral cavity and is reported to cause meningitis, endocarditis and bacteraemia [127] . It may show an inhibitory

effect on in vitro growth of GAS [130, 131]. Probiotic effects of *Streptococcus salivarius* in pharyngeal streptococcal and viral infections have been studied, but no difference between the control group and the treated group has been noted [132]. Other studies about probiotics in AT or PTA prevention have not been published. Probiotics such as *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium animalis subsp. lactis* BB-12 (BB-12) have been observed to decrease both the plaque index (criteria for evaluation of oral hygiene by measuring dental plaque) and gingival index (criteria for evaluation of periodontal disease based on severity and localization of lesion) without affecting microbiota [133].

1.12.5 Effects of smoking on oral microbiota

Effects of smoking on oral microbiota are controversial. In some studies smokers have had alterations in oral bacterial composition compared to nonsmokers [134, 135]. However, opposite findings have also been reported [136]. The resting whole-mouth salivary flow rate is known to have an important role in oral hygiene. An Iranian study group showed that salivary flow rate is significantly lower in smokers and this could be one mechanism explaining the higher rate of oral diseases in smokers. [137]. Klug et al. compared the bacteria of pus aspirates of PTA patients between smokers and nonsmokers, but observed no differences between groups [46]. No studies about the role of oral microbiota in PTA formation exist.

1.13 Treatment of PTA

No international clinical practice guidelines are available for the treatment of PTA [55, 138]. Needle aspiration, ID, and abscess tonsillectomy are all efficient as first line treatments [139]. Antimicrobial therapy is recommended to all patients. Antimicrobial therapy is, however, ineffective without adequate surgical drainage. [28, 36]. Outpatient drainage is most often sufficient. Extraperitonsillar spread, observed with computed tomography, and prior recurrence of AT are indications for TE. [31].

1.13.1 Prior antibiotic treatment

Before diagnosis, 21–63% of PTA patients have been treated with antibiotics [21, 33-35, 40, 45, 48, 55, 56, 85]. Prior antibiotic treatment may have an influence on the possibility of obtaining a positive culture in PTA. It can also impact which bacteria grow in the culture or the number of growing bacteria. A trend towards more frequent isolation of anaerobes in specimens in PTA patients taking prior antibiotics has been observed. [34, 40, 50]. It is also shown that if systemic antibiotics

are administered to healthy individuals, there is no measurable concentration of antibiotics in the tonsillar surface, but in patients with GAS tonsillitis, the antibiotic concentration on the surface of tonsillar tissue is measurable and doses are therapeutic [140].

There is no consensus whether prior antimicrobial treatment prevents the development of PTA. In some studies, the number of PTAs decreased after prior antimicrobial treatment [13, 63]. On the other hand, other studies have not demonstrated differences in the likelihood of having PTA between patients with and without prior antibiotics. [27, 45, 62]. In a subgroup analysis of one study (comparing ´pharyngitis´ patients to ´tonsillitis´ patients), it was speculated that prior antimicrobial treatment could be beneficial only to patients with tonsillitis, but not to patients with acute beginning of symptoms and unilateral throat pain [62].

1.13.2 Antimicrobial treatment of PTA

There are many different national guidelines about the treatment of PTA [21, 33, 34, 40, 45, 48, 55, 56, 85]. In Finland, 30% of PTA patients were initially treated with oral antibiotics, whereas the corresponding number in Sweden is 91%, in Norway 53%, and in Denmark 18% [138]. In Scandinavia, Great Britain, and Israel, the most commonly used antimicrobial agent is penicillin, with or without metronidazole. However, in Spain and New Zealand, amoxicillin/clavulanic acid is the primary antimicrobial agent of choice [21, 33, 45, 49, 138]. Clindamycin has also been used [51]. Wikstén et al. have shown that penicillin alone is as effective as the combination of penicillin and metronidazole. Metronidazole causes more diarrhoea and nausea and does not prevent renewal, so it is not recommended in adult outpatient PTA cases. [55].

1.13.3 Antimicrobial resistance

Amoxicillin/clavulanic acid, penicillin, and macrolides are the most commonly prescribed antimicrobial agents in PTA. Some causative bacteria of PTA carry a resistance to penicillin in the laboratory, but these patients are nonetheless cured with penicillin. [141]. GAS still has a 100% sensitivity to penicillin; FN is also susceptible to penicillin. Resistance of GAS to macrolides varies remarkably geographically. In Northern Ireland it has been reported to be even 26%, when in Finland it has been 2–3% in 2017. [40, 50, 113]. So penicillin should be used as a first line antimicrobial treatment because, in addition to its clinical efficacy, it has a narrow spectrum of activity and no resistance [50].

1.13.4 Steroids

Steroids might enhance recovery after PTA but there are only a few studies about the subject. Chau et al. observed a small relief of pain during the first 24 hours after corticosteroid administration. Differences, however, disappeared in 48 hours. [139, 142, 143]. In a large study Johnston et al. reported that steroids were prescribed to 9% of PTA patients, but they did not examine the effectiveness [49]. In AT, a single low corticosteroid dose provides pain relief without any adverse effects [142, 144].

1.13.5 Outpatient treatment

In Finland, 50% of adult PTA patients are treated as inpatients and 50% as outpatients, whereas in other Scandinavian countries the number of outpatients is higher.

A needle aspiration and ID are shown to be equally effective methods in managing PTA. In UK 60% of physician preferred needle aspiration primarily and 25% favoured ID [2, 139]. Needle aspiration has been criticised for removing the pus from only one area of the abscess. After needle aspiration as well as after ID, approximately 10–20% of patients get a re-collection of pus. [2, 145, 146]. ID is a bit more definitive but also more painful [2, 139]. Aspiration is also recommended to be undertaken prior to ID so that a physician can localise the abscess [49]. In a recent study it has been shown that both hospitalization time and the need for reopening the abscess are decreased in patients with primary ID.

In Scandinavia, 80% of physicians routinely reopen the abscess cavity daily or every other day after ID [138]. In contrast, in New Zealand reopening is performed in 24% of cases after clinical examination and estimation of the need for reopening [49]. Reopening of the abscess cavity, use of broad-spectrum microbial agents or being an outpatient or inpatient has no effect on the probability of TE in the future. 25% of PTA patients undergo TE during the next 5 years. Young age and earlier tonsillar infections increase the risk of later TE. [56].

1.13.6 Tonsillectomy (TE)

In children aged 0 to 14 years, the primary treatment of PTA is nearly always abscess TE. This is due to the fact that procedures under local anaesthesia are not possible because of poor cooperation. In adults, abscess TE is quite rare in most of countries and in-office procedures are preferred.

Abscess TE is shown to be a safe procedure. It requires only one recovery period compared to TE after recovery time which requires two recovery periods. Therefore, abscess TE (TE performed in an acute phase of PTA) reduces the overall need of recovery time and sick leave [2, 139].

TE includes a risk of severe, potentially fatal complications, such as postoperative haemorrhage and dehydration. Patients with comorbidities, prior PTA, and several antibiotic prescriptions during the past few years are at a higher risk of developing complications. Thus, management of TE should be preceded by solid indications [2, 139, 147, 148]. When targeted to the right patients, TE appears to save costs and has a curative effect on a patient's quality of life. [147].

2   AIMS OF THE STUDY

The aim of this study was to clarify the aetiology of PTA and the role of minor salivary glands in the controversial aetiology of PTA.

The specific aims were:

1. Identify bacteria and clinical factors which might potentially promote the renewal of PTA. (Study I)

2. Histologically analyse and compare peritonsillar tissue of AT, CT and PTA patients and verify the presence of minor salivary glands and possible signs of infection. (Study II)

3. Find out whether the serum amylase levels are elevated in PTA patients, which could prove the role of minor salivary glands in PTA. (Study III)

4. Find out whether the serum amylase levels could be used in differential diagnostics of PTA. (Study III)

5. Find out whether symptoms, clinical findings and serum CRP levels in AT and PTA patients are linked to a specific bacterial aetiology. (Study III)

6. Explore whether AT, PT, and PTA are associated with changes in oral microbiota (Study IV)

7. Explore whether smoking, alcohol consumption and oral hygiene have an effect on saliva bacteria of PTA patients. (Study IV)

3   MATERIALS AND METHODS

All four studies in the current thesis included patients. All patients had visited the Department of ORL - Head and Neck Surgery, Helsinki University Hospital, Finland.

3.1   Ethical consideration (studies I-IV)

The Ethics Committee of Helsinki and Uusimaa Hospital District approved all four studies (I: 224/13/03/02/2009, II: 51/13/03/02/2016, III and IV: HUS/1760/2016). All patients and healthy volunteers gave their informed written consent [149]. Patient recruitment and data processing was in accordance with Helsinki Declaration and Good Clinical Practice [150].

Table 3. The study characteristics of Studies I-IV

	Study I	Study II	Study III	Study IV
Prospective study	X	X	X	X
Time	2010-2011	2012, 2015	2012, 2015, 2017	2017-2018
Number of patients (n)	180	278	200	200
Bacteria samples	X [b]		X [a]	X [a]
Blood samples			X [x]	X [x]
Saliva samples				X [c]
Histology samples		X		
Prospective clinical status			X	X
Questionnaire	X	X	X	X
Patient charts evaluated	X	X	X	X [y]
Earlier publications	[55, 151]	[149]	[149] [z]	

[a] bacterial culture
[b] microarray
[c] Flow cytometry
[x] Serum C-Reactive protein (CRP) , Serum amylase (S-Amyl)
[y] only patient group
[z] only reference group data

3.2 Study I

A total of 180 consecutive patients suffering from PTA were prospectively recruited in the emergency department of ORL at HUH between February 2010 and April 2011. 18 of 180 patients had a renewal of PTA (renewal group). Patients without renewal formed the recovery group (n=162). There is one publication earlier from the same material. In that earlier study the aim was to investigate whether combining metronidazole and penicillin improves recovery from PTA and whether metronidazole prevents PTA recurrences. [55].

Inclusion criteria of the Study I were diagnosed PTA and age over 18. Exclusion criteria were allergy to penicillin or metronidazole, use of metronidazole during the previous month, pregnancy, breastfeeding, renal or liver insufficiency, alcohol abuse, participation in another clinical trial at the time, patient's condition requiring in-patient care, TE scheduled within the next 30 days, or performing military service. All patients had given their informed written consent when participating in the study. All patients answered a questionnaire concerning smoking habits, duration of symptoms before admission, and prior antibiotic treatment. All patients' medical records were analysed and all symptoms of renewal (soreness of throat, swallowing problems, mouth opening difficulties, fever, general condition) and hospital visits during the prior two months were reviewed.

All patients were treated with ID. Before ID the peritonsillar tissue was anaesthetised and pus was aspirated with a 5 ml syringe and a long 25-gauge needle. The bacterial samples of aspirated pus were collected and analysed in a laboratory by using a microarray. The microarray method is described accurately in two earlier publications [55, 151]. Patients were randomised to get either V penicillin 1 million IU x3x10 and metronidazole 400mg x3x7 or V penicillin 1 million IU x3x10 and placebo. Routine laboratory tests, such as total blood count, CRP or radiological imaging, were not completed.

Statistical calculations were performed by NCSS (Kaysville, Utah, USA) 2009 statistical analysis software using the Chi-Square, Fisher´s exact and Mann-Whitney U-tests. *P* values <0.05 were considered significant.

3.3 Study II

In this prospective study, tonsil samples of 278 patients who had undergone TE at the ORL clinic of HUH in 2012 and 2015 were collected and evaluated. In all these 278 patients tonsillectomies were indicated because of the diagnosis of recurrent AT, CT, or PTA, and the tonsils were collected in the first place for a human papilloma virus study. The aim of the original study was to analyse the prevalence of high-risk HPV and the genetic alterations in non-malignant tonsils, such as

tonsillitis [149]. 124 tonsil samples were selected for the current study (Study II) from the original 278 patients. Selection was made so that all PTA patients fulfilling the inclusion criteria were included. For the two control groups (AT and CT), we selected all patients with recurrent AT and CT in the same age range as PTA patients (18.47–69.48). Hence, we got three similar groups with the same size and age range. Patients under 18, previous tonsillotomy, and insufficient amount of tonsil tissue were excluded (Figure 5). We examined all patients' medical records from the hospital's data management system. All patients filled in a questionnaire about their general overall health, medications, and smoking habits. TE was typically performed approximately six months (mean 6.25 months, median 6 months, range 1–13 months) after the diagnosis and decision to have TE. During TE, both tonsils were resected but only one tonsil was collected as a specimen for the study. Right after TE, the tonsils were fixed in formalin, cut in 2–4 sections depending on tonsil´s size and embedded in paraffin. 4µm slides were cut from paraffin embedded tissue blocks and stained with haematoxylin and eosin, for histological examination. Histological examination was performed by two independent physicians. A total of 114 tonsils were available for analysis after the former process. We analysed and prepared 2 to 4 slides per patient. All slides were examined for the presence of minor salivary glands and the location of the minor salivary glands. We also analysed and reported inflammation, degenerative and fibrotic changes of minor salivary glands, as well as histological changes in the salivary ductus. Inflammation was histologically defined as leukocyte infiltration into minor salivary glands and divided into periductal and acinar inflammation, depending on the specific location of leukocytes. The experienced pathologist analysed the number of leukocytes varying from no leucocytes to plenty of leukocytes. Statistical calculations were performed by NCSS 8 (Hintze, J. PASS 11. NCSS, LLC. Kaysville, Utah; 2011. www. ncss.com. Accessed September 21, 2012)) 2012 statistical analysis software using the Chi-Square test. *P* values <0.05 were considered significant.
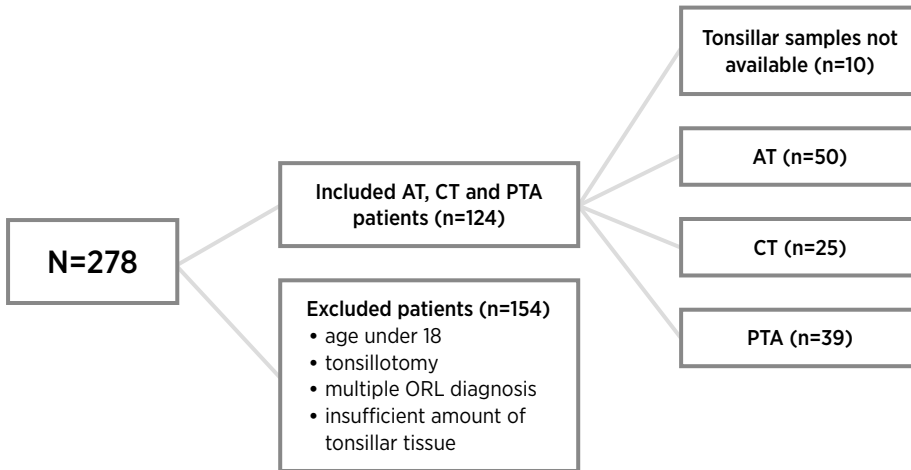
Figure 5. Flow chart study II.

AT, acute tonsillitis; CT, chronic tonsillitis; PTA, peritonsillar abscess

3.4 Study III

The material consisted of two groups of prospectively collected patients: the acute group (n=112) and the reference group (n=98). In the acute group, the patients were suffering from throat pain and were referred to the ORL emergency department at HUH between February and October in 2017. The reference group consisted of patients who were scheduled for TE due to AT (n=55) or PTA (n=43) at the ORL clinic of HUH during 2012 and 2015. Patients in the reference group were originally recruited for another study (human papillomavirus study) [149]. Exclusion criteria in the acute group were age under 15 and a known pancreas disease. In the reference group, exclusion criteria were age under 18 and pancreas disease. Patients in both groups were divided into two subgroups based on clinical diagnosis: AT and PTA (Figure 6). Serum CRP and amylase levels were analysed in all patients. In the reference group the blood samples were collected approximately 7 months (mean [±SD]; 7.18 [5.6] months) after the acute infection. All patients filled out a questionnaire about their smoking habits and overall health. Patients in the acute group also answered questions about their current disease, prior antibiotics, smoking habits, alcohol consumption, and earlier tonsillar or peritonsillar infections. The treating physician filled out a questionnaire concerning the acute group patients' oral hygiene (good/poor) and tonsillar findings (tonsillar erythema/tonsillar exudates/no findings/tonsillar erythema and exudates). In addition, in the acute group, PTA patients were treated with ID. Pus aspirate samples of the acute group PTA patients and throat swabs collected from the acute

group AT patients were sent to the laboratory for bacterial culture. Local laboratory Helsinki University Hospital Laboratory Services (HUSLAB) grew pus samples under both aerobic and anaerobic conditions and throat swab cultures from AT patients under only aerobic conditions. Both cultures were grown according to the laboratory´s standard methods for diagnostic samples. Correlations between smoking, alcohol consumption, signs and symptoms of infection, laboratory tests and bacterial samples were analysed. For statistical analysis, bacteria findings were first divided into five groups: 1) *Streptococcus betahaemolytica* (GAS, group B, C or G *Streptococci*) 2) SAG, 3) FN 4) FN + SAG and 5) other bacteria (Included: *Haemophilus influenzae,* mixed oral flora, *Fusobacterium nucleatum, Staphylococcus aureus, Prevotella spp., Neisseria meningitides,* other aerobes, other anaerobes). FN and FN+ SAG groups were further combined for statistical analysis because of the small number of patients in the groups. The acute and reference groups were first analysed separately and then compared. Calculations were performed by NCSS 8 statistical software (Hintze, J. PASS 11. NCSS, LLC. Kaysville, Utah; 2011. www. ncss.com. Accessed September 21, 2012). Numerical variables were analysed with the Mann-Whitney U-test and Kruskal-Wallis one-way ANOVA. The chi-square test was applied to compare nominal variables. Spearman rank correlation was applied to two numerical variables with non-normal distribution. Logarithmic transformation was applied and Pearson correlation with confidence intervals was calculated to compare correlation coefficients between groups. *P* values <0.05 were considered to be statistically significant.
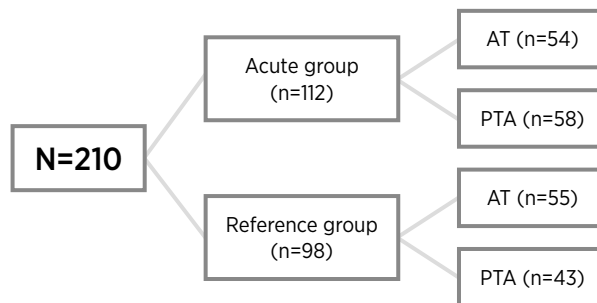

Figure 6. Flow chart of study III.

AT, acute tonsillitis; PTA, peritonsillar abscess

3.5 Study IV

The material consisted of two groups of prospectively collected patients: the patient group (n=148) and heathy volunteers (n=52). In the patient group, patients having throat pain and fulfilling the inclusion criteria were recruited and evaluated at the emergency department of ORL of HUH between February and October in 2017. Healthy volunteers were recruited between March and April in 2018 from students and hospital staff. Exclusion criteria of patient group were age under 15 and a known pancreas disease. In healthy volunteers, exclusion criteria were acute throat pain, acute or chronic tonsillar infection, recent (during previous two months) antibiotic treatment, and age under 15. Participants in the patient group were divided into three groups based on clinical diagnosis: AT, PT and PTA.

The patients received paraffin tablets to stimulate saliva secretion and the saliva was collected in 2 minutes. The analysis of salivary saples were performed by GutGuide (https://gutguide.com). The amount of 2.5 ml saliva was used for bacterial analysis. Concentrations of bacteria were analysed according to a previously published method with minor modifications [152]. Briefly, to isolate bacteria, saliva was aliquoted in 1.5 ml Eppendorf tubes and centrifuged at 13,000 rpm for 6 minutes at room temperature. After that, the supernatant was discarded, and pellets were suspended in 200 µl of phosphate-buffered saline (PBS). Thereafter, we combined and centrifuged the suspensions. Supernatant was removed and the pellet was suspended in 1000 µl of 4% paraformaldehyde in PBS (w/v) to fix the bacteria. Glass beads were added to the bacterial suspensions and samples were combined and incubated on a shaker at 4°C overnight. To prepare bacterial stocks, samples were centrifuged at 13,000 rpm for 10 min at RT. After that, supernatant was removed, and pellets were washed twice with PBS. At the end, the bacterial pellet was suspended in 200 µl PBS and an equal volume of 94% ethanol. The samples were kept at -20°C until analysis.

Flow cytometry (FCM; BD FACSCalibur) was used to analyse the total bacteria concentration. To analyse the total amount of bacteria, 4 µl of bacterial stock was diluted in 4 ml of PBS and samples were vortexed and sonicated. After that 300 µl of bacterial suspension was mixed with 3 µl of Sytox Orange (Molecular Probes, Eugene, Oregon) DNA stain (Ex/Em 547/570 nm) for FCM analysis to separate bacteria from nonbacterial material.

To analyse the bacterial species-specific FCM, 15 µl of bacterial stock sample was hybridised at least 12 hours with 16S rRNA-targeted CY5 indocarbocyanine (Ex/Em 646/662 nm Molecular Probes) -labelled oligonucleotide probes against 1. *Porphyromonas gingivalis* [153] 2. *Fusobacterium nucleatum* (FN) [154] 3. *Streptococcus mutans* (*S. Mutans*) [154] 4. Narrow Streptococcus probe (NSP; *Streptococcus anginosus* (SAG), *Streptococcus oralis* and *Streptococcus mitis)* [155] and 5. Broad Streptococcus probe (BSP) [156] (Figure 7). Before FCM, 4

μl of Sytox Orange was added to each sample. The Ribosomal Database Project database (http://rdp.cme.msu.edu/html) was used to analyse the sequence match of the probes with the target species.

Concentrations of the bacteria were analysed using TrueCount® tubes (Beckton Dickinson, San Jose, CA) containing an exact number of fluorescent microbeads. 300 μl of each sample was added to a TrueCount® tube and FCM analysis was performed until 2% of the microbeads had been observed. Bacterial samples hybridised with Cy5-labelled probes were analysed using the FL4 detector (661/16 nm). Then the samples were stained with Sytox Orange DNA stain by FL2 detector (585/42 nm). Two parallel samples from each bacterial stock sample were run. The mean, standard deviation and coefficient of variation of bacterial concentrations were measured.

From the patient group, serum CRP and amylase levels were analysed in HUSLAB with an accredited, standard method for analysing serum CRP and S-Amyl levels. The blood samples of the patient group were analysed from fresh blood while the blood samples of the reference group were analysed from frozen serum samples. All PTA patients were treated with ID. Pus aspirate samples of the PTA patients as well as throat cultures from the AT and PT patients were sent to the laboratory for bacterial culture. A local laboratory (HUSLAB) tested both aerobic and anaerobic bacteria from pus samples and aerobic bacteria from throat swabs from AT and PT patients. Both cultures were grown according to the laboratory´s standard methods for diagnostic samples. The patient group filled out a questionnaire about their smoking habits, current disease, prior antibiotics and overall health, alcohol consumption, and earlier tonsillar or peritonsillar infections. The treating physician filled out a questionnaire concerning the patient group participants' oral health (good/poor) and tonsillar finding. Oral health was evaluated by inspection. Healthy volunteers answered questions about recent throat infections, recent antibiotics during the past two months, smoking, oral hygiene (self-evaluated), and alcohol consumption. Alcohol consumption was considered alcohol abuse if the patient reported being intoxicated at least five times in one month. The limit was relatively high compared to Finnish Current Care Guidelines [157].

Correlation between smoking, alcohol consumption, laboratory tests, pus or superficial bacterial samples, and salivary bacterial samples were analysed. Calculations were performed by NCSS 8 statistical software (Hintze, J. (2012). NCSS 8. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com). Amounts of total salivary bacteria were divided with 10^6 for statistical reasons. Log transformation was used on a variable to make data conform more closely to the normal distribution. Numerical variables were analysed with a Mann-Whitney U-test and Kruskal-Wallis one-way ANOVA. A chi-square test was used to compare nominal variables. *P* values <0.05 were considered to be statistically significant.

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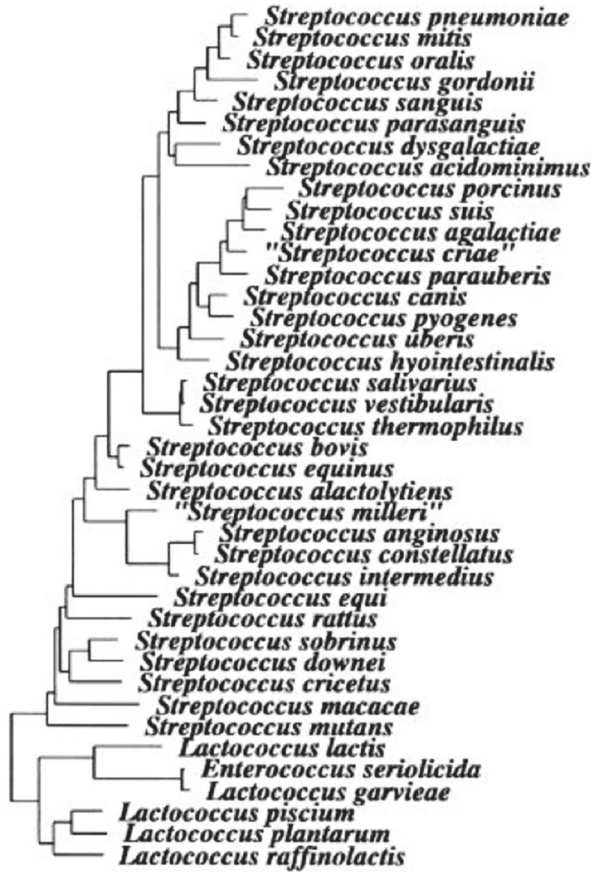
Figure 7. The target group of the Broad Streptococcus probe. The figure (chart B) represents the bacteria species observed with Broad Streptococcus probe [156].

4   RESULTS

4.1   Demographics (Studies I-IV)

4.1.1   Study I

Characteristics of patients in Study I are presented in Table 4.

Table 4. Characteristics of peritonsillar abscess (PTA) patients in renewal group and recovery group in Study I.

Characteristics	Study I Renewal group (n=18)	Study I Recovery group (n=162)
Age, years Mean	34.8	34.2
Male, n(%) Female	13 (72) 5 (28)	85 (52) 77 (48)
Smoker, n(%)	7 (39)	65 (40)
Prior antibiotics n(%) Yes	7 (39)	48 (30)
Duration (days) of symptoms prior PTA, n(%) 1-4 days >4 days	9 (50) 9 (50)	47 (29) 115 (71)

PTA, peritonsillar abscess

4.1.2 Study II and Study III

Characteristics of patients in Studies II and III are presented in Table 5. In Study III 3.4% (2/58) of PTAs were bilateral and none of the patients had a complication during the first three months.

Table 5. Characteristics of patients with acute tonsillitis (AT), chronic tonsillitis (CT) and peritonsillar abscess (PTA) in Studies II and III.

Characteristics	Study II AT patients (n=50)	Study II CT patients (n=25)	Study II PTA patients (n=39)	Study III Acute group PTA patients (n=58)	Study III Acute group AT patients (n=54)	Study III Reference group PTA patients (n=43)	Study III Reference group AT patients (n=55)
Age, years							
Median	26.03	32.19	23.97	36	28.5	36	28.5
Range	19-50	20-49	18-69	16-65	15-86	18.5-69.5	18.7-60.3
Male, n(%)	35 (70)	5 (20)	16 (41)	36 (62)	21 (39)	18 (42)	17 (31)
Female	15 (30)	20 (80)	23 (59)	22 (38)	33 (61)	25 (58)	38 (69)
Smoker, n(%)	20 (40)	7 (28)	16 (41)	24 (42.1)	22 (40.7)	20 (46.5)	21(38.2)
Ex-smoker	5 (10)	3 (12)	12 (31)	12 (21)	12 (22)	12 (28)	5(9)
No information				1			
Alcohol consumption, n(%)							
Yes	NA	NA	NA	36 (62)	31 (57)	NA	NA
No				21 (36)	23 (43)		
No information				1 (2)			
Prior antibiotics more than 24 hour, n (%)							
Yes	NA	NA	NA	10 (17)	9 (17)	NA	NA
No				48 (83)	45 (83)		
No information							
Duration (days) of symptoms prior PTA, n (%)							
days	NA	NA	8[1]/14[2]	16 (28)	28 (52)	NA	NA
>3 days			25[1]/10[2]	42 (72)	26 (48)		
No information			6[1]/15[2]				
Unilateral throat pain, n(%)							
Yes	NA	NA	13[1]/19[2]	54 (93)	31 (61)	NA	NA
No			17[1]/6[2]	4 (7)	20 (39)		
No information			9[1]/14[2]		3		

Characteristics	Study II AT patients (n=50)	Study II CT patients (n=25)	Study II PTA patients (n=39)	Study III Acute group PTA patients (n=58)	Study III Acute group AT patients (n=54)	Study III Reference group PTA patients (n=43)	Study III Reference group AT patients (n=55)
Fever, n(%)							
Yes	NA	NA	NA	38 (66)	41 (76)	NA	NA
No				20 (35)	13 (24)		
Common cold symptoms, n(%)							
Yes	NA	NA	NA	16 (28)	23 (44)	NA	NA
No				42 (72)	29 (56)		
No information					2		
Earlier tonsillar infections, n(%)			*)				
AT	50 (100)	0	14(36)	13 (23)	12 (22)	NA	NA
CT	0	25(100)	6 (15)	1 (3)	1 (3)		
PTA	0	0	25(64)	8 (14)	3 (6)		
None	0	0	3 (8)	35 (61)	38 (70)		
No information			2 (5)	1			
Tonsillar findings, n(%)							
Exudates	NA	NA	NA	16 (28)	6 (30)	NA	NA
Erythema				26 (46)	16 (30)		
Both				7 (12)	21 (40)		
None				8 (14)	0 (0)		
No information				1			
Oral hygiene, n(%)							
Good	NA	NA	NA	52 (91)	48 (91)	NA	NA
Poor				5 (9)	5 (9)		
No information				1	1		

PTA, peritonsillar abscess; AT, acute tonsillitis; CT, chronic tonsillitis; NA, not available

[1] patient´s first PTA episode
[2] patient´s second PTA episode
*) some patients had all AT, CT and PTA in clinical history

4.1.3   Study IV

Characteristics of patients of Study IV are presented in Table 5.

Table 6. Characteristics of patients with AT (n=54), PT (n=36), PTA (n=58) and healthy volunteers (n=52)

Characteristics	AT (n=54)	PT (n=36)	PTA (n=58)	Healthy volunteers (n=52)
Age Mean[±SD] Median Range	30.5 [±12.0] 28.5 15-86	36.2 [±18.3] 28.5 17-85	35.7 [±12.6] 36 16-65	37.4 [±11.0] 25.8 19-67
Gender, n (%) Male Female	21 (38.9) 33 (61.1)	18 (50.0) 18 (50.0)	36 (62.1) 22 (37.9)	19 (36.5) 33 (63.5)
Smoking, n (%) Yes No Ex-Smoker	22 (40.7) 20 (37.0) 12 (22.2)	6 (17.1) 21 (60.0) 8 (22.9)	24 (42.1) 21 (36.8) 12 (21.1)	0 (0) 52(100.0) 0 (0)
Alcohol abuse, 5 or more times a month intoxicated n (%)	4 (7.4)	1 (3.4)	5 (8.6)	0 (0)
Prior antibiotics for more than 24 hours, n (%)	9(16.7)	7(20.6)	10(17.2)	No prior antibiotics
Oral hygiene, n (%) Good Poor No information	48 (90.6) 5 (9.4) 1	32 (94.1) 2 (5.9) 2	51 (91.1) 5 (8.9) 2	52 (100.0) 0

PTA, peritonsillar abscess; AT, acute tonsillitis; CT, chronic tonsillitis.

4.2 Bacterial findings (Studies I and III)

Bacteria samples were taken in Studies I and III. In Study I, analysis on aspirated pus of PTA patients was performed by the microarray assay. In Study III, aspirated pus of PTA patients as well as throat swabs of AT and PT patients were analysed by bacterial culture. The identified bacterial species are presented in Table 7. In Study I, in 20 samples two different bacterial species were detected. In Study III, according to HUSLAB protocol, clinically significant bacterial findings were reported by the laboratory; other findings were reported as normal flora, mixed aerobes, and mixed anaerobes.

Table 7. Bacterial findings in Study I and Study III.

Bacteria	Study I PTA (n=180) n (Number of specimens with another species)	Study III PTA(n=57)	Study III AT (n=54)
Group A streptococci	40 (4)	13	22
Group C or G streptococci		1	2
Streptococcus anginosus	12 (10)	9	
Fusobacterium necrophorum	44 (5)	3	
Haemophilus influenzae	14 (8)		2
Fusobacterium nucleatum		1	
Staphylococcus aureus	4 (1)	2	
Prevotella spp.	30 (10)	2	
Neisseria spp.	2	1	
Coagulase negative staphylococci	4		
Streptococcus viridans group	6 (1)		
Streptococcus pneumoniae	4		
Aggregatibacter aphrophilus	2		
Peptostreptococcus spp.	2		
Streptococcus dysgalactiae subsp. *equisimilis*	2 (1)		
Bacteroides fragilis group	1		
Enterobacter cloacae	1		
Lactobacillus delbrueckii subsp. *bulgaricus*	1		
Streptococcus anginosus and Fusobacterium necrophorum		3	
Haemophilus influenzae and *Fusobacterium nucleatum*		1	
Mixed aerobes		1	
Mixed anaerobes		7	
Mixed oral flora		12	28
No samples analysed	11	1	

PTA, peritonsillar abscess; AT, acute tonsillitis

4.2.1   Study I

18 (10%) of PTA patients (renewal group) had a renewal episode during the follow-up period (2 months). None of the patients in the renewal group had GAS as the only finding, whereas in the recovery group, this was the case in 40 patients (p =0.014). SAG was observed in 5/18 (28%) of the renewal group's bacterial samples whereas in patients without a renewal (recovery group), SAG was found in 7/162 (4%) of the bacterial samples (p=0.003). In addition, 75% of patients under the age of 25 had FN as a pathogen. There was no difference in age (p=0.722), smoking habits (p=0.837), or gender (p=0.110) between the groups. 72% of the renewal group were men, when in the recovery group the number was 52%. Patients over the age of 40 comprised 39% of the renewal groups and 31% of the recovery group. All patients with SAG were nonsmokers. None of the renewals occurred during the summer months (June–August). Specific characteristics of the renewal group are presented in Table 8.

Table 8. Characteristics of the renewal group (n=18)

Bacteria	Gender	Age (years)	Renewal (days)[a]
FN	F	18	1
FN	F	19	14
FN	M	20	15
FN	M	21	15
FN	M	22	17
FN	M	22	14
FN	F	25	2
FN	M	46	1
FN	M	64	4
SAG + PO	M	24	42
SAG + PO	M	40	14
SAG + PO	M	40	2
SAG + PO	M	47	1
SAG + PO	M	57	1
Other	F	21	19
Other	F	31	5
Other	M	53	6
Other	M	60	1

FN, *Fusobacterium necrophorum*; SAG, *Streptococcus anginosus* group; O, *Prevotella oris;* M, male; F, female

[a] Renewal is presented as number of day between primary episode and renewal of peritonsillar abscess.

In subgroup analysis, we observed that in patients over 40 years the renewal happens after a shorter period compared to younger patients with a PTA renewal (p= 0.013). All these older patients with a quick renewal were male when in younger group only half of the patients were male (p=0.036). In the group of older patients, SAG was detected as a dominant pathogen in 50% of samples (4/8), when in the group of younger patients only 1/10 had SAG as a major bacterium (p=0.118).

4.2.2 Study III

For the statistical analysis bacteria findings were divided into five groups: 1) *Streptococcus betahaemolytica* (GAS, group B, C or G *streptococci*) 2) SAG, 3) FN, 4) FN + SAG and 5) other bacteria (Included: *Haemophilus influenzae,* mixed oral flora, *Fusobacterium nucleatum, Staphylococcus aureus, Prevotella spp., Neisseria meningitides,* other aerobe, other anerobes; see Table 9). To get reliable statistical analysis, FN and FN+SAG had to be combined. Bacterial samples were taken only from the acute group (n=112) and the comparison was performed between AT and PTA patients.

Table 9. Bacterial findings in patients with AT or PTA.

Bacteria n (%)		AT[a] (n=54)	PTA (n=56)
Betahaemolytic streptococci		24 (44.4)	16 (22.2)
	Group A *streptococci*	22 (40.7)	13 (18.1)
	Group B, C or G *streptococci*	2 (3.7)	3 (4.2)
Streptococcus anginosus group		0 (0)	11 (15.3)
Haemophilus influenzae		2 (3.7)	1 (1.4)
Neisseria meningitidis		0 (0)	1 (1.4)
Staphylococcus aureus		0 (0)	3 (4.2)
Other aerobic bacteria		0 (0)	0 (0)
Fusobacterium necrophorum			5 (6.9)
Other anaerobic bacteria[b]			22 (30.6)
Mixed regional microbiota		28 (51.9)	13 (18.1)

AT, acute tonsillitis; PTA, peritonsillar abscess

[a] Only aerobic culture was done from patients with acute tonsillitis (AT).

[b] Other anaerobic bacteria isolated: anaerobic Gram-negative rods (n=14), *Prevotella* species (n=2), *Fusobacterium* species other than necrophorum (n=4), anaerobic mixed flora (n=2)

In AT or PTA group, there were no differences in S-Amyl and CRP levels between bacterial groups (AT: p= 0.620 and p=0.331; PTA: p=0.925 and p= 0.203).

Among PTA patients who were on antimicrobial treatment before the diagnosis, SAG was significantly more frequent than other bacteria (p= 0.0374). AT patients with betahaemolytic *Streptococci* (mainly GAS) in the bacterial culture had a shorter duration of symptoms before contacting the hospital, compared to patients with other bacteria in culture (p=0.0125). In the PTA group, no association between bacteria and duration of symptoms was noted (p=0.628). We did not observe an association between certain bacteria and patients' symptoms (unilaterality, lack of common cold, fever) either in the AT or PTA group (AT p=0.472, p=0.123 and p=0.618; PTA p=0.232, p=0.891, p=0.731). Neither the history of tonsillar infections (AT p=0.484 and PTA p=0.816) nor tonsillar findings (AT p=0.653 and PTA p=0.057), had an association with bacteria findings. In AT or PTA groups oral hygiene had no impact on bacterial findings (p= 0.803 and p=0.420).

In a subgroup analysis, SAG was found in significantly older patients (mean age 43.4 years) than bacteria from other bacterial groups (betahaemolytic *Streptococci* 31.8 years, other bacteria 33.6 years, combined FN and FN +SAG 26.0 years, p= 0.0219). Seven out of nine (77.8%) patients with SAG were male.

4.3 Pathologic-anatomic diagnosis (PAD) (Study II)

Tonsil samples of 114 patients were histologically analysed in Study II. Patients were divided into three groups based on indication of TE: i.e. AT, CT and PTA. Histological findings were compared between the groups.

4.3.1 Study II

In Study II we identified histologically minor salivary glands from 67.5% (77/114) of the tonsil samples. The majority of the minor salivary glands were located superficially near the tonsil, but they were also observed deeper, inside the muscle tissue in 17.5% (20/114) of tonsil samples. Between the AT, CT, and, PTA groups, there was no difference in the presence of minor salivary glands (p=0.372). Infection of minor salivary glands was observed in 94.8% (n=73) superficially located glands but in only 15% (n=3) of the glands that were located deeper, inside the muscle tissue (Figure 8).

Muscle tissue   Minor salivary gland
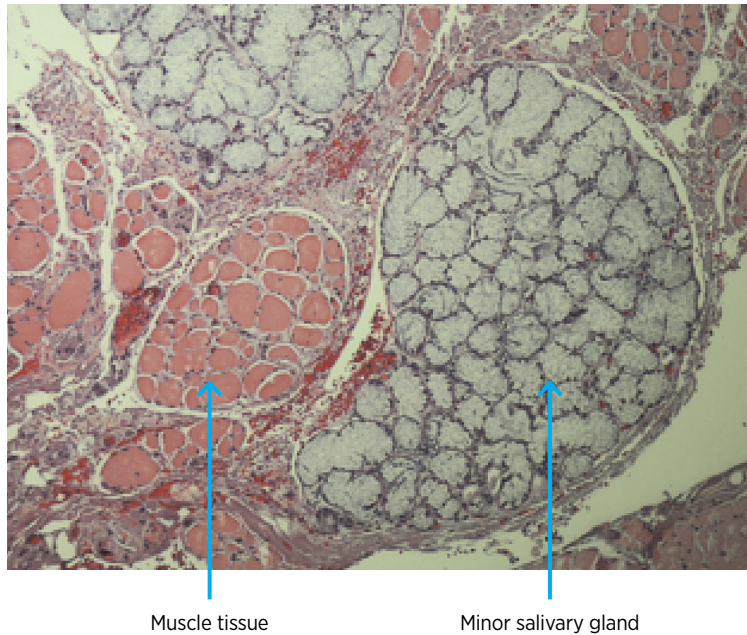
Figure 8. The deeper-located minor salivary gland without any signs of inflammation inside the muscle tissue. Photo: Sanmark/Hagström.

Periductal inflammation, i.e. intense invasion of leukocytes around the ductus draining from the minor salivary gland, was demonstrated in 82.1% of the PTA patients´ samples, 57.1% of AT patients´ samples, and 42.9% of CT patients´ samples (p=0.011) (Figure 9).

The amount of acinar inflammation, i.e. invasion of leukocytes between the clusters of gland cells, did not differ between the groups (PTA 50%, AT 60%, and CT 57.1%) (p=0.725). The number of previous PTAs had no effect on the presence of minor salivary glands (p=0.970) or periductal inflammation (p=0.211).

Only one tonsil was analysed from every patient and in 21/39 of the patients, the analysed tonsil was from the same side as the previous abscess. There was no difference in the presence of salivary glands (p=0.170) or inflammation signs (p=0.413) between the tonsils from the abscess side and from the non-abscess side.

Minor salivary gland

Ductus of the minor salivary gland

Periductal inflammation (invasion of leukocytes)
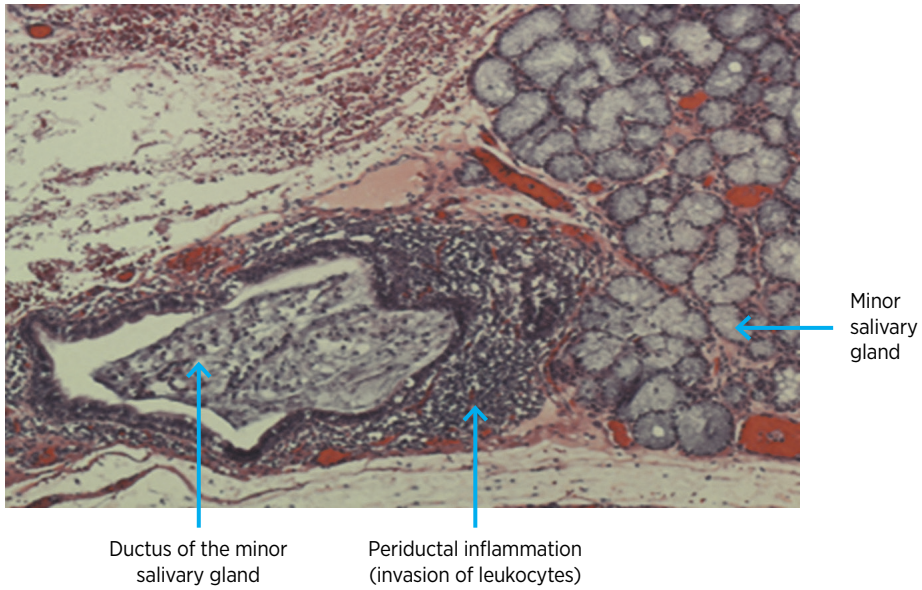
Figure 9. Periductal inflammation (blue dots) around the ductus of the minor salivary gland. Acinar inflammation is seen on the right side of the picture. Photo: Sanmark/Hagström.

Fibrosis was evident in 20 specimens and degeneration in 8 specimens. In 62 (54.5%) specimens, actinomyces-like bacteria were observed. The presence of actinomyces-like bacteria did not differ between AT, CT, and PTA patients (p=0.220) (Figure 10).

Tonsillar tissue

Actinomyces-like bacteria

The tonsillar crypt
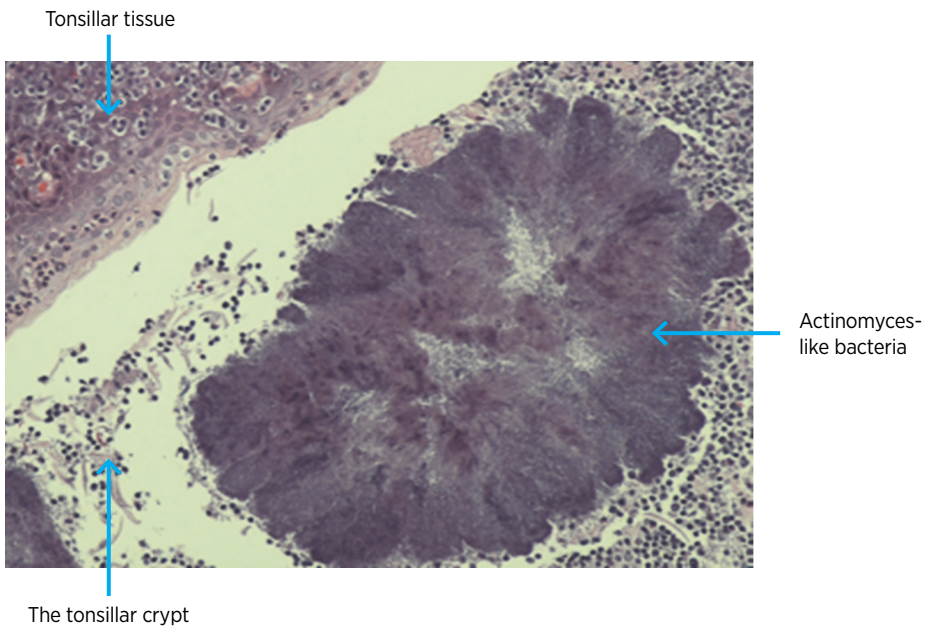
Figure 10. Actinomyces-like bacteria in tonsillar crypt. Photo: Sanmark/Hagström.

Patients' smoking habits were not associated with the presence of superficial or deeper salivary glands (p=0.964 and p=0.171), periductal or acinar inflammation of salivary glands (p=0.758, p=0.115), nor the presence of actinomyces-like bacteria (p=0.988).

4.4 Laboratory results (Study III and IV)

Laboratory tests, more specifically S-Amyl and CRP, were analysed from all patients in Studies III and IV. In Study III the blood samples were taken during acute infection in the acute group and in the post-infectious stage in the reference group. Signs and symptoms of infections were recorded and evaluated only from the acute group, so analyses of the association between the signs and symptoms of the infection, and S-Amyl and CRP levels were performed only in this group.

4.4.1 Correlation of serum amylase (S-Amyl) and CRP in the acute and reference group

In the acute group, a significant negative correlation was noted between S-Amyl and CRP levels in both AT and PTA patients (AT r=-0.3475, PTA r= -0.353) (Figure 11).

Figure 11. Correlation between serum amylase (Amyl) and CRP levels in patients with a) acute tonsillitis (r=-0.519) and b) peritonsillar abscess (r=-0.353).

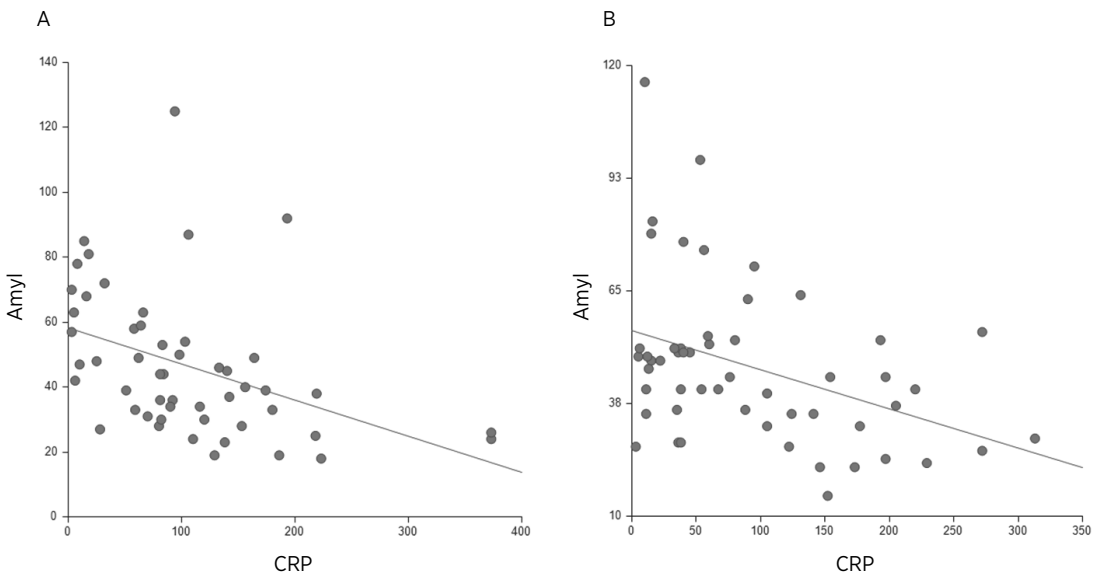In the reference group, PTA patients had a slightly negative correlation (r= -0.160) and in AT patients, a positive correlation (r=0.125) but these correlations were not significant. There was a significant difference in correlations between the acute and reference groups (acute group r= 0.4248 CI [-0.2516, -0.5688], reference group r=0.0112, CI [-0.1868, 0.2082]) (Figure 12).
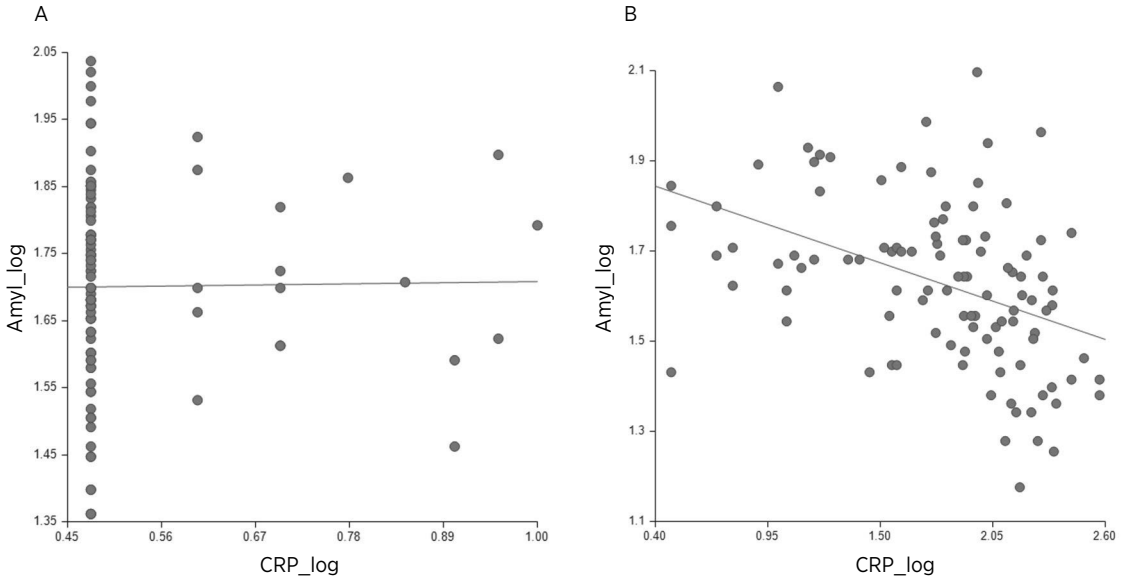
Figure 12. Correlations between serum amylase (Amyl) and CRP in reference group (a) and in prospective group (b).

S-Amyl and CRP are changed to logarithm numbers so that they are distributed normally before calculating correlations.

In the acute group neither alcohol consumption nor smoking habits had any effect on S-Amyl levels in AT or PTA patients (p=0.750, p=0.205). In the reference group patients, smoking habits had no influence on S-Amyl levels either (p=0.625). S-Amyl was significantly lower in AT (median 41 U/l, range 15–125 U/l) and PTA (median 44 U/l, range 15–116 U/l) patients of the acute group compared to the AT (median 50 U/l, range 23–105 U/l) and PTA (53 U/l, range 28–109 U/l) patients in the reference group. Differences were significant in both groups AT (p=0.017) and PTA (p=0.026). CRP level in the acute group both in AT (median 87 mg/l, range 3-373 mg/l) and PTA (median 60mg/l, range 3-313 mg/l) patients was significantly higher than in the reference group's AT (median 3mg/l, range 3-10 mg/l) and PTA (median 3mg/l, range 3-9 mg/l) patients. Differences were significant in the AT group (p<0.0001) and the PTA group (p<0.0001). In the acute group, there were no differences in S-Amyl or CRP levels between AT and PTA groups (p=0.767 and p=0.501).

4.4.2 Association of laboratory test results with signs and symptoms

AT patients without fever had a higher S-Amyl and lower CRP value than patients with fever (65.5 U/l vs 37.5 U/l, p=0.0121 and 15mg/l vs 104.5mg/l, p=0.00037). In the PTA group, there was no difference in S-Amyl or CRP levels between the febrile and non-febrile patients (p=0.637 and p=0.239). AT patients with shorter duration of symptoms had higher CRP levels than patients with longer duration of symptoms (94 mg/l vs. 80mg/l, p=0.063), but in S-Amyl levels there was no difference (p=0.650). In the PTA group, there were no differences in S-Amyl or CRP levels when comparing patients with a different duration of symptoms (p=0.655, p=0.720). AT patients with bilateral symptoms had higher CRP (110 mg/l vs. 75.5mg/l, p= 0.031) and lower S-Amyl levels (30.5 U/l vs 46U/l, p=0.033) than patients with unilateral throat pain. In PTA patients, the difference was not significant (CRP:120.5mg/l vs 60mg/l, p= 0.522. S-Amyl: 32.5U/l vs 44U/l p=0.175). PTA patients with common cold had higher S-Amyl than those without common cold symptoms (51 U/l vs 41 U/l, p=0.0096). In CRP levels, there was no difference (36mg/l vs 82mg/l, p=0.0755). In AT patients, there was no difference in S-Amyl or CRP levels between patients with or without common cold (p=0.914 and p=0.575). Poor oral hygiene had no effect on amylase or CRP levels (p=0.627 and p=0.680). History of tonsillar infections (recurrent AT, CT, PTA) did not cause changes in S-Amyl or CRP levels in AT or PTA patients either (AT patients p=0.151 and p=0.971. PTA patients p=0.852 and p= 0.410).

4.4.3 Association of laboratory test results and tonsillar findings

Eight PTA patients had no signs of infection (tonsillar erythema, tonsillar exudates, and both) in their tonsils. These 8 PTA patients had significantly lower CRP levels (median 15 mg/l, range 3-40mg/l) than PTA patients with tonsillar findings (median S-CRP 60mg/l, range 3-313 mg/l, p=0.00063). In S-Amyl level the difference was not significant (p=0.19) (Figure 13).

Eight patients were significantly older (median 50.5 years) than other PTA (median 34 years) patients (p=0.006). Bacteria culture findings of PTA patients without tonsillar findings are presented in Table 9.
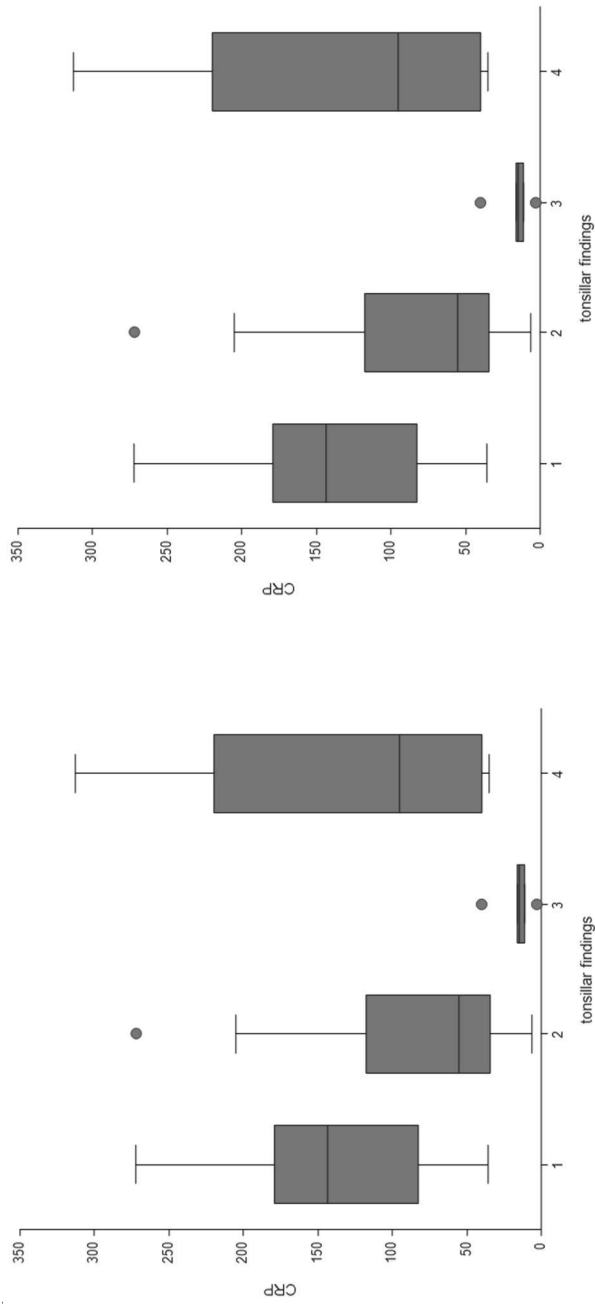
Figure 13. CRP (a) and amylase (Amyl) (b) levels in PTA patients with different tonsillar findings.

1, tonsillar erythema; 2, tonsillar exudates; 3, no findings; 4, tonsillar erythema and exudates.

Table 9. Bacterial species cultured from PTA patients without signs of infection in tonsils.

Bacteria species	PTA patients without tonsillar findings (n=8)
Staphylococcus aureus and Gram-negative bacillus	2
Gram-negative bacillus	2
Fusobacterium nucleatum	1
Coagulase-negative *Staphylococci* and coliform bacillus	1
Group A *Streptococci*	1
Group A *Streptococci* and gram-negative bacillus	1

PTA, peritonsillar abscess

In AT patients, tonsillar findings (tonsillar erythema, tonsillar exudates and both) were not associated with S-Amyl or CRP levels (p= 0.306 and p=0.363).

4.4.4 Association of laboratory results and salivary samples

No correlation between the amount of saliva bacteria and serum CRP (p=0.429) or S-Amyl (p= p=0.941) was noted. Neither in subgroup analysis between different salivary bacteria and serum CRP and S-Amyl were correlations observed.

4.5 Salivary bacteria (Study IV)

We observed no difference in the amount of total salivary bacteria between AT, PT and PTA groups (p=0.273) or between patients with acute throat infection (patient group) and healthy volunteers (p=0.104) (Figure 14).
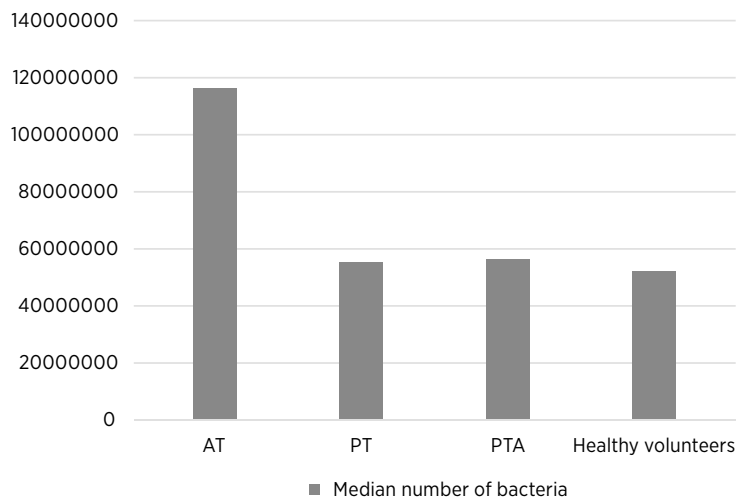
Figure 14. The median concentration (**/ml**) of saliva bacteria in AT, PT, and PTA patients, as well as in healthy volunteers

PTA, peritonsillar abscess; AT, acute tonsillitis; PT, peritonsillitis

Distribution between bacteria species in AT, PT, and PTA patients (patient group) and healthy volunteers is demonstrated in Figure 15. Healthy volunteers had a significantly higher amount of POGI (p=0.00003) (Median amount of bacteria AT: 0.257×10^6; PT: 0.0426×10^6; PTA 0.0523×10^6; Healthy volunteers 0.3715×10^6).

Figure 15. The median concentration (/ml) and distribution of saliva bacteria in A) AT, B) PT, C) PTA patients and D) healthy volunteers

A
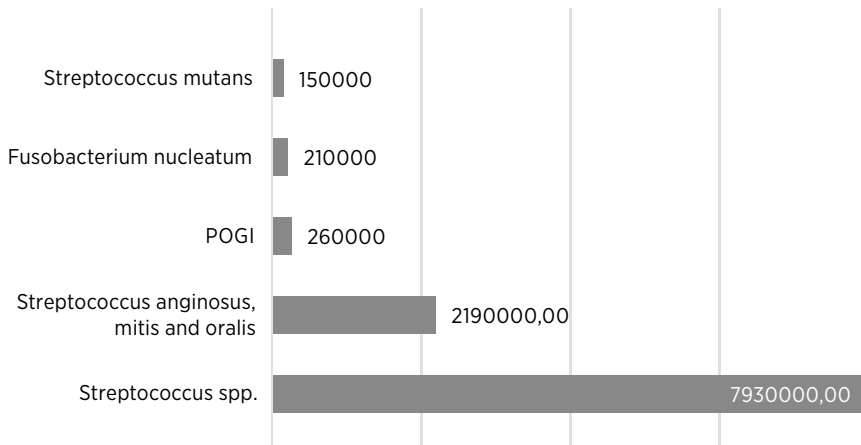
AT, acute tonsillitis; POGI, *Porphyromonas gingivalis*
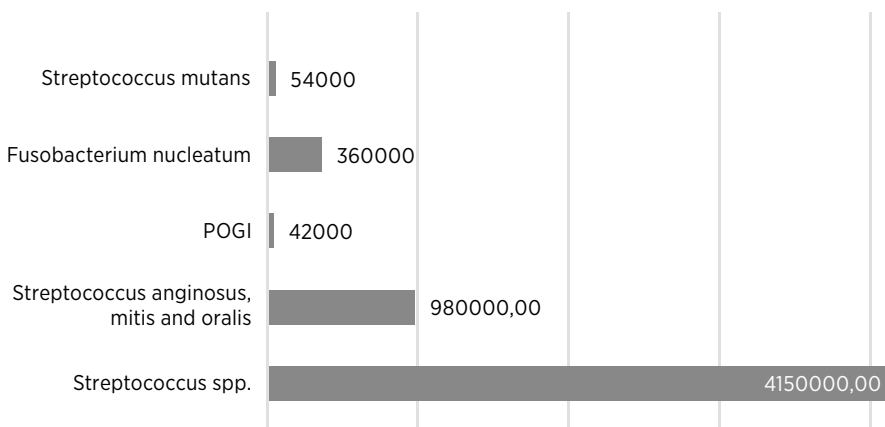
B

AT, acute tonsillitis; POGI, *Porphyromonas gingivalis*

C

Streptococcus mutans	76000
Fusobacterium nucleatum	120000
POGI	52000
Streptococcus anginosus, mitis and oralis	1290000,00
Streptococcus spp.	3680000,00

AT, acute tonsillitis; POGI, *Porphyromonas gingivalis*

D

Streptococcus mutans	244000
Fusobacterium nucleatum	316000
POGI	372000
Streptococcus anginosus, mitis and oralis	813000,00
Streptococcus spp.	3750000,00
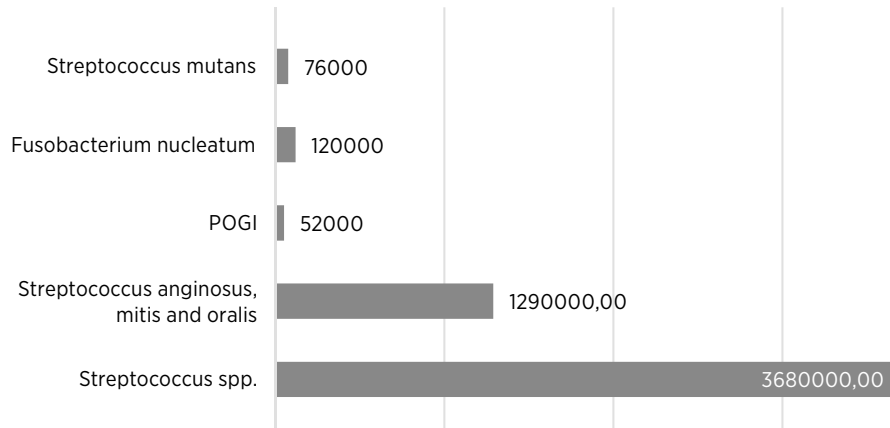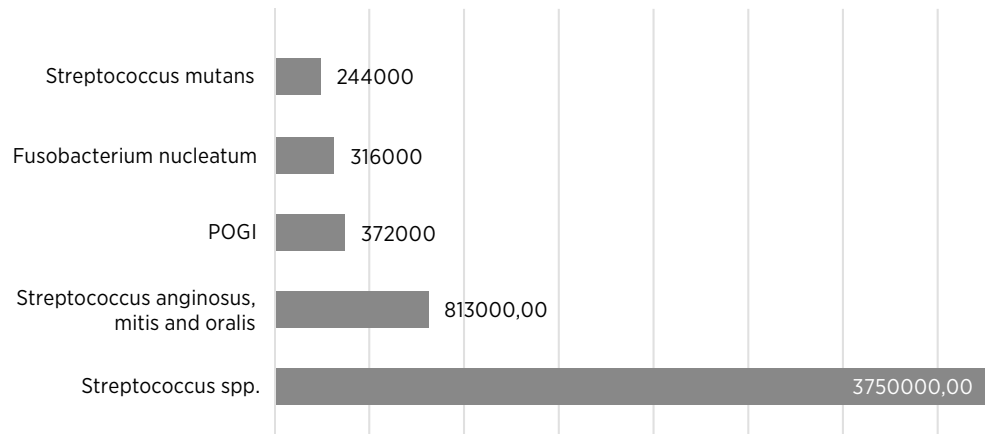
POGI, *Porphyromonas gingivalis*

Smokers had a significantly (p=0.00074) lower rate of *Fusobacterium nucleatum* in their saliva compared to nonsmokers and ex-smokers (median number: smokers 0.77×10^5, nonsmokers 2.1×10^5 and ex -smokers 5.41×10^5). Ex-smokers had a significantly higher amount of *Streptococcus* group bacteria compared to smokers and nonsmokers (p=0.028) (median number smokers: 3.2×10^6, nonsmokers 4.5×10^6, ex-smokers 14.6×10^6). Patients' smoking status had no effect on the amount of SAG (p=0.068), POGI (p=0.483), or *S. mutans* (p=0.164). The distribution of saliva bacteria is demonstrated in Figure 16.

Figure 16. The median concentration (/ml) distribution (%) of saliva bacteria in A) smokers, B) nonsmokers and C) ex-smokers

A

SMOKERS

POGI, *Porphyromonas gingivalis*

B

NON-SMOKERS

POGI, *Porphyromonas gingivalis*

C

EX-SMOKERS

205300, 1%
273733, 1%
541000, 3%

3914310, 20%

14603390, 75%

- ■ Streptococcus spp.
- ■ POGI
- ■ Streptococcus mutans
- ■ Streptococcus anginosus, mitis and oralis
- ■ Fusobacterium nucleatum
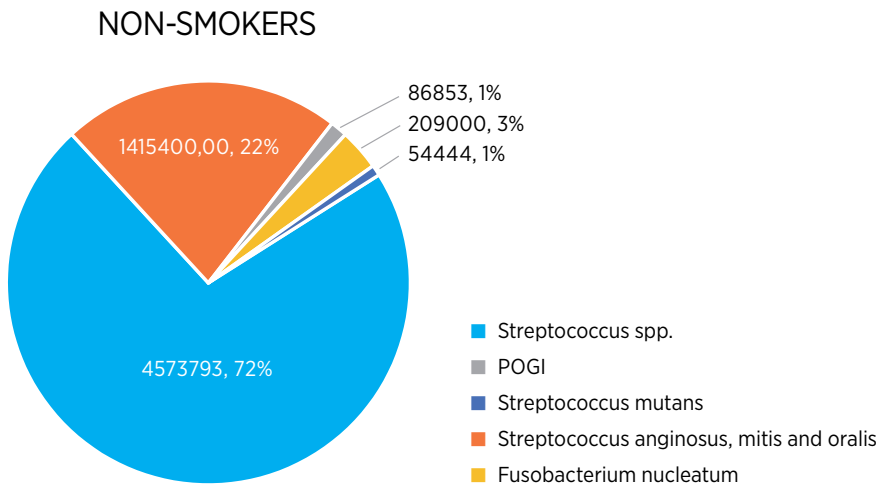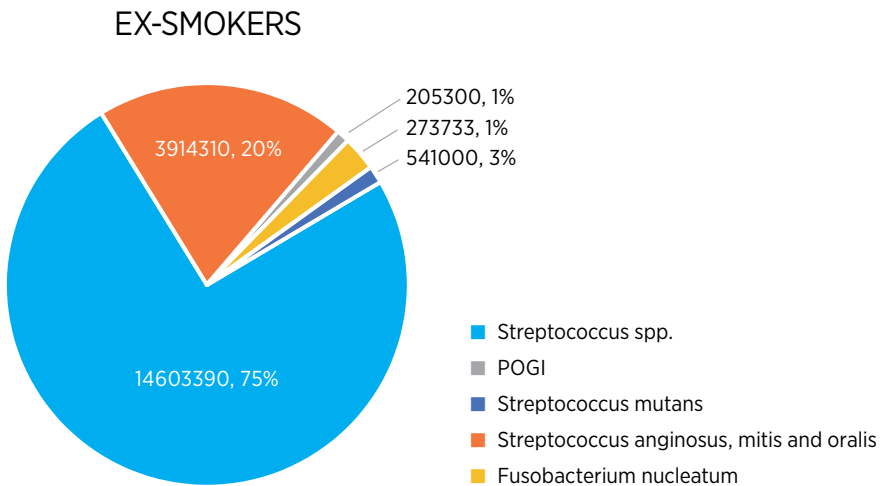
POGI, *Porphyromonas gingivalis*

The total amount of bacteria did not differ between patients with different smoking status (p=0.0866). In a subgroup analysis, ex-smokers in the AT group had a significantly higher total number of bacteria (p=0.0428), group of SAG, mitis and oralis (p=0.049) and *Fusobacterium nucleatum* (p=0.0013) compared to smokers and nonsmokers. In other saliva bacteria, there was no significant difference between patients' smoking habits. In PT or PTA groups, significant differences were not noted either in the amount of saliva bacteria in patients with different smoking habits.

Alcohol consumption did not affect the total amount of saliva bacteria (p= 0.141), but those patients who don´t consume any alcohol had higher amounts of SAG*, mitis and oralis* group (p=0.046) and POGI (p=0.036) in their saliva. When analysing different diagnostic groups (AT, PT and PTA), PT patients who did not use alcohol had a higher amount of POGI than those PT patients who used alcohol (p=0.019). There were no differences in the AT or PTA group.

Patients' oral hygiene (good/poor) had no effect on the total number of salivary bacteria (p=0.621). Distribution between bacteria in patients with different oral hygiene is presented in Figure 17. Prior use of antibiotics didn´t affect to the total amount of saliva bacteria (p=0.656) or specific bacteria species.

Figure 17. The concentration (/ml) and distribution of saliva bacteria in throat infection patients with A) good B) poor oral hygiene

A
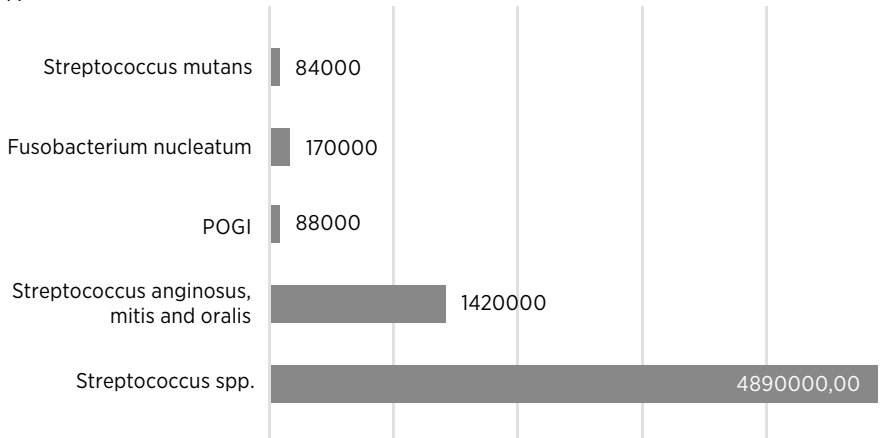
POGI, *Porphyromonas gingivalis*

B
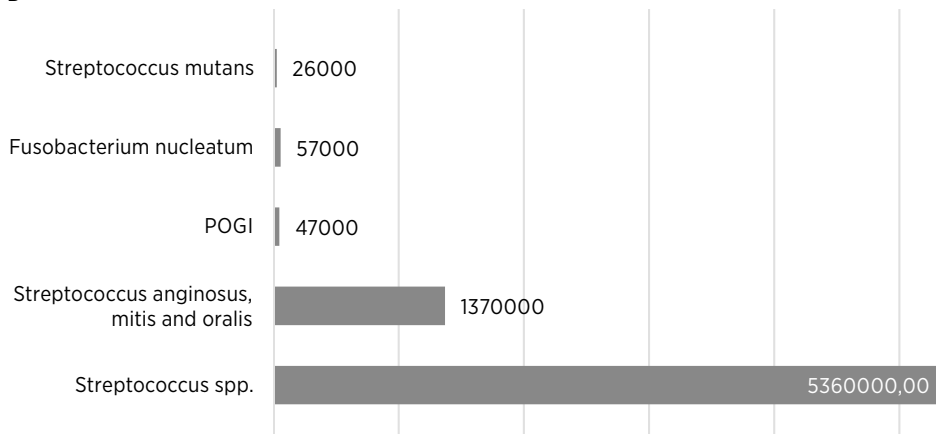
POGI, *Porphyromonas gingivalis*

No correlation between saliva bacteria and serum CRP (p=0.429) or S-Amyl (p=0.941) was noted. There was no correlation between age and salivary bacteria either. Earlier tonsillar infection had no effect on salivary bacteria (p=0.941). Patients with *Streptococcus* in their throat or pus sample had no more *Streptococcus* in their saliva than patients with other pathogens in their throat or pus sample (p=0.291). The same results came out with SAG (p=0.811). Other bacteria groups stayed so small that reliable analysis could not be run.

5   DISCUSSION

In this thesis we clarified the aetiology of PTA and showed that it is possible that PTAs do not always begin as a complication of AT, but as an infection of minor salivary glands. Our study also showed that the changes in concentration or distribution of saliva bacteria do not explain the higher rate of PTAs in patients with periodontal disease or who are smokers. We identified the minor salivary glands in most of the tonsil specimens collected after TE due to recurrent AT, CT, or PTA and showed that patients with PTA had significantly more intense periductal infection than patients with AT or CT. We also observed that neither smoking habits nor oral hygiene has any effect on the number or distribution of salivary bacteria in patients with tonsillar infection.

We also showed with an adequate number of patients that PTA patients are not a uniform group of people but there are subgroups with different bacterial findings, different clinical pictures, and different laboratory test results. We demonstrated some subgroups of PTA patients with different bacterial findings, different clinical pictures, and different laboratory test results: 1) SAG predisposes for renewal of PTA whereas GAS is not related to renewal episodes. 2) The renewal episodes were observed especially in two subgroups: older males with SAG and young women with FN. 3) Some PTA patients had no tonsillar findings in clinical examination whereas all AT patients had tonsillar erythema, tonsillar exudates, or both. 4) PTA patients presenting without tonsillar erythema and tonsillar exudates had significantly lower S-CRP levels than PTA patients with tonsillar findings.

5.1   Patient features

Altogether patient features, such age and gender of PTA patients in our Studies I, II, III and IV, are in line with the literature.

In Study II age distribution was similar to that shown in the literature. Compared with the literature, a slight difference in age distribution in Studies I, III and IV can be explained by the fact that the incidence of PTA is highest in teenagers, and age over 18 (and age over 15 in Study III reference group) was our inclusion criteria [3, 34, 41]. In Studies I, III (acute group), and IV the distribution of gender was similar to that presented in literature but in Study II, as well as in the reference group in Study III, females predominated over males [21, 41]. Smoking has been associated with increased risk of PTA and in previous studies, as many as 30% of PTA patients have reported daily smoking. We also observed a higher smoking rate compared to the general population in all our studies. In Finland 13% of all

adults were smoking daily in 2013 (15% of males and 12% of females) [46, 48, 158]. We also observed a high number of ex-smokers among PTA patients in Studies II, III, and IV [46, 48, 91]. The number of PTA patients treated with antibiotics before diagnosis has varied between studies, but our Studies I, II and III adapt to the literature, as well as in the duration of the symptoms before contacting the hospital [21, 33, 34, 40, 45, 48, 55, 56, 85].

In Study II, AT and CT patients´ age distribution cannot be analysed reliably because the groups were comprised based on age range. Equal range and median age are, however, reported in literature. In Studies III and IV patients are a little older. The most likely reason is that the highest incidence of AT is between 5 to 15 years of age and in our studies these patients were excluded [3, 11, 20, 21, 159] . It has earlier been shown that smoking and alcohol consumption can elevate CRP and S-Amyl levels [46, 160]. In Study III we did not observe such elevation in CRP or S-Amyl levels in smokers or in patients with alcohol consumption. Poor dental hygiene has also been proposed to predispose to PTA. PTA patients have been shown to suffer considerably more often from periodontal diseases than patients with RT. In Study III, only 9.1% of patients had poor dental hygiene and it was not dependent on diagnosis (PTA, AT).

In this thesis we examined whether patients' features, such as age and gender, are associated with certain pathogens or the course of disease.

5.2 Bacterial findings

Our major finding in Studies I and III was that SAG predicts a renewal, especially a rapidly proceeding renewal, of PTA and is found in older male patients. GAS was not observed to be related to renewal episodes. This strengthens the theory about different aetiological factors causing PTA.

In Study I, FN was the most prevalent pathogen in PTA patients, GAS was the second and *Prevotella spp*. was the third. In Study III, the order was different as GAS was the first, SAG the second, and FN the third. In a recent Danish study, FN was the most prevalent pathogen in PTA. In the same study, prevalence of GAS was the second highest and group C *Streptococcus* the third [40].

Over 20 years ago, a Finnish study group expressed similar results about the tendency of renewal in PTAs caused by SAG (43%) and FN. They also found that GAS (25%) did not cause renewals as frequently as other bacteria [57]. Anaerobic, and mix of aerobic and anaerobic bacteria also increase the risk on recurrence [57, 58, 145]. Based on these facts and our findings, bacterial culture, and microarray assay, microbiology may have a role in filtering PTA patients with a higher likelihood of renewal. For these patients, abscess TE as a first line treatment could be beneficial. On the other hand, for patients with GAS, aspiration or ID is

a favourable treatment. Bacterial culturing has not been regarded as a beneficial tool when planning the treatment of PTA [50]. One relevant reason for this is the slowness of the bacterial culture as well as the difficulty of taking samples. When the method has no use in everyday circumstances in hospitals and health-care centres, it leads to lack of protocols to choose adequate treatment based on bacterial findings. So physicians do not necessarily know how to benefit from the results of bacterial culture. Nowadays, when novel bacteria systems such as microarray assay have been developed and also proved to be reliable and accurate, perhaps the role of bacteria identification in treatment should be reconsidered. Use of bacterial findings in targeting the right treatment to the right patient could save costs and prevent renewals.

We also showed that patient characteristics may predict the renewal. Recently, FN has been reported to be the major bacteria causing PTA among young adults. This is in line with our results also. [40, 46, 88, 91, 104, 115]. GAS also appears more likely in younger patients [87]. PTA in older patients differs in many ways from PTA in young adults. Signs of PTA are milder, and fever and trismus are rare. On the other hand, the duration of symptoms as well as recovery time are longer and comorbidities are more common. [35, 76, 87]. Also, in our Study I, the course of disease as well as the bacterial findings in older patients differed from those in younger patients, being more intense and faster renewal of the disease. Based on our studies, older male patients need more active treatment, outpatient controlling, or reopening of abscesses. The choice of antibiotics is known to have no influence on the probability of renewal [55]. Maybe daily reopenings or abscess TE should be an option for these patients.

Antibiotic treatment does not necessarily alter the ability to obtain a positive culture in PTA but there is a trend towards more frequent isolation of anaerobes in specimens in PTA patients with prior antimicrobial therapy [34, 40, 50]. On the other hand, from patients who have not received prior antibiotics, pathogenic bacteria have been isolated more frequently [35]. In Study III, 5/9 (55.6%) of PTA patients with prior antibiotics had SAG in bacterial culture and no AT patients with prior antimicrobial treatment had pathogenic bacteria in bacterial culture.
In our studies we specified bacteria only by the species and not the different serotypes of the bacteria. Examples of different serotypes are the M protein (emm) types in GAS. M protein is an important virulence factor of GAS by intermediating antiphagocytic activity, decreasing the opsonisation, adhering to host tissue and invading the cells. GAS with the lack of M protein is readily phagocytosed, and the antiphagocytic potential of GAS is serotype-specific [161]. The most common serotypes causing severe infections are M1 and M3. M protein is decreasing the ability to opsonise by binding complement regulators factor H and FH-like protein 1 [162]. A Finnish research group has shown that certain allotypes of factor H and FH-like protein 1 are associated with weakened binding of GAS to these complement

factors, and thus also weakened virulence of GAS. This finding suggests that certain gene variants protect from GAS infections such as tonsillitis and PTA. [163-165]. The same group has also shown the association between the psoriasis risk allele and increased rate of recurrent streptococcal tonsillitis [166]. According to these findings, the serotype of bacteria and the genotype of the host can also have impact on the course of disease such as tonsillitis and PTA. These factors should also be taken into account when studying the different aetiological factors of tonsillitis and PTA as well as when examining novel treatments of streptococcal diseases.

5.3 PAD findings

In Study II, tonsils were analysed histologically. We had two main findings: presence of the minor salivary glands in a majority of the patients and significant periductal infection in PTA patients.

The minor salivary glands were demonstrated in most (67.5%) of the tonsil specimens of AT, CT, and PTA patients. The majority of the minor salivary glands were located superficially near the tonsil capsule, but we identified minor salivary glands also deeper, inside the muscle tissue. While we analysed only one tonsil per patient, Kraitrakul et al. has previously analysed both tonsils, and detected the minor salivary glands in 96.4% of patients (either one of the tonsils). In a Kraitrakul et al. study, some patients had minor salivary glands in only one tonsil; the presence of minor salivary glands in one tonsil varied between 87% and 91%, which is in line with our findings. [5]. We are the first to report deep-located minor salivary glands.

In Study II, we found that PTA patients have significantly greater periductal infection (infiltration of leukocytes around the draining duct of the minor salivary gland) of the minor salivary glands compared to AT or CT groups. Undefined (acinar/periductal) inflammation of the minor salivary glands in PTA patients has been demonstrated in a few earlier studies [4, 7]. Kraitrakul et al. have also reported a slight inflammation in the periductal space in histologically examined tonsil specimens, but not in their 3 PTA patients. In PTA patients they observed neither any fibrotic nor degenerative changes. [5]. In our study, we were able to identify fibrotic and degenerative changes in tonsil specimens. Statistical analysis remained unreliable because of the small sample size.

We observed actinomyces-like bacteria in 55% of patients and there were no differences between subgroups (AT, CT, PTA). Previously, when analysed from pus, the prevalence of actinomyces-like bacteria in PTA patients has been reported to be 23% [28]. Histological analysis in PTA patients has not been reported, but in CT and sleep apnoea patients, *actinomyces spp.* have been observed in 36–41% of patients. These numbers are a little lower than in our data. [167, 168]. We

did not find any single factor exposing the actinomyces-like bacteria. In a recent study, a lower proportion of actinomyces-like bacteria of all bacteria in smokers was noted [169].

5.4 Laboratory test findings

Laboratory test (S-Amyl and S-CRP) results were analysed in Studies III and IV (S-Amyl normal reference 28–100 U/l, S-CRP normal reference 0.05–3 mg/l). As the main findings, we showed a negative correlation between S-Amyl and CRP and also identified the group of 8 PTA patients without signs of infection in their tonsils. These patients had significantly lower CRP and they were significantly older than other PTA patients.

5.4.1 Correlation of serum amylase (S-Amyl) and CRP levels

An inverse correlation between S-Amyl and CRP levels was found only in the acute group. This indicates that the activation of minor salivary glands during the infection is transient and is related to infection. No difference in S-Amyl levels between acute group patients with or without a history of recurrent AT or PTA episodes was noted. This observation differs from the El Saied et al. study, which demonstrated significantly lower pus amylase in patients with a recurrent PTA. They did not report S-Amyl levels in patients with recurrent PTA [8]. In the recent Estonian study, the research group reported increased pus amylase levels in some of the PTA patients (13%). The median S-Amyl level in PTA patients was 45.3 U/l and range 20–108 U/l), which is in line with our results. [170]. The Estonian group did not observe the tonsillar findings of the patients or report the correlation between CRP and S-Amyl levels. Nor did they report the subgroup analysis of the 12 patients with the elevated pus amylase levels. This study, however, strengthens our findings about different subgroups of PTA patients with potentially different causative agents of the PTA.

We did not observe any difference in CRP levels between AT and PTA patients. This is in line with Tachibana et al. findings [36]. We did not observe the difference in S-Amyl levels, either, between AT or PTA patients. Earlier El Saied et al. detected a difference in both serum and pus amylase levels between PTA patients and deep neck abscesses and also PTA patients and dental abscesses. [8, 9].

5.4.2 Association of laboratory test results between signs and symptoms

We observed that afebrile AT patients had lower CRP and higher S-Amyl levels than febrile patients. One could even speculate that salivary gland activation sometimes also occurs in AT, although this hypothesis has not been presented earlier [20, 171]. AT patients with unilateral symptoms had lower CRP and higher S-Amyl levels than patients with bilateral throat pain. This could suggest some kind of activity in the minor salivary glands.

5.4.3 Association of laboratory test results and tonsillar findings

Eight PTA patients had no signs of infection in their tonsils in the clinical examination. In contrast, all AT patients had tonsillar erythema, tonsillar exudates or both. PTA patients without tonsillar findings in a clinical examination had significantly lower CRP levels than other PTA patients. We also noted that these 8 patients without tonsillar findings were older than other PTA patients. The fact that PTA is encountered also in patients without tonsillar findings suggests that AT is not necessarily always a pre-stage of PTA. Patient characteristics, CRP level and clinical findings can be used to differentiate PTA patients with different aetiological factors causing PTA. [3, 4, 13].

The growth of GAS and *Staphylococcus aureus* in bacterial cultures, as well as low CRP levels, have been demonstrated earlier in patients with parotitis. Saarinen et al. showed that a majority of patients suffering from parotitis had elevated S-Amyl levels, but only a few patients demonstrated elevated CRP levels. These analogous findings between our eight PTA patients and patients with a confirmed infection of major salivary glands support strongly the conclusion that PTA in these eight patients is caused by the infection of minor salivary glands. [18, 37-39]. In our study, the elevation of S-Amyl level in PTA patients without tonsillar findings was not significant. One possible explanation is the small size of minor salivary glands compared to major salivary glands, which prevents the elevation becoming substantial. The S-Amyl reference values are also validated for pancreatic diseases, not salivary gland infection, and don´t reflect directly the activity of minor salivary glands.

5.5 Saliva bacteria findings

In Study IV, we showed that smoking habits and oral hygiene had no effect on the number or distribution of saliva bacteria. Although the role of periodontal disease in the development of PTA has been speculated about widely in literature,

the association between PTA and periodontal disease has been shown in only one study. Georgalas et al. showed that the prevalence of periodontal disease is elevated in PTA patients compared to patients with CT. In addition, the overlapping of the microbiological profile of these two diseases has been noticed. The pathogenesis has, however, stayed unclear; it has even been speculated that perhaps poor oral hygiene could be only an indicator of low socioeconomic status, smoking, and the patient's overall health rather than a cause of PTA itself. [3, 12, 172].

In our study, we observed no increase in the number of saliva bacteria during acute infection compared to the samples of healthy volunteers. This observation differs from the Brook et al. study, where they showed a 10- to 1000-fold increase of saliva bacteria during AT compared to samples from the same patients after acute infection. In the same study, a significant increase in *Fusobacterium nucleatum* antibodies after infection was also noted .[121].

Smoking has been conclusively shown to be associated with an increased risk to PTA in both genders. As many as 30% to 60% of patients with PTA have reported daily smoking, which is a significantly higher number than the smoking rate in the general population. [36, 46, 48, 49, 66]. Also, the number of ex-smokers among PTA patients is high: 18% [49]. The exact mechanism through which smoking promotes PTA is, however, unclear. The higher incidence of *Streptococcus viridans* isolates (including SAG) has been reported in smokers suffering from PTA [48]. In the other study, however, no differences in PTA pathogens between smokers and nonsmokers were reported [46].

Effects of smoking on oral microbiota are controversial. In some studies smoking have been shown to change the oral microbiota [134, 135]. However, opposite findings have also been reported [136]. We observed that smokers had a lower number of *Fusobacterium nucleatum* and ex-smokers had a higher number of *Streptococcus* in their saliva. Our findings differ from Moon et al. and van Winkelhoff et al. and their observations that smokers have a higher number of *Fusobacterium nucleatum* in subgingival samples. On the other hand, earlier studies analysed only patients with periodontal disease, so the results are not completely comparable. [125, 126]. Another mechanism, not related to saliva bacteria, has also been speculated upon. Torre et al. have shown histological alterations in tonsils´ lymphoid and non-lymphoid compartments in smokers, which could reflect that smoking alters the immune responses and predisposes smokers to PTAs. Similar, but milder, changes were observed in patients with CT. RT is known to predispose to PTA, but no studies about the role of CT are published. [35, 36, 67]. On the other hand, an Iranian study group showed that salivary flow rate is significantly lower in smokers and this could be the one mechanism of the higher rate of oral diseases in smokers [137]. Our findings do not support the hypothesis that smoking promotes PTA by changing the oral microbiota.

5.6   Strengths and Limitations

This thesis consists of four separate studies. Every study has its own limitations, but the limitations differ between the studies, which can be regarded as the strength of this thesis. The only limitation associated with all our studies is that patients were recruited by several of the nurses and physicians in the Emergency Department of ORL in HUH and also examined and treated by several physicians. On the other hand, this reflects the real circumstances in hospitals and health care centres in Finland and makes the results more generalizable. The fact that several physicians recruited and examined the patients had the most important impact on results in Study III. The evaluation of tonsillar findings (erythema, exudates) was based on the ear, nose and throat physician's subjective observation. Due to the prospective nature of the study (physicians had a structured examination form which included the questions about the tonsillar findings), as well as the physicians' substantial experience on tonsillar diseases, the evaluation can be considered reliable.

The microarray assay used in Study I for bacteria determination is non-standardised. The method is, however, shown to have sufficient accuracy and reliability in bacterial identification. [55, 151]. Patients had two different antibiotic treatments, which can be considered as a biasing factor but as shown also in the original study, it had no effect on recovery or results [55]. The microarray method is based on duplicating the bacteria and thus it also observes the dead bacteria, whereas the bacterial culture method notices only the living bacteria. In addition, because the laboratory´s standard methods for pus samples and throat swabs were used, only the colonies large enough to cause disease and bacterial species with pathogenic features were answered from cultures. Thus, comparing the bacterial findings between the studies in which different methods have been used is not completely reliable.

In Study II, the tonsils were collected a mean of six months after the acute infection episode which, especially concerning the PTA samples, can be regarded as a limitation. Because the specimens were collected originally for another study and were not marked during TE, location of the glands in the peritonsillar space (i.e. the lower, upper, or middle portions of the peritonsillar space) could not be analysed accurately and they could only be classified as deep or superficial. Kraitrakul et al. have demonstrated the presence of the minor salivary glands in upper, lower and middle portions of peritonsillar space. We were also able to observe the minor salivary glands all over the peritonsillar space, supporting the findings of Kraitrakul et al. [5].

In Study III, we analysed only the aerobes from throat swabs of AT patients which can be considered as a limitation. Bacterial cultures were grown according to the laboratory´s standard methods for diagnostic samples. From PTA patients' pus samples both aerobes and anaerobes were analysed, also with the laboratory´s

standard methods, and no request to analyse specific bacteria was done. The small size of bacterial groups (FN, FN+SAG) also limited the statistical analysis. We did not exclude AT patients with earlier PTA (n=3) from the acute group. The number of these patients is, however, low (n=3) in our material and we reviewed the patient charts and observed no PTA episodes during the few months before the current episode. So the influence on results is minor and not significant. The evaluation of tonsillar findings (erythema, exudates) was based on subjective observation. Evaluation was made by ear, nose and throat physicians, and due to the prospective nature of the study as well as the physicians' large experience of tonsillar diseases, the evaluation can be considered reliable. This can, however, be considered as a limitation of this study. The acute group and reference group were collected originally for different studies, and in the acute group, we had age under 15 years as an exclusion criterion, but in the reference group all patients under 18 were excluded.

In study IV, a physician, not a dentist, evaluated patients´ oral hygiene. No standardised evaluation scale or index was used in the dental status. This can be regarded as a limitation. In a Georgalas et al. study, periodontal status was evaluated by an oral surgeon using the Periodontal Index of Treatment Needs (CPITN) [12]. 49 of 200 saliva samples did not become hybridised which means that a probe did not recognise any bacteria from the salivary sample. The degree of oral hygiene between the different diagnosis groups could not be statistically analysed because of the small number of patients with poor oral hygiene in certain diagnosis groups. Prior antibiotics had no effect on hybridisation (p=0.348). It can be speculated whether previously ingested food or blood blended with saliva prevented the hybridisation. None of the healthy volunteers smoked or had alcohol abuse, so these parameters could not compare with the patient group.

5.7 Future aspects

The aetiology of PTA and especially the role of minor salivary glands needs to be researched more in the future. The histology of tonsillar tissue and minor salivary glands should be analysed from the patients who have undergone an abscess tonsillectomy. The larger study about the clinical findings of PTA patients—and especially the higher sample size of those patients without tonsillar findings—and their laboratory results could give us more knowledge about the different subgroups of PTA patients. Also, the larger study of PTA patients' S-Amyl levels could strengthen our observations about the amylase levels of PTA patients. The development of technology could also make possible finding the markers suggesting a greater genetic predisposition to PTA, like certain HLA-molecules or from bacteria, like M proteins of GAS.

6   CONCLUSIONS

The present studies examined the aetiology of PTA and showed that it is not as unambiguous as previously thought. There are various subgroups of PTA patients with different patient characteristics, symptoms, and clinical examination findings, as well as bacterial and laboratory test results. Some PTA patients´ disease seems not to begin as a complication of AT but as an infection of minor salivary glands. The renewal rate and severity of the symptoms differ between subgroups. Smoking, alcohol consumption, oral hygiene, and acute throat infection have no influence on salivary bacteria findings, and the role of periodontal diseases in developing PTA is questionable. At least the mechanism seems not to be changes in oral microbiota. In the future, more studies about different aetiological factors of PTA and, especially, diagnostic tools to differentiate aetiological factors are needed to target the treatment, especially TE, more accurately to certain subgroups of PTA patients.

The following findings clarify the aetiology of PTA and may act as diagnostic tools to separate different aetiological factors:

1. SAG predicts renewal of PTA while GAS is not detected in patients with renewal of PTA.

2. SAG predominates and abscess recurs after a shorter period among male patients over 40

3. The minor salivary glands are identified in peritonsillar space in PTA patients and the histological signs of peritonsillar inflammation are more intense than in patients with AT or CT.

4. Not all patients with PTA have signs of infection in tonsils in clinical examination. These patients have significantly lower CRP levels than other PTA patients

5. A negative correlation between CRP and S-Amyl levels is observed during the acute infection. In post-infectious condition, correlation is not noted.

6. Smoking, oral hygiene, and acute throat infection (AT, PT, PTA) have no influence on the number of saliva bacteria.

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