Peritonsillar abscess: clinical aspects of microbiology, risk factors, and the association with parapharyngeal abscess

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This doctoral dissertation is based upon the following peer-reviewed publications referred to in the text by Roman numerals:

I: **Ehlers Klug T**, Rusan M, Fuursted K, Ovesen T. Fusobacterium necrophorum: most prevalent pathogen in peritonsillar abscesses in Denmark. Clin Infect Dis 2009; 49:1467-72. doi: 10.1086/644616.

II: **Klug TE**, Henriksen JJ, Fuursted K, Ovesen T. Significant pathogens in peritonsillar abscesses. Eur J Clin Microbiol Infect Dis 2011; 30:619-27. doi: 10.1007/s10096-010-1130-9.

III: **Klug TE**, Henriksen J, Rusan M, Fuursted K, Ovesen T. Bacteremia During Quinsy and Elective Tonsillectomy – An Evaluation of Antibiotic Prophylaxis Recommendations to Patients Undergoing Tonsillectomy. J Cardiovasc Pharmacol Ther 2012; 17:298-302. doi: 10.1177/1074248411423023.

IV: Rusan M, **Klug TE**, Henriksen JJ, Ellermann-Eriksen S, Fuursted K, Ovesen T. The role of viruses in the pathogenesis of peritosnillar abscess. Eur J Clin Microbiol Infect Dis 2012; 31:2335-43. doi: 10.1007/s10096-012-1573-2.

V: **Klug TE**, Rusan M, Clemmensen KK, Fuurted K, Ovesen T. Smoking promotes peritonsillar abscess. Eur Arch Otorhinolaryngol 2013; 270:3163-7. doi: 10.1007/s00405-013-2474-4.

VI: **Klug TE**, Fischer AS, Antonsen C, Rusan M, Eskildsen H, Ovesen T. Parapharyngeal abscess is frequently associated with concomitant peritonsillar abscess. Eur Arch Otorhinolaryngol 2014; 271:1701-7. doi: 10.1007/s00405-013-2667-x.

VII: **Klug TE**. Incidence of peritonsillar abscess: the influence of season, age, and gender. Eur J Clin Microbiol Infect Dis 2014; 33:1163-7. doi: 10.1007/s10096-014-2052-8.

VIII: **Klug TE**, Henriksen JJ, Rusan M, Fuursted K, Krogfeldt K, Ovesen T, Struve C. Antibody development to Fusobacterium necrophorum in patients with peritonsillar abscess. Eur J Clin Microbiol Infect Dis 2014; 33: 1733-9. doi: 10.1007/s10096-014-2130-y.

Abbreviations

AUH	Aarhus University Hospital
CRP	C-reactive Protein
CT	Computer tomography
EBV	Epstein-Barr virus
ENT	Ear-Nose-Throat
FN	*Fusobacterium necrophorum*
GAS	Group A streptococci
ID	Incision and drainage
IFA	Indirect fluorescent antibody
IM	Infectious mononucleosis
LS	Lemierre´s syndrome
PCR	Polymerase chain reaction
PPA	Parapharyngeal abscess
PTA	Peritonsillar abscess
RADT	Rapid antigen detection test

1. Introduction

1.1 Background

The palatine tonsils constitute a major component of the lymphoid tissue in Waldeyer´s ring [1]. With a strategical location in the oropharynx (Figure 1.1), they are exposed to both inhaled and ingested antigens and perform localized immune functions [2]. This exposure to potential pathogens and involvement in local processing of microorganisms may be the basis for the high incidence of tonsillar infections.

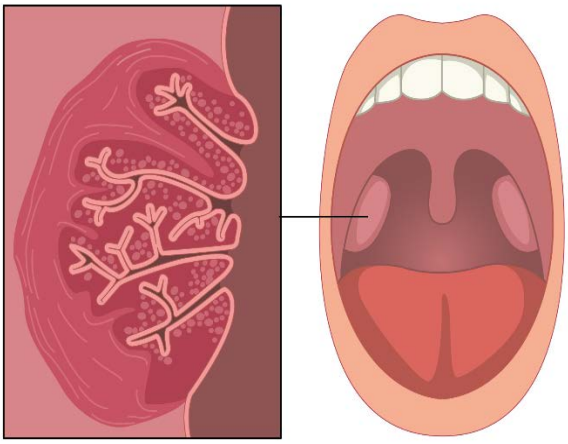

Figure 1.1. Tonsillar anatomy.
The palatine tonsils are located in the oropharynx. The surface of the tonsils is lined by stratified squamous epithelium. Multiple tonsillar crypts are formed by deep invaginations of the epithelium into the tonsil. The crypts increase the surface area for interactions between antigens and the nodular lymphoid tissue beneath the epitheium. The tonsils are separated from the underlying musculature by a capsule, which is firmly coherent to the tonsillar tissue by multiple septae. (Copy right Kommunikationsafdelingen, Aarhus University Hospital)

Tonsillar infections
Acute tonsillitis is a highly prevalent and costly condition that affect children and adults. In the United States, 18 million patients sought care for acute tonsillitis in 1996, making it the sixth leading reason for physician consultations [3]. An estimated four to six times more individuals may not seek medical care for a sore throat [4]. The condition may be even more prevalent in Denmark, as approximately 425,000 rapid streptococcal antigen detection tests (RADT) are performed by Danish general practitioners, annually, in order to diagnose infection with Group A Streptococci (GAS) [VII].
Peritonsillar abscess (PTA), or quinsy, refers to a collection of pus located between the tonsillar capsule and the pharyngeal constrictor muscle. It is commonly regarded as a complication of acute tonsillitis although some researchers have found evidence pointing to a role of the salivary glands in the soft palate (Weber´s glands) in the pathogenesis of this condition [5-6].
Quinsy is a medieval English word describing any throat infection, especially tonsillitis. It is taken from the Latin quinancia, which again is derived from the Greek cynanche. The ancient Greeks decribed all inflammations of the throat and neck by the single term cynanche. Hippocrates reserved the term for internal inflammations only. Cynanche and quinsy were used to describe tonsillitis until the turn of the 19th century, when quinsy became synonymous with PTA. Although the prognosis is said to have been generally good, lethal complications are reported in the literature over the last three centuries. In England, the number of annual deaths due to PTA varied between 110 and 623 in the period 1848 to 1876 [7]. Nowadays, lethal outcome of PTA is extremely rare. However, despite improvements in living conditions and general health (including oral hygiene), the ease of access to medical care, and the invention and widespread use of antibiotics, PTA is still relatively frequent (approximately 2000 cases annually in Denmark) [I].

Signs and symptoms of peritonsillar abscess
The usual history of patients with PTA is three to six days of progressive sore throat, pain on swallowing (that often become unilateral) to the point of being unable to eat, ear pain, malaise, and trismus [I, 8-13]. Approximately 40% of patients have received antibiotic therapy within the week prior to presentation [I]. Patients are commonly found obviously ill, uncomfortable, dehydrated, and in pain [14]. Oropharyngeal examination typically reveals tonsillar exudates and asymmetric, indurated peritonsillar swelling with deviation of the tonsil and uvula towards the midline [8]. Other common findings are tender and enlarged cervical lymph nodes, trismus, muffled voice, and fever [8, 15]. Significant upper airway obstruction is seldom [14].
At the Department of Otorhinolaryngology, Head & Neck Surgery, Aarhus University Hospital (AUH), we noticed that a typical PTA patient complained of aggravation of sore throat over the previous four days and had a negative RADT performed a couple of days previously. Because GAS is generally considered the only significant bacterial pathogen in the oropharynx, most Danish practitioners rely on the RADT and patients are not prescribed antibiotics if the test is negative.

Differential diagnoses of peritonsillar abscess
Severe cases of acute tonsillitis (including infectious mononucleosis (IM)) share many symptoms and findings of PTA including sore throat, pain on swallowing, ear pain, malaise, discomfort, dehydration, tonsillar exudates, tender and enlarged cervical lymph nodes, and fever. The diagnosis may be even more difficult to differentiate from acute peritonsillitis, where the infection has spread to the peritonsillar tissue, but without abscess formation. In acute peritonsillitis additional trismus and asymmetric peritonsillar swelling with deviation of the tonsil and uvula can be found, but the peritonsillar induration is absent or less pronounced. Empirically, it seems apparent that acute tonsillitis, acute peritonsillitis, and PTA are entities within a continuous spectrum, and an infection may progress through these three stages if left untreated or even despite antibiotic treatment. Minor salivary gland tumors, parotid gland tumors within the deep lobe, and tonsillar neoplasms (primarily lymphomas and carcinomas) may be associated with prominence of one or both tonsils with peritonsillar swelling. However, patients typically lack symptoms of acute infection (rapid onset of sore throat, fever, tender cervical lymph nodes etc.) and these tumors are generally easily differentiated from PTA by ENT specialists. Of note, simultaneous PTA and tonsillar malignancy has been described in a number of case reports [16].

Complications of peritonsillar abscess
Complications of PTA are relatively rare. They include parapharyngeal abscess (PPA) [VI, 17-19], upper airway obstruction [20-23], Lemierre´s syndrome (LS) [24-29], necrotizing fasciitis [30-31], mediastinitis [32-34], erosion of the internal carotid artery [35-37], brain abcess [38-39], and streptococcal toxic shock syndrome [40].

Diagnostic tools in peritonsillar abscess

The differentiation between acute peritonsillitis and PTA relies on the result of a needle aspiration (+/- pus), although false negative results may be due to inadequate technique or atypical abscess location. Alternatively, contrast-enhanced computer tomography (CT) or ultrasound can be used to visualize a PTA. Because these radiological examinations are not useful in the drainage of a PTA, at our institution they are only performed if PPA or other complications are suspected. Instead, after a day without improvement despite intravenous antibiotic treatment, patients are examined and treated (typically with tonsillectomy) under general anaestesia. PPA located in the proximity of the tonsillar bed (most often derived from tonsillar or dental infections) may be difficult to differentiate from PTA until surgical exploration. As an ENT surgeon, I have multiple times performed acute tonsillectomy on patients with PTA and after removal of the tonsil and abscess discovered a concomitant PPA located laterally to the constrictor muscle and in close proximity to the PTA. This finding lead me to believe that PPA and PTA is often closely related and may share significant pathogens. However, this dual abscess formation was not well-described in the literature.

Microbiology of peritonsillar abscess

GAS is the only bacterium, which is recognised as pathogen in PTA. However, studies of PTA aspirates show that the majority of abscesses develop without evidence of GAS involvement (see chapter 4.1.3). A possible role for anaerobes has been proposed by several researchers, but there was a lack of convincing evidence for individual pathogens [41]. Hence, approximately 80% of PTA patients have an invasive infection with undefined bacterial pathogens and the majority of patients have had symptoms and signs of tonsillar infection the days prior to abscess development. Identification of significant pathogens, other than GAS, is necessary to deploy this great potential for improvement in timely antibiotic treatment with avoidance of infectious spread and abscess development. Furthermore, complications secondary to PTA may be avoided or better controlled if more pathogens in PTA are identified.

Surgical treatment of peritonsillar abscess

Hippocrates performed what is thought to be insicion of PTAs in the 4th century BC. The first complete acute tonsillectomy (à chaud) for the treatment of a PTA was performed by Chaussaignac in 1859. It was not until a number of publications in the period 1910 to 1935 [42-44] confirmed the safety and efficacy of the procedure that it was adopted by a number of surgeons in Europe and the United States as the routine method for PTA treatment. It is difficult to estimate the percentage of PTA patients who were treated with acute tonsillectomy or incision and drainage (ID) over time in Denmark, Europe, and elsewhere. However, in the 1960´s and 70´s, a great number of surgeons advocated for the advantages of tonsillectomy à chaud (Bateman (1959)[45], Volk (1960)[46], Beeden (1970)[47], Brandow (1973)[48], Lee (1973)[49], Bonding (1973)[50], and Yung (1976)[51], Templer(1977)[9], Muller (1978)[52]) and they probably convinced many centers to routinely treat PTA patients with acute tonsillectomy. In the 1980´s and 90´s multiple studies showed high and comparable efficacy rates of ID as well as needle aspiration alone, and there was a strong trend towards a more conservative treatment without removal of the tonsils in the acute phase [10-11, 53-59].

Currently, there are three accepted methods of draining PTAs: needle aspration, ID, and acute tonsillectomy. None of these interventions has been identified as the optimal surgical treatment. Acute tonsillectomy can be performed safely and requires only a brief hospitalization [52]. The procedure secures complete drainage of the abscess regardless of position relative to the tonsil and removal of the infectious load within the tonsils. Hence, there is no need for postoperative antibiotic therapy in uncomplicated cases [60]. Moreover, the risk of future recurrent acute tonsillitis or PTA is (almost) abolished. However, there is a risk of post-tonsillectomy hemorrhage [61], the costs are higher than those of ID or needle aspiration [62], and surgical and anaestetic specialists are required. Most patients can avoid admission if treated with ID or needle aspiration, but will typically need outpatient follow up and antibiotic therapy. In patients treated with ID or needle aspiration, a high incidence of pus can be detected in patients undergoing routine tonsillectomy in the days after initial drainage [63]. This finding supports post-drainage antibiotic therapy in order to minimize the risk of abscess recurrence and spread of infection in patients treated without removal of the tonsils. Studies of needle aspiration and ID have shown resolution rates in the range of 82% to 100% without significant difference between the two procedures [8, 10-11, 54-59]. Stringer et al found that patients tolerate needle aspiration better than ID [54]. However, Nwe et al found higher rates of improvement in trismus and ability to swallow water in the hours after treatment in patients undergoing ID (100% and 92%, respectively) compared to needle aspiration (38% and 8%, respectively) [64]. Both procedures require patient cooperation, which can be difficult to obtain, especially in children and teenagers.

The preferred surgical procedure for abscess drainage and the indications for more aggressive treatment differs greatly in different parts of the world or even within countries [65-70]. In Denmark and some centers in Germany, tonsillectomy à chaud is the choice of surgical treatment in the majority of patients [I, 66-67]. Centers in France, Canada, Brazil, Japan, Korea, and Nigeria report using ID as the primary treatment [71-76] while centers in Ireland [77], Sweden, Norway, Finland [70], Italy [78], Israel [79-80], USA [69], New Zealand [81], and Singapore [82] use either ID or needle aspiration alone. In 2001, Mehanna et al carried out a postal questionnaire reporting on the initial method of treatment among 101 surgeons in United Kingdom [83]. Sixty percent of surgeons performed needle aspiration alone, 25% ID, and only one percent preferred acute tonsillectomy. Furthermore, five percent treated the patients with intravenous antibiotics alone (the authors did not state the treatment modality for the remaining nine percent). In some countries, the approach to PTA seems even more conservative. In Taiwan, Wang et al reported on 28,837 patients with PTA [84]. Fiftyone percent of patients were treated with needle aspiration, 8% with ID, and 41% with antibiotics alone. In another study from Taiwan, Hsiao et al described eight (of 56) children treated with antibiotics alone, who recovered smoothly, but with prolonged admission and two of them with concurrent parapharyngeal space involvement [85]. Qureshi et al reported on the management of pediatric PTA in the United States from 2000 to 2009 [69]. They found increased rates of ID / needle aspiration (they were not able to differentiate the two

treatment modalities) from 26% in 2000 to 34% in 2009 and a corresponding decrease in acute tonsillectomy (13% to 8%) and acute tonsillectomy after initial ID (10% to 7%). Fiftyone percent of patients had no surgical intervention performed throughout the period. A similar, but more radical, de-escalation of surgical intervention from acute tonsillectomy to ID was recently decribed in a German center [65]. Two studies have been conducted exploring if PTA can be treated with medical management alone. In patients treated with intramuscular penicillin for a day followed by oral penicillin, Tucker reported that 32% of abscesses were eventually incisized, 14% bursted spontaneously, and 54% were not drained [86]. More recently, Lamkin et al reported recovery of 94 of 98 PTA patients treated with medical therapy alone (one dose of intravenous cefazolin followed by oral cephalexin) and without complications [87]. However, these studies reporting on PTA patients without recovery of pus carry the great problem that the diagnosis relied on clinical examination and some patients may have just had acute peritonsillitis without abscess formation. Overall, there seems to be a trend towards less invasive surgical approaches to PTA treatment with conservation of the tonsils, needle aspiration instead of ID, and in some cases even antibiotic treatment without surgical intervention. Traditions, favorable outcomes with quick recovery, and rare serious complications serve as reasons for the aggressive approach to PTA treatment in Aarhus and the rest of Denmark today. While researchers of PTA microbiology in other parts of the world can perform studies on pus aspirates or tonsils from highly selected cases only, the frequent use of acute, bilateral tonsillectomy almost unique to Denmark, made it possible to carry out a comparative study of the tonsillar core tissue microbiology from PTA patients and electively tonsillectomized patients (cohort B, chapter 3.2).

Antimicrobial treatment of peritonsillar abscess

In addition to surgical drainage, antimicrobial therapy is generally recommended [88]. The preferred antibiotic regimen varies between countries and centers. Wiksten et al reported on the results of a questionnaire sent to 81 chief physicians in four Nordic countries [70]. Penicillin, either oral or intraveneous, was the first choice for 65% of responders. In Denmark, combined treatment with penicillin and metronidazole was preferred by 58% of responders. This combination was also the most commonly preferred by 302 consultants in United Kingdom in 2009 [89]. However, multiple antibiotic regimens are reported in recent literature including (but not exclusive to) intravenous penicillin alone [68], amoxicillin with clavulanic acid with or without metronidazole [77, 90], cefuroxime and metronidazole [91], and clindamycin [92]. In a retrospective study of 103 patients, Kieff et al found that penicillin alone did not lengthen the admission or increased the risk of complications compared to broad-spectrum antibiotics [92]. The different antibiotic regimens may illustrate the lack of knowledge concerning the significant pathogens in PTA and that most clinicians regard bacteria other than GAS of importance and therefore do not rely on penicillin alone.
Lastly, two studies have found support for a single dose of intravenous glucocorticoid in patients treated with needle aspiration, with improvements in analgesia (short term) and a shortened hospital stay [93-94].

1.2 Challenges in studying the microbiology of peritonsillar abscess

There are multiple challenges, which need to be addressed, when studying PTA microbiology. Some challenges are derived from the fact that multilple factors influence the microbiological findings and others are related to the complexity of the infection.

Diagnostic precision

All patients studied should suffer from the same diagnostic entity. However, differential diagnostic problems are common in PTA patients because of the resemblance to acute tonsillitis and acute peritonsillitis, which are probably entities within a continuous spectrum. Clinical examination and radiological modalities are imperfect in the differentiation between acute peritonsillitis and a minor (or even medium-sized) PTA. Hence, detection of pus is important for diagnostic reliance in studies of PTA. In 15-20% of cases, the PTA is located at the lower tonsillar pole and it is very difficult or even impossible to perform an aspiration in local anaestesia [45, 47-48, 50-51, 95]. It is unexplored if the pathogenesis and microbiology are associated with the location of the abscess, but this may be the case, if the Weber´s glands play a significant role. The optimal diagnostic confidence and reduction of location bias can be achieved, when an acute tonsillectomy is performed. During the acute tonsillectomy an aspiration can be done from a lower pole abscess.

Number of patients

The number of patients is not a challenge specific to studies of PTA microbiology, but should, of cource, be considered and depending on the type of study and the issue(s) in focus, the number of patients needed can be difficult to achieve.

Specimen material, collection, handling, and transportation

The quality of the specimens submitted to the microbiology laboratory is critical for optimal evaluation. Aseptic aspiration of pus is the natural material for examination for microorganisms in patients with PTA. However, if the microbiological findings are to be compared to the "normal flora" for study purposes, comparable materials must be obtained. In acute tonsillitis and PTA, the stratified squamous epithelium of the tonsil is ulcerated and invaded by neutrophils. It is unknown if there are quantitative or qualitative differences in the microbiology between the crypt and surface epithelium (see Figure 1.1) in patients with acute tonsillitis and PTA, but studies have shown differences in the bacteriology of the tonsillar surface and the core in patients with recurrent tonsillitis and tonsillar hypertrophy [96-98]. It is very likely that the micro-environment (e.g. oxygen concentration, immunocells, immunoglobins, and cytokines) of the surface differs from that of the tonsillar crypts. These differences are likely to affect the microbiology. We anticipated that the crypt (core) bacteriology resembles that of pus as the micro-environment in the crypts is likely more similar to that of the abscess than the tonsillar surface. Moreover, the tonsillar core is located closer to the abscess than the surface. Collection of specimens should be done by appropriate swabs (surface) and collected in appropriate containers (tissues and pus). Concerns regarding freezing of specimens are described in chapter 7 (study II).

Methods for detection and quantification of microorganisms

With only one exception [99], all researchers have used bacterial culture methods in studies of PTA. The advantages of culture-based microbiology include the possibility of quantification and

antibiotic susceptibility testing. The disadvantages include poor or complete lack of growth of fastidious or antibiotic-exposed bacteria. In general, more bacterial strains can be detected and identified when using Polymerase Chain Reaction (PCR)-based techniques compared to conventional culture methods [100]. However, PCR techniques do not provide information on the viability and resistance patterns of the identified bacteria and PCR studies are expensive because of the abundance of potential bacterial species present in PTA. A novel method is microarray, which may provide even more information regarding fastidious bacteria or bacteria in small numbers. However, these methods do not address the major problem of determining which bacteria are significant pathogens. Quantification of bacteria may provide important information concerning the significance of the cultured bacteria in question. Quantification can be done using the classical dilution streak technique, preferable from pus (and not swabs). In the detection of virus, PCR is the method most commonly used, due to relatively high sensitivities and specificities. Alternatives are culture and detection of antigens and antibodies.

Normal tonsillar flora
The tonsils are normally heavily and diversely colonized. Frequently, bacteria thought to have pathogenic potential and importance can also be found in non-infected and healthy tonsils [101-103]. Hence, tonsillar infections may be derived from the normal flora (opportunistic infections).

The polymicrobial nature of PTA
A polymicrobial mixture of aerobes and anaerobes can be obtained from the majority of PTA aspirates, if cultures include both aerobic and anaerobic incubation [II, 53, 104-108]. Concurrent Epstein-Barr virus (EBV) infection is also an occasional finding. The fact that multiple bacteria can be obtained in PTA patients from an area that is normally heavily colonized raises the questions: which bacteria are true pathogens, which bacteria enhance the infection or provide an environment for pathogens to thrive in, which bacteria are insignificant bystanders, which bacteria are just contamination of the specimen as the needle passes through the mucosal surface and peritonsillar tissue, and what additional factors contribute to initiation of active infection by opportunistic pathogens? A conceptual framework for how and when an agent has pathogenic significance is discussed in detail in chapter 1.3. Risk factors for PTA development are described in chapter 5.

Change in significant pathogens during PTA development
During the period of infection and abscess development (typically four to seven days), a change in significant pathogens may occur. E.g. GAS could initiate the infectious process and other microorganisms, e.g. anaerobes, could thrive and "take over" the infection once an abscess is formed. This theoretical transition has not previously been studied.

Influence of current or recent antibiotic treatment
Inclusion of patients with antibiotic treatment prior to collection of specimens may also alter the microbiologic findings and camouflage the true pathogens. Some studies report no significant difference in bacterial flora between patients with recent antibiotic treatment and those without [53, 108-109]. Other researchers report increased recovery rates of anaerobes in patients receiving antibiotic treatment [110-111], decreased recovery of GAS [112], decreased quantity of streptococci [107], or decreased isolation rates of both aerobes and anaerobes [73]. Overall, the majority of researchers find significant alterations in bacterial recovery rates in patients taking antibiotics prior to culture.

Risk factors / host factors and demography
In contrast to acute tonsillitis, the risk factors for PTA have not been well characterized. The frequency of GAS in sore throat patients is highly related to patient age [113] (and therefore age is included in the McIsaac criteria to estimate the likelihood of GAS-positive acute tonsillitis). Furthermore, differences in tonsillar core bacteriology between children and adults with recurrent tonsillitis have been documented [114]. Hence, differences in patient age may also influence PTA culture results. Working at an Ear-Nose-Throat (ENT) department, you quickly get the impression that teenagers and young adults predominate among PTA patients. However, older patients may be managed at private ENT practices more often than younger patients (who often refuse treatment under local anaesthesia) and Aarhus County harbors the highest concentration of university students in Denmark, leaving the questions if this empiric notion regarding age is correct and to which extent does it influence culture results? No studies of age- and gender- stratified incidence rates have previously been published. Other than a characteristic age relation, PTA seems to affect both genders and all social groups with or without a history of previous tonsillar infections and in all seasons. The incidence of acute tonsillitis fluctuates with the seasons [115]. This variation is caused by fluctuations in both virus and GAS. However, no previous studies have been conducted studying the seasonal variation in microbiological findings in PTA patients. Some degree of geographical variation may exist. Differences in climate, antibiotic pressure, and socioeconomic factors are likely to influence the tonsillar microbiology. Bacterial changes over time (decades) have been reported for patients with recurrent tonsillitis and it is likely that PTA microbiology also have changed over time [116-117]. Lastly, tobacco smoking has been shown to alter the local bacterial flora [118-119] and is associated with increased risk of PTA (see chapter 5.1).
If risk factors for PTA development were identified, hypotheses for the found associations may be generated and possible interventions for prevention may be recognised.

1.3 Pathogenic significance

A cornerstone of this thesis is to explore the pathogenic significance of bacteria species and virus isolated in PTA. A pathogen is defined as a disease-producing agent. In 1890, Koch stated four criteria (postulates), which should be fulfilled before a microorganism could be considered the cause of a disease:
1. The microorganism must be present in all organisms suffering from the disease, but should not be identified in healthy organisms.
2. The microorganism must be recovered from a diseased host and grown in pure culture.
3. The microorganism from the pure culture must cause disease when inoculated into a healthy experimental organism. 4. The microorganism must be re-isolated from the new host and shown to be identical to the originally isolated agent.

However, only a minority of infectious diseases have only one

causative agent and relatively few microorganisms can be classified as always pathogenic (e.g. Rabies virus, *Plasmodium* species). Instead, most microorganisms are able to establish disease only under well-defined circumstances. None of the known potential pathogens in PTA is able to fulfill Koch´s criteria and it seems unlikely that such an agent exists. We are left with studies and findings that indirectly point to the pathogenic importance of specific microorganisms.

Nowadays, it is acknowledged that pathogenicity is not only a matter of the presence of specific species or strains, but also depends on the relative densities of these microorganisms [120]. Moreover, while some bacteria cause disease by their presence, other bacteria are associated with pathogenic processes by their absence [120]. *What makes a pathogen, is the addition, or deletion, of metabolic capabilities in the symbiome (the organismal ecosystem complete with the eukaryotic host and all of its associated microbiomes (an interacting group of microorganisms that share an ecological niche within the host)) that results in a disruption of homeostasis* [120]. Ideally, one would have to map all interactions between all microorganisms and the host at the site of infection in order to define microorganisms with pathogenic significance. The scale of such a project would demand the integration of multiple scientific approaches and massive resources. The studies in this thesis exploring the pathogenic significance of bacterial species and viruses can be seen as a few pieces in a puzzle depicting the role of all microorganisms in PTA.

Different research areas with potential to explore the pathogenic significance of individual microorganisms include (but are not exclusive to) host immunology (locally and systemically in healthy individuals and in patients during disease and health), microbiome mapping (qualitative and quantitative measures in patients and healthy subjects), core genome and supragenome sequencing of the recovered microorganisms, specific immunologic responses and non-specific inflammatory responses, *in vivo* studies, and histological studies. With the equipment and resources available, we chose a clinical approach and carried out microbiological studies using specimens obtained from individuals with well-defined diseases. Our studies are founded on the following principles to suggest pathogenic significance of a candidate agent in PTA development: (1) frequent and repeated recovery from the area of infection, (2) growth in relative abundance to other microorganisms (or even pure growth), (3) more frequent recovery and / or in relative abundance compared to the same ecological niche (the tonsils) in individuals without signs of infection (normal flora), (4) specific immunologic response towards the microorganism, (5) measurements suggesting enhanced inflammatory response.

Lastly, as PTA is often perceived as a complication of acute tonsillitis, it seems fair to assume that the establishment of pathogenic significance in acute tonsillitis for a particular microorganism, may suggest a role for that agent in the development of PTA. Some of the above-mentioned principles (number 1, 2, and 4) are recognized by most, if not all, researchers of PTA microbiology. Studies based on principles number 3 and 5 have, currently, only been conducted by our group in relation to PTA research. These novel approaches to identify significant pathogens carry many limitations (e.g. identifying normal flora), but they also seem logical and may provide important information (e.g. deduct insignificant remnants of the normal flora from the list of significant pathogens).

The complexity of the mechanisms behind the development of PTA is further highlighted by the existence of additional contributing factors (chapter 5). These contributing factors may influence the risk of PTA through changes in anatomy, the tonsillar microflora, the local immune response to colonizing microorganisms or local infection (acute tonsillitis), or the systemic immune response. These mechanisms are largely unexplored, but highlight that causation is not “one-dimensional”.

2. Hypotheses and aims

After performing a comprehensive review of the literature regarding PTA, three unclarified fields were identified prior to this thesis:

1. The significant pathogen(s) are unknown in approximately 80% of patients with PTA. Despite this these patients are (internationally) treated with de-escalated surgical surgical intervention and increasing reliance on appropriate antibiotic treatment. Acknowledgement of additional pathogens (to GAS) may be important in the prevention of PTA development, in the identitification of an optimal antibiotic regimen, and for the avoidance of complications.
2. Risk factors for the development of PTA are largely unexplored, but their identification is relevant for the development of preventative strategies.
3. Controversies exist regarding the optimal surgical approach and antibiotic regimen for patients with PPA, in part due to a lack of studies exploring the clinical characteristics and microbiology of PPA patients.

We made three hypotheses:

1. In addition to GAS, *Fusobacterium necrophorum* (FN) and possibly other microorganisms are significant pathogens in PTA.
2. Smoking, age, gender, and seasons influence PTA development.
3. PPA development is often closely linked to acute tonsillitis and PTA and these entities share common pathogens.

Hence, the aims of this thesis were:

1. To explore the microbiology of PTA with a special attention to FN.
2. To elucidate whether smoking, age, gender, and seasons are risk factors for the development of PTA.
3. To characterize patients with PPA, explore the relationship between PPA and PTA, identify the pathogens associated with PPA, and review our management of PPA.

3. Patients and methods

Three cohorts of patients constitute the basis for the eight studies of the thesis. An overview of the cohorts and the relationship between the studies is presented in Table 3.1.

Table 3.1. Overview of the patients and methods used and the relationship between the studies of the thesis.

Cohort	A	B	C
Design	Retro	Prospective Cohort comparison	Retro
Period	2001-2006	2005-2009*	2001-

Setting	AUH		Aarhus County	AUH, Aalborg University Hospital, and Holstebro Hospital				2011 AUH
Study Topics	I Bacterial flora and Inflammatory response	V Smoking	VII Epide-miology	II Bacterial flora	III Bacteremia during tonsillectomy	IV Viral flora	VIII Antibody response	VI PPA
Groups	PTA			PTA + elective tonsillectomy				PPA
No. pts	847		1,620 **	36+80		25+55	16+48	63

Abbreviations: Retro: Retrospective. AUH: Aarhus University Hospital. PPA: Parapharyngeal abscess. PTA: Peritonsillar abscess. Pts: patients.
* None of the patients included in 2005 were used in study IV (due to loss during storage).
** Consists of cohort A with addition of patients treated for PTA at Randers Hospital, private ENT practices in Aarhus County, and in the outpatient clinic at AUH (773 patients).

3.1 Cohort A

Cohort A constitutes of all 847 patients with PTA admitted to the Department of Otorhinolaryngology, Head & Neck Surgery, AUH, a tertiary clinic, in the period 2001-2006. In study VII, additional information concerning the numbers of patients treated for PTA over this same period at Randers Hospital, private ENT practices in Aarhus County, and in the outpatient clinic at AUH was also acquired. For these 773 patients information regarding age, gender, and date of diagnosis were obtained, but the bacterial culture results were unavailable.

In study I, routine culture results and blood work results (available from 760 of 847 patients admitted to AUH) were used to make comparisons in inflammatory markers between patient groups with different bacterial findings. Data were extracted from the medical records.

In addition to the data obtained in study I, we extracted information concerning tobacco smoking behavior from the medical records (available for 679 (80%) patients) in study V. Age- and gender-stratified data on smoking habits in the Danish population (from the same six years) from *the Annual Smoking Habit Survey* were provided by the Danish Cancer Society. Using these data sets, we calculated age-stratified odds ratios of smoking for patients with PTA compared to the general Danish population. Using age- and gender-stratified population data for Aarhus County (for the same six years) from Statistic Denmark, we calculated whether smoking could potentially explain the observed difference in PTA incidence between males and females.

Using information on age, gender, and season from all centers in Aarhus County (1,620 patients) and age- and gender-stratified population data for Aarhus County from Statistic Denmark, I explored the age- and gender-stratified incidence rates and the seasonal variation of PTA in study VII. Furthermore, the gender-, age-, and seasonal-stratified microbiology of PTA was explored using the 847 in-patients at AUH only.

3.2 Cohort B

Cohort B constitutes of 36 patients that underwent acute bilateral tonsillectomy due to PTA and 80 patients (controls) admitted for elective tonsillectomy due to either recurrent tonsillitis (30 patients), tonsillar hypertrophy (20 patients), both recurrent tonsillitis and tonsillar hypertrophy (20 patients), or halitosis or persistent sore throat syndrome (10 patients). None of the controls had symptoms or signs of infection or inflammation at the time of surgery. Patients were included in the study between November 2005 and February 2009 at three ENT-departments: AUH, Aalborg Hospital, and Holstebro Hospital. The handling of specimens was performed identically and independent of location. At AUH, 11 PTA patients and 18 controls were included, at Aalborg Hospital 22 PTA patients and 43 controls were enrolled, and at Holstebro Hospital three patients with PTA and 19 controls were included. All patients were included by Jens-Jacob Henriksen or Tejs Ehlers Klug. In this time period, we were under education to become ENT specialists and shifted place of work according to a planned schedule that included rotations at the three ENT departments. Hence, the study followed our work schedule. The three departments had the same indications for acute and elective tonsillectomy during the period.

The patients in cohort B were used for the studies II, III, IV, and VIII. Unfortunately, some specimens were lost during storage and as a consequence only 25 / 16 PTA patients and 55 / 47 controls were included in study IV and VIII, respectively. Each of the studies were executed according to individual power estimations, which were considered for all of the studies and power calculations were performed whenever possible.

All bacterial and viral analyses were carried out at the Department of Clinical Microbiology, AUH. Blood culture bottles (study III) were incubated between two and seven hours after they were obtained. Tonsillar tissue, tonsillar surface swabs, and pus specimens were stored at minus 80^{o}C until the bacteriologic (study II) and virus (study IV) investigations were performed. The tonsils were placed in sterile plastic containers separately without the use of cryoprotectant at the time of surgery and left untreated until culture and subsequent viral analysis. Bacterial cultures were performed in March 2009. Hence, specimens were stored between one month and three years and three months. Virus analyses were carried out in the period September 2010 to July 2011 and antibody analyses were performed in November 2013. The culture media and techniques used are described in the corresponding articles.

Only patients aged 8 to 30 years were included in the study. At the ENT department, AUH, is was mandatory to send tonsils removed from patients older than 30 years for histological examination to rule out underlying malignancy. PTA is rare in patients younger than 8 years and they may harbor other bacteria than patients in high incidence age groups. Hence, we speculated that inclusion of a few very young patients could obscure our findings. This argument may also apply to older patients. Moreover, it would have been difficult to obtain patients older than 30 years for the elective tonsillectomy group.

Study II is the only study in the literature comparing bacterial findings in PTA patients with clinically non-infected tonsils [II]. To make such a comparison, comparable materials must be obtained

and we speculated that tonsillar core tissues constitute the most appropriate specimens for cultures for the clinically non-infected tonsils. We hypothesised that potential pathogens recovered from aspirated pus would also be present in the tonsillar core on the side of the abscess. The limitations and concerns regarding the use of tonsillar tissue for comparison and the use of tonsils from electively tonsillectomzed patients as controls are discussed in chapter 6.2.

3.3 Cohort C

Cohort C constitutes of all 63 patients with PPA admitted to the ENT Department, AUH, from 2001 to 2011. Clinical diagnosis of PPA was based on symptoms and signs of deep neck infection with visual detection of pus laterally to the pharyngeal constrictor muscle. Similarly, the diagnosis of PTA was based on visual detection of pus between the tonsillar capsule and the pharyngeal constrictor muscle. Patients with and without concurrent PTA were compared with regards to symptoms and clinical and biochemical findings. We used the term "parapharyngeal abscess (PPA)" to represent abscesses surgically recovered laterally to the pharyngeal constrictor muscle and well knowing that only a minority of the patients had an abscess radiologically localised to the parapharyngeal space. Hence, "parapharyngeal abscess" was used as a clinical (surgical) term and different to "parapharyngeal space abscess" (radiologic term). Culturing and identification of bacteria were performed as part of the routine procedures.

4. Microbiology of peritonsillar abscess

4.1 Bacteriology

4.1.1 Overview of studies

Tables 4.1.1.1 and 4.1.1.2 give a simplified overview of PTA microbiology based on 32 studies, from the last 42 years, with a focus on PTA pus bacteriology. The diversity of aerobic and anaerobic findings is much greater than illustrated in Table 4.1.1.2, as recovery rates are only presented for the 10 most commonly recovered bacteria. Most likely, the microbiological diversity found in PTA reflects the normal bacterial diversity of the oropharynx and of the tonsils in particular [101-103].

As illustrated in the two tables there is much inconsistency between the studies on PTA microbiology. The average number of isolates per specimen varied from 0.5 to 7.7. Aerobes were recovered from 18 to 98% of patients. When anaerobic culture was performed (in 30 of 32 studies), anaerobes could be isolated in 8 to 100% of patients and mixed aerobic and anaerobic flora was found in 0 to 81% of patients.

When considering only prospective studies that define PTA bacteriology and focus on culturing all aerobes and anaerobes whether believed to be pathogenic significant or not, more consistent results are reported [II, 53, 73, 104-107, 122-123, 125, 127, 138]. Disregarding the studies by Sugita et al and Sunnergren et al [123, 138], more than two isolates per specimen were obtained in the other ten studies. Aerobes were recovered in 60 to 91% of patients, and anaerobes in 63 to 100% of patients [II, 53, 73, 104-107, 122, 125, 127].

Only bacterial findings in PTA pus specimens are displayed in Table 4.1.1.2. Hence, variations cannot be explained by differences in sampling location. Great variations in handling and culturing methods exist across the studies. In some studies (semi)-quantitative cultures were done [I, II, 53, 107, 110-111, 121-122], and some researchers (study I and II included) have used bacterial growth as an indicator of significance and reported only those cultures with moderate to heavy growth [I, V, VII, 110-111].

The interpretation of bacteriologic findings in the studies described in Table 4.1.1.1 and 4.1.1.2 is also complicated by the fact that all but four studies [II, 106, 122, 125] pool patients with and without prior antibiotic treatment. Studying 760 culture results from 847 patients, we found significantly decreased positive culture rates among patients with antibiotic treatment (45%) compared to patients without preadmission antibiotics (59%) ($P<0.001$, Fisher´s exact test) [I]. The recovery rates were significantly decreased for both FN (36% vs 44%) and GAS (26% vs 33%) among antibiotically treated patients compared to non-treated patients ($P=0.0029$ and $P=0.0093$, respectively, Fisher´s exact test). Antibiotic treatment was not associated with significant differences in clinical or biochemical findings at admission.

As described in chapter 1.2, many other factors may play a role for the observed discrepancies. None of the studies have addressed a potential shift in significant pathogens during the cource of the infection, nor possible variations due to the seasons, patient age, and smoking status.

Moreover, none of the studies address the obvious problem that some of the detected microorganisms may have been derived from the adjacent heavily colonized area.

4.1.2 Potential pathogens

With reference to the considerations for determining pathogenic significance of individual microorganisms, key findings supporting a role for various pathogens in PTA are outlined below.

4.1.2.1 Group A Streptococci

GAS is widely recognized as pathogen in PTA by clinicians and researchers. The background for this common view relies, most likely, on the fact that GAS is a well-documented pathogen in acute tonsillitis, and it is widely believed that PTA is a complication of acute tonsillitis. Furthermore, GAS is a well-known pathogen in other human infections (e.g, erysipelas, sepsis, necrotizing fasciitis) with well-described virulence factors (e.g streptolysin O and S).

GAS has been established as the most frequent and significant bacterial pathogen in acute tonsillitis for decades. It has been recovered significantly more frequently among patients with acute tonsillitis than healthy controls [139-140] and antibiotics reduce the duration of symptoms [141-147] and the risk of acute rheumatic fever [148-149]. Moreover, the development of GAS-specific antibodies (anti-streptolysin O, anti-hyaluronidase, and anti-deoxyribonuclease B) during the weeks following GAS-positive acute tonsillitis cases has been documented by several researchers [142, 150-152].

These important observations form the background for the fact that cultures and speciation in PTA studies are consistently done with a focus on GAS detection. Keeping that in mind, GAS is the only bacterium that has been consistently detected in PTA aspirate studies with recovery rates between 10% and 50% (Table 4.1.1.2). Regardless of the focus on GAS, this consistency also suggests that GAS is a true pathogen in PTA. The average GAS isolation rate based on the 29 studies presented in Table 4.1.1.2 (three studies did not specify beta-hemolytical streptococci grouping [126, 129, 131]) is 22%.

Table 4.1.1.1. Studies on peritonsillar abscess bacteriology published 1974-2015.

Study	Cultures (no)	Mean age (years)	(semi)-quanti-fication	Prior anti-biotics (%)	Average isolates / specimen	Cultures positive for aerobes (%)	Cultures positive for anaerobes (%)	Mixed ae-robes & anaerobes (%)
Sprinkle et al, 1974 [121]	6	15	Q	NS	1.5	67	67	50
Flodström & Hallander, 1976 [122]	37	NS	SQ	No	2.4	84	76	60
Brook, 1981 [104]	16	10	No	Yes (69%)	7.7	81	100	81
Sugita et al, 1982 [123]	30	NS	No	Yes	1.4	50	77	27
Jokinen et al, 1985 [105]	41	28	No	Yes (49%)	3.0	83	90	61
Stegehuis & Schousboe, 1986 [124]	42	24	No	Yes (66%)	2.0	79	52	36
Tunér & Nord, 1986 [106]	40	30	No	No	2.1	NS	NS	NS
Haeggström et al, 1987 [125]	10	NS	No	No	2.1	60	70	30
Jokipii et al, 1988 [107]	42	28	SQ	Yes (55%)	3.2	91	67	62
Brook et al, 1991 [108]	34	32	No	Yes (53%)	3.1	82	94	76
Snow et al, 1991 [126]	91	29	No	Yes (59%)	0.7	49	21	16
Savolainen et al, 1993 [127]	85	21	No	Yes (54%)	4.2	87	85	NS
Jousimies-Somer et al, 1993 [53]	124	21	Q	Yes (57%)	4.4	86	82	70
Muir et al, 1995 [109]	46	25	No	Yes (30%)	2.2	70	74	59
Prior et al, 1995 [110]	44	29	SQ	Yes (61%)	4.1	13	84	0
Mitchelmore et al, 1995 [111]	53	30	SQ	Yes (61%)	3.3	25	81	9
Lilja et al, 1997 [128]	51	18	Q	NS	1.3	92	65	61
Matsuda et al, 2002 [129]	466	32	No	Yes	1.3	83	NS	NS
Sakae et al, 2006 [73]	30	24	No	Yes (63%)	2.3	83	63	60
Gavriel et al, 2008 [130]	295	28	No	NS	0.5	NS	NS	NS
Sunnergren et al, 2008 [138]	83	27	No	Yes (31%)	0.8	40	37	NS
Megalamani et al, 2008 [131]	60	NS	No	NS	0.7	65	NS	NS
Gavriel et al, 2009 [132]	281	32	No	NS	0.5	28	24	NS
Segal et al, 2009 [133]	126	13	No	Yes (33%)	0.5	43	8	8
Hidakaet al, 2011 [134]	65	35	No	Yes (61%)	1.4	85	31	26
Hsiao et al, 2012 [85]	45	13	No	NS	2.1	NS	71	NS
Takenaka et al, 2012 [135]	57	NS	No	Yes (37%)	1.5	37	72	21
Albertz & Nazar, 2012 [136]	69	24	No	Yes (100%)	1.6	62	36	NS
Mazur et al, 2015 [91]	45	31	No	Yes (47%)	1.1	98	16	11
Suzuki et al, 2015 [137]	89	NS	No	NS	2.6	NS	NS	NS
Klug et al, 2009 [I]	**760**	**21**	**SQ**	**Yes (38%)**	**0.6**	**32**	**29**	**6**
Klug et al, 2011 [II]	**36**	**18**	**SQ**	**No**	**3.7**	**89**	**92**	**81**

Abbreviations: Q: Quantification. SQ: Semi-quantification. NS: not specified.

Notes. In the category "prior antibiotics" it is unlikely that studies with the label "not specified" have exclusively included non-treated patients, as these were all retrospective evaluations. Mean ages and percentages of patients treated with antibiotics prior to culture may be slightly imprecise in studies where cultures were only performed on a subset of the patient cohort. The results of study I and II are highlighted.

Table 4.1.1.2. Recovery rates (in %) of potential pathogens from pus specimens in studies of peritonsillar abscess bacteriology published 1974-2015.

Study	GAS	GCS/ GGS	*Staphylo-coccus au-reus*	Hemo-philus sp.	*Viridans strepto-cocci*	FN	Fuso. sp.	Bacte-roides sp.	Pepto-strepto-cocci	Prevo-tella sp.
Sprinkle et al, 1974 [121]	33	17	17			17		67		
Flodström & Hallander, 1976 [122]	43	14	11	8			43	22	38	
Brook, 1981 [104]	25		19	19	81	13	81	100	63	
Sugita et al, 1982 [123]	28			2			9	2	30	
Jokinen et al, 1985 [105]	24	20	12	7	39	7	2	39	29	
Stegehuis & Schousboe, 1986 [124]	12	24	2	10	43		7	31		
Tunér & Nord, 1986 [106]	50	5	5	5	43		20	38		
Haeggström et al, 1987 [125]	10	10	10		20		30	40	30	
Jokipii et al, 1988 [107]	24	19	14	7	40	7	5	43	36	
Brook et al, 1991 [108]	29	18	18	21	40		32	62	47	
Snow et al, 1991 [126]		31	3	1	5		2	6	4	
Savolainen et al, 1993 [127]	49	20	2	15	9	34	25	NS	29	
Jousimies-Somer et al, 1993 [53]	45	8	2	11	37	38	28	16	35	59
Muir et al, 1995 [109]	26	17	4	2	11		2	15		
Prior et al, 1995 [110]	10			2	48		36		52	
Mitchelmore et al, 1995 [111]	11		4	4	53	8	36	4	66	81
Lilja et al, 1997 [128]	16	16	8	8	26	8	6	4	2	
Matsuda et al, 2002 [129]		10			74					
Sakae et al, 2006 [73]	20	3	9	3	60		6		40	47
Gavriel et al, 2008 [130]	20	2	1	1	3		1	2	4	9
Sunnergren et al, 2008 [138]	24	6	2		4			17	3	3
Megalamani et al, 2008 [131]		23	12	3						
Gavriel et al, 2009 [132]	21	2	1	1	3		1	2	5	8
Segal et al, 2009 [133]	23	5	2	1	1					
Hidakaet al, 2011 [134]	14				31		5	2	12	14
Hsiao et al, 2012 [85]	20		4		49	4	38		16	20
Takenaka et al, 2012 [135]	20	2	4	5	40		26	12		22
Albertz & Nazar, 2012 [136]	23						30	38	33	
Mazur et al, 2015 [91]	29	2	2	2	49	3				16
Suzuki et al, 2015 [137]	8		1	1			13		2	29
Klug et al, 2009 [I]	**19**	**5**	**2**	**1**	**3**	**25**	**0.4**	**0.2**	**1**	**0.1**
Klug et al, 2011 [II]	**19**	**17**	**6**		**64**	**58**	**17**			**75**

Abbreviations: GAS: Group A streptococci. GCS/GGS: Group C and G streptococci. Sp.: Species. FN: *Fusobacterium necrophorum*. Fuso. spec.: Fusobacterium species.

Notes. "Fuso sp." includes *Fusobacterium nucleatum* and Fusobacteria only specified to species level. "GCS / GGS" includes Streptococci Group C and G and Beta-hemolytical streptococci non-group A and B. Recovery rates of "aerobes", "anaerobes", "bacteroides sp.", "peptostreptococci", and "prevotella sp." can be overestimates (especially when frequently recovered) as two or more species from each bacterial category may have been recovered from one patient (this information was not provided in all studies). Two reasons for Bacteroides being very common in some of the earlier studies and much less frequently isolated in the later studies are the facts that some Bacteroides species were re-grouped under Prevotella and others under Porfyromonas. This also explains why Prevotella species were isolated only in studies after 1993. The results of study I and II are highlighted.

Thus, GAS only explains a minority of PTA cases and other pathogens are most likely also of importance. Of note, we found complete concordance between growth of GAS from pus aspirates and tonsillar core tissue [V]. This finding suggests that GAS may not be involved in cases where the bacterium was not recovered from the abscess and the percentage of cases with involvement of GAS is probably not significantly underestimated.
A few PTA studies have been conducted using quantitative or semi-quantitative measurements [I, II, 53, 107, 111, 121-122, 128]. Jousimies-Somer et al found that 77% of GAS, 66% of the *Streptococcus milleri* group, and 65% of FN were isolated at concentrations > 10^4 cfu/mL [53]. Though not written explicitly, the interpretation must be that these frequent findings of abundant growth signify pathogenic importance. Unfortunately, the frequencies of heavy growth were not reported for other isolates and the significance of the quantitative findings is therefore difficult to interpret. Of note, Jousimies-Somer et al´s quantitative findings concerning GAS are in opposition to the findings by Lilja et al [128]. In study II, 85% (6/7) of GAS and 70% (19/27) of Beta-hemolytic streptococci recovered from tonsillar cores of PTA patients were isolated as heavy growth (4+) [II]. However, the semi-quantitative findings in controls were similar (80% of GAS and 67% of Beta-hemolytic streptococci had a heavy growth pattern) suggesting that these bacteria are also frequently present in heavy growth. A quantitative approach using a predefined volume of aspirated pus or tonsillar tissue is probably a better method than our semi-quantitative measurements in the search of evidence for pathogenic significance of individual bacteria.
Fourteen of the 32 studies (in Table 4.1.1.2) described growth of pure culture from PTA pus specimens [I, II, 73, 91, 105, 107-108, 110-111, 122-123, 125-126, 128]. GAS has been isolated in pure culture from a total of 48 patients [II, 73, 91, 107-108, 110-111, 122-123, 125, 128]. In addition, Jousimies-Somer reported 21 (out of 124) cases of beta-hemolytical streptococci growth in pure culture [53]. Combining all bacterial strains, only 93 cases of pure culture have been described in the literature. Compared to the large number of patients (3,329) included in these studies, the isolation of one single pathogen seems a rare event. Moreover, prospectively conducted studies using a more elaborate culture set-up, report polymicrobial findings in nearly all cases [II, 53, 104-108, 127]. Nevertheless, although a rare event, pathogenic significance of GAS is suggested by the fact that it has been found in pure culture in multiple, independent studies.
Flodström and Hallander measured antistreptolysin O, antistreptozyme, and antideoxyribonuclease titres in serum samples obtained at the time of the first visit and three weeks later in 30 patients with PTA [122]. They classified every case into a high titre group and a low titre group, combining the results from the three anti-streptococcal antibody tests. However, it was not stated how many of the 22 patients in the high titre group had significant anti-streptococcal antibody development between the two serum samples. Out of 16 patients with detection of beta-hemolytical streptococci in the PTA aspirate, 15 patients fell into the high titre group (all because of rise >50% in antistreptolysin O titre or antistreptolysin O values >300). The remaining 14 patients without beta-hemolytical streptococci in the abscess showed high antistreptolysin O titre in six cases and high antideoxyribonuclease titre in one case. Although not stated directly, I assume that none of these 14 patients had significant anti-streptococcal antibody development between the two serum samples. In addition to anti-FN antibody, we also measured anti-streptococcal antibodies in the same serum samples from PTA patients and controls [VIII]. Two PTA patients (both FN-negative) grew GAS and three patients grew beta-hemolytical streptococci Group G (two FN-positive) from the tonsillar core at the side of the abscess [VIII]. The one GAS-positive patient had doubling of antideoxyribonuclease B levels between serum 1 and 2 and the other patient had a four-fold increase of antideoxyribonuclease B levels and doubling of antistreptolysin O levels. No differences in anti-streptococcal antibody levels were found among the three Streptococci Group G-positive patients. Of PTA patients that were FN-positive (n=11) six patients had equal levels of both antistreptolysin O and antideoxyribonuclease B in the two serum samples, two patients had a doubling of antistreptolysin O titres (and equal antideoxyribonuclease B levels), two patients had doubling of antideoxyribonuclease B (and equal antistreptolysin O levels), and one patient had a decrease in antideoxyribonuclease B levels. These findings of no significant anti-streptococcal antibody development in FN-positive patients, support the hypothesis that FN can act as pathogen without streptococcal co-infection.
Lastly, we detected GAS significantly more frequently in tonsillar core tissue from the abscessed side of PTA patients compared to tonsillar core tissue from clinically non-infected patients (P=0.046; Fisher´s exact test) [II].

4.1.2.2 *Fusobacterium necrophorum*

Fusobacterium necrophorum (FN) is a gram-negative, obligate anaerobic, pleomorphic rod. The subspecies recovered in human infections (subsp. funduliforme) is less virulent than the subspecies commonly found in animals (subsp. necrophorum) [153]. Hence, the two strains are very different in terms of pathogenicity, morphology, biological activities, and biochemical properties [153]. Multiple toxins have been identified as virulence factors in the pathogenesis of human FN infections. They include adhesins, leukotoxin, endotoxin, haemolysin, and proteolytic enzymes [153]. Interestingly, FN is the pathogen most commonly associated with LS. The majority of patients complain of sore throat prior to the development of LS and the tonsils are believed to be the primary focus of infection in these patients [24].
Fusobacteria have been recovered from PTA aspirates by multiple researchers (see Table 4.1.1.2), but only a minority have specified their Fusobacterium findings [53, 85, 104-105, 107, 111, 127-128]. Because the recovery of FN was only one among many other bacteria, researchers have paid very little attention to their Fusobacterium findings in PTA aspirates. Apart from the studies within this thesis, FN has only been suggested to be pathogenic significant in PTA by Jousimies-Somer et al [53]. The Finnish group found FN in 38% of PTA aspirates and highlighted that 65% of FN were isolated at high concentrations (> 10^4 cfu/mL) and that FN was recovered from 93% of pus cultures with solely anaerobic growth (the exact numbers were not stated) [53].
In study I, FN was recovered from 25% of PTA patients in routine cultures. Patients with FN displayed significantly higher neutrophil counts (P<0.001, t-test) and C-reactive protein (CRP) values (P=0.01, t-test) at admission, than did FN-negative patients [I]. These associations indicated a greater inflammatory response in FN-positive patients and suggest that FN may be responsible for this enhanced inflammation and a significant pathogen in PTA.
In an attempt to explore the pathogenic significance of FN and other hitherto unrecognized potential pathogens, we enrolled 36 patients with PTA and 80 patients undergoing elective tonsillectomy in a comparative study [II]. Compared to core tissue from

clinically non-infected tonsils (elective tonsillectomy patients, 24%), FN was detected significantly more frequently in core tissue from the abscessed side of PTA patients (56%) (P=0.001; Fisher´s exact test) [II].

To further substantiate our FN findings, we developed an immunofluorescence-based method for the detection of anti-FN antibodies in humans. We applied this method on acutely obtained and convalescent sera from the same PTA patients and controls as in study II [VIII]. Unfortunately, some of the serum samples were lost during storage before analyses were performed. Serum samples from 16 PTA patients (one patient missing serum sample 1) (mean age 18.1 years, median 17 years, range 9-29 years) and 48 electively tonsillectomized patients (one patient missing serum sample 2) (mean age 18.9 years, median 18.5 years, range 8-30 years) were analysed for the presence of anti-FN antibodies. We found a two-fold or more increase in anti-FN antibody levels in eight of 11 (73%) PTA patients with recovery of FN from pus aspirates (FN-positive patients) and in none of four FN-negative PTA patients, which was statistical significantly different (P=0.026, Fisher´s exact test) [VIII]. The development of anti-FN antibodies in FN-positive PTA patients was also significantly different from that of all and FN-positive electively tonsillectomized patients (P<0.001 and P=0.014, respectively, Kruskal-Wallis test).

Studies have also implicated FN in acute tonsillitis. Until recently, only a few retrospective studies had been conducted studying the prevalence of FN in throat swabs from patients with acute pharyngitis, acute tonsillitis, recurrent acute tonsillitis, or persistent sore throat [154-158] (Table 4.1.2.2). Four of the studies did not include a control group and are therefore very difficult to interpret, because FN is part of normal tonsillar flora [155, 157, 159-160]. In two studies, swabs were taken from healthy individuals for comparison [154, 156]. Aliyu et al. detected FN DNA in ten of 100 swabs from patients with sore throat, but in none of 100 controls [154]. Unfortunately, the mean age of the controls was 40 years, which was significantly higher than that of the patients (25 years) and this may account for the lack of FN DNA findings among healthy individuals. Also using PCR, Jensen et al found FN DNA in 29 (48%) swabs from patients with non-streptococcal acute tonsillitis [156]. That was significantly more frequent compared to control swabs (21%) from female student nurses [156]. Moreover, FN DNA was found in significantly higher quantities among patients than controls.

Within the last year, four additional studies have been conducted exploring a possible role of FN in acute tonsillitis [161-164] (Table 4.1.2.2). In a retrospective study of throat swabs taken from 486 subjects in 2009, Jensen et al isolated FN from 27.8% of patients with either acute, recurrent, or chronic tonsillitis, which was significantly greater than in the non-tonsillitis group (5.6%) [162]. In a well-performed prospective study, Hedin et al recovered FN from 15% of 15-45 year old patients with acute tonsillitis which was significantly more than in equally aged healthy controls (3.1%) [161]. Using PCR methodology, Centor et al detected FN in 20.5% of patients and 9.4% of asymptomatic students. These findings made the authors conclude that the data clearly document that FN causes pharyngitis in persons aged 15 to 30 years [163]. Lastly, Kjærulff et al found FN in 16% of cultures from patients with acute tonsillitis compared to 9% in healthy controls [164]. A metaanalysis of the data from the studies with a control group shows a convincing association between FN and acute tonsillitis, as FN was recovered from 21.2% (CI95, 18.7-23.8%) of patients compared to 7.6% (CI95, 5.8-9.7%) of healthy controls (P<0.001, chi2). Of note, culture (using selective FN agar plates) seems as sensitive (20.3% (108/532)) as PCR (22.2% (105/473)) in the recovery of FN from tonsillar swabs from patients with acute tonsillitis. Hedin et al and Centor et al found higher mean Centor scores (a clinical score system invented to estimate the likelihood of GAS-infection in patients with acute tonsillitis) in FN-positive patients compared to patients without recovery of potential pathogens, which also supports the belief that FN is a significant pathogen in uncomplicated acute tonsillitis [161, 163]. However, no studies have been conducted showing immunological response in FN-positive patients. Furthermore, it is unknown if antibiotic treatment of FN-positive patients with acute tonsillitis reduces the duration and intensity of symptoms or the risk of complications. In conclusion, there is solid evidence of an association between FN and acute tonsillitis. However, to rule out that FN may be an insignificant bystander to infection with other significant pathogens, additional studies are needed to establish FN as a clinically important pathogen in acute tonsillitis (e.g. studies of FN-positive patients showing a specific antibody response and effect of timely antibiotic treatment directed against FN).

Table 4.1.2.2. Studies on recovery rates of *Fusobacterium necrophorum* (FN) in patients with acute pharyngitis / tonsillitis.

Study	Method	Number		FN recovery rate (number)		P[a]
		Patients	Controls	Patients	Controls	
Batty [155]	Culture	248[b]	0	9.7% (24)		
Amess [157]	Culture	1157	0	4.9% (57)		
Price [158]	Culture	NS[c]		4.5% (?)		
Eaton [160]	Culture	502	0	7.4% (37)		
Aliyu [154]	PCR	100	100	12.0% (12)	0% (0)	0.0012
Jensen [156]	PCR	61[d]	92	47.5% (29)	20.7% (19)	<0.001
Hedin [161]	Culture	220	128	15.0% (33)	3.1% (4)	0.001
Jensen [162]	Culture	212[e]	176	27.8% (59)	5.6% (10)	<0.001
Kjaerulff [164]	Culture	100	100	16.0% (16)	9.0% (9)	0.199
Centor [163]	PCR	312	180	20.5% (64)	9.4% (17)	0.0014
All[f]		1,005	776	21.2% (213)	7.6% (59)	<0.001

4.1.2.3 Additional bacteria

The studies exploring the pathogenic significance of bacteria other than GAS and FN are pooled in one paragraph, because the evidence for involvement of these other bacteria and the number of studies are limited.

Brook et al studied 19 children with PTA for the presence of antibodies to four bacteria commonly found in oral flora (*Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans* (now called *Aggre-*

gatibacter actinomycetemcomitans)) using enzyme-linked immunosorbent assays (ELISA) [165-166]. Serum levels were measured at day 1 and 42 to 56 days later. An increase (at least a doubling) in antibody levels to *P. intermedia* was observed in 11 of the 13 serum samples obtained from patients from whom this bacterium was found in the abscess, and in one of the six patients where this bacterium was not recovered. Similarly, an increase in antibody levels to *F. nucleatum* was detected in 11 of 14 *F. nucleatum*-positive (based on pus cultures) patients, and in none of *F. nucleatum*-negative patients. No development of antibodies to *P. gingivalis* and *A. actinomycetemcomitans* was found.
In order to validate the specificity of our Indirect fluorescent antibody (IFA) assay, we tested five selected sera (with high or low FN antibody levels) for antibodies against *F. nucleatum*. All sera tested negative (<16), except one serum with high FN antibody level (256), which also had a low *F. nucleatum* antibody level (16) suggesting a very low level of cross-reactivity [VIII]. Furthermore, we isolated Fusobacterium non-necrophorum (probably *F. nucleatum*) significantly less frequently and in significantly lighter growth in the tonsillar cores of PTA patients compared to the tonsillar cores of the non-infected controls [II].
In addition to GAS and FN, several other bacteria play an infrequent, but documented role in acute tonsillitis, which may indirectly suggest pathogenic significance in PTA development:
Large colony forming beta-hemolytic streptococci Group C and G have been recovered from tonsillar surface swabs from acute tonsillitis patients with higher frequency than from healthy controls [167-169]. Antibiotic treatment in these cases has been shown to have a positive effect on the course of the illness [147] and a specific antibody response has been documented [151, 170].
In Sweden, *Arcanobacterium haemolyticum* was found in 2% (the authors did not state the exact number of patients) of throat swabs from patients with sore throat. This bacterium was 10 times less frequent in swabs from healthy individuals (n=550) [171]. In another study, specific antibody development was detected in seven of eight patients with *A. haemolyticum*-positive acute tonsillitis [172]. In addition, two case reports reported pure growth of *A. haemolyticum* in PTA aspirates [173-174].
Several studies suggest that *Mycoplasma pneumoniae* is a pathogen in acute pharyngitis (especially in cases with recurrent infection) among young children [140, 175]. Furthermore, in cases of recurrent tonsillitis caused by *M. pneumonia*, tonsillectomy seems to be more efficient in preventing recurrent infection, than in cases with other microbiological origins [176]. However, the prevalence of acute tonsillitis due to *M. pneumoniae* is largely unknown and the bacterium does not seem to play a role in adolescents and adults. *Chlamydia pneumoniae* has also been detected in some children with acute tonsillitis in a few studies, but the role of this atypical bacterium in the pathogenesis of this disease remains poorly characterized [175, 177].
In addition to the review of individual potential pathogens in PTA, some researchers have drawn attention to the role of anaerobes, but have not been able to specify which species carry significance: (1) Prior et al and Mitchelmore et al classified the patients into three arbitrary groups on the basis of semi-quantification and whether the isolated bacteria were aerobic or anaerobic [110-111]. Prior et al concluded that anaerobic infection is an important factor in PTA and Mitchelmore et al concluded that, from a microbiological point of view, a clear distinction could be made between aerobic and anaerobic infections. (2) Flodström et al interpreted aerobes found in sparse amounts in polymicrobial mixtures of aerobes and anaerobes as minor contamination, and therefore disregarded these findings [122]. (3) Using a quantitative method, Lilja et al found that the median value of the overall bacterial count was approximately 100 times higher in abscesses with mixed bacterial flora (7,000 x 10^5 per ml pus) than in those with GAS alone (80 x 10^5 per ml pus) [128]. (4) Lastly, Jokipii et al reported that anaerobes were isolated as confluent growth (++++) in 57% of specimens compared to 24% of specimens for non-anaerobes [107]. When bacteria seen as only one or few colonies (+) were ignored, anaerobic and non-anaerobic isolates were equally frequent and quantitatively the most important were Streptococci, Bacteroides, Peptostreptococci, and Fusobacteria. Jokipii et al concluded that semi-quantitative culture might be useful in the microbiological analysis of PTA, reducing the pool of potentially significant bacteria to the four species mentioned above.

4.1.3 Tonsillar surface bacteriology and bacteremia during quinsy tonsillectomy

The tonsillar surface bacteriology in PTA patients is not well described [112, 122, 125]. Flodström and Hallander found that 15 of 16 (94%) GAS isolates from PTA aspirates were also present in tonsillar and /or nasopharyngeal surface swabs [122]. They did not report concordances for other bacterial isolates. Haeggström et al recovered the same bacteria from surface swabs (nasopharyngeal and tonsillar) and PTA aspirates in two of 10 patients (GAS and *Streptococcus pneumonia*, respectively) [125]. Hoffmann et al, only studying GAS, detected this bacterium from tonsillar surface swabs in nine of 13 (69%) patients with GAS-positive PTA aspirates [112]. Performing a RADT on PTA pus aspirates, this test was positive in only five of 13 (38%) culture-positive cases. Hence, in the detection of GAS, tonsillar surface swabbing seemed more sensitive than performing a RADT on pus aspirates.
In study II, we obtained tonsillar surface swabs from both the side of the abscess and the contra-lateral side. Hence, this study is the first to give a more nuanced picture of the tonsillar surface bacteriology in PTA patients (Table 4.1.3.1). Moreover, we were able to describe tonsillar surface swab concordance rates between the two sides (abscess and contralateral side) and evaluate the reliability of swabbing the tonsillar surface in order to detect bacteria growing in the abscess (Table 4.1.3.1).
We found that 85% of aerobes and 80% of anaerobes could be isolated from both tonsillar surfaces (in each individual). Similarly, 83% of GAS and 100% of FN were isolated in concordance. These high concordance rates were also seen between the contra-lateral tonsillar cores (aerobes 85%, anaerobes 84%, GAS 100%, FN 95%) and indicate that both tonsils are infected in PTA patients. The surface swabs were reliable (85% detection rate) at detecting aerobes in PTA pus aspirates, but they were not reliable at detecting anaerobes (65%) including FN (56%) (Table 4.1.3.1).

Table 4.1.3.1. Bacterial isolation rates from the tonsillar surface on the side of the peritonsillar abscess (PTA side), concordance rates between surface swabs from the PTA side and the contra-lateral side (AT side), and the percentage of isolates that were detected in both the tonsillar cores and surface swabs from the PTA side are given for 36 PTA patients in study II.

	PTA side tonsillar	Concordance between	Percentage of isolates from PTA side tonsillar

Bacteria	surface swabs	surface swabs from PTA side and AT side	cores that were also detected in PTA side surface swabs
Aerobic			
BH strept			
Group A	18%	83%	100%
Group C	9%	100%	100%
Group G	6%	100%	67%
Not grouped	6%	100%	33%
Viridans strep.	94%	93%	98%
S. aureus	24%	64%	50%
Coagualse-negative staphylococci	9%	67%	50%
E. corrodens	3%	100%	50%
Neisseria species	73%	85%	96%
M. catarrhalis	3%	0%	
Corynebacterium sp	36%	76%	60%
Total aerobic		85%	85%
Anaerobic			
F. necrophorum	27%	100%	56%
Fusobacterium sp	33%	83%	56%
Prevotella sp	79%	80%	70%
Other anaerobes	9%	25%	100%
Total anaerobic		80%	65%

Abbreviations: BH: Beta-hemolytical. Strep.: Streptococci. Sp: species.

The concordance rates between tonsillar cores and surfaces were higher in acutely infected tonsils compared to non-infected tonsils. Our findings question whether FN can be reliably diagnosed using cultures from surface swabs in patients with acute tonsillitis. It has not been explored whether PCR or other more sensitive detection methods (performed on surface swab samples) are more appropriate in making the diagnosis.

Even though bacteremia during elective tonsillectomy has been well documented (in 7 to 40% of patients) [178-187] and bacteremia during quinsy tonsillectomy has not previously been studied, the European Society of Cardiology recommends antibiotic prophylaxis for patients at high risk of infective endocarditis, who undergo acute tonsillectomy due to PTA but not for patients undergoing elective tonsillectomy [188]. In order to have a more complete picture of PTA bacteriology and to evaluate these recommendations, we obtained blood cultures during the tonsillectomy of the same 36 patients with PTA and the 80 patients admitted for elective tonsillectomy on which we have performed extensive tonsillar microbiological studies [III]. We found a trend towards less frequent bacteremia during quinsy tonsillectomy (in 56% of patients) than during elective tonsillectomy (73%) (P=0.089, Fisher´s exact test). Similarly, there was a trend for fewer isolates in blood cultures obtained during quinsy tonsillectomy (average 0.9 isolates per patient) than during elective tonsillectomy (average 1.3 isolates per patient) (P=0.14, Wilcoxon rank sum test). This trend of less (frequent and severe) bacteremia during quinsy tonsillectomy was supported by our finding of significantly fewer blood culture bottles that were positive for each strain during quinsy tonsillectomy (1.1 bottles on average) compared to elective tonsillectomy (1.7 bottles on average) (P<0.001, Spearman rank correlation). However, the average time to detect bacteria in blood cultures was significantly (P=0.003, Wilcoxon rank sum test) higher in bottles obtained during elective tonsillectomy (19.1 hours) than in bottles taken during quinsy tonsillectomy (13.7 hours). Taken together, the frequency and severety of bacteremia during elective tonsillectomy seems to be at least as high as during quinsy tonsillectomy.

Viridans group streptococci and FN were the most frequently isolated bacteria from the blood cultures of PTA patients. FN was detected significantly (P=0.004, Fisher´s exact test) more often (22%) and viridans group streptococci significantly (P=0.026) less often (28%) during quinsy tonsillectomy compared to elective tonsillectomy (FN 4% and viridans group streptococci 48%). Our blood culture observations confirm the excess of FN in PTA and the diminishment of the normal tonsillar flora including viridans group streptococci, *Staphylococcus aureus*, and *Hemophilus influenzae*. An overview of the bacterial isolation rates for the tonsillar surface and core, PTA pus aspirates, and blood cultures from the 36 PTA patients is provided in Table 4.1.3.2.

Table 4.1.3.2. Bacterial isolation rates for the tonsillar surface and core (abscess side), peritonsillar abscess (PTA) pus aspirates, and blood during acute tonsillectomy in 36 PTA patients.

Bacteria	Tonsillar surface	Tonsillar core	PTA pus aspirate	Blood
Aerobic				
BH streptococci				
Group A	18%	19%	19%	6%
Group B		3%		
Group C	9%	8%	6%	
Group G	6%	9%	6%	
Not grouped	6%	6%	6%	
S. pneumoniae		3%		
Viridans streptococci	94%	89%	69%	28%
S. aureus	24%	33%	6%	3%
Coagulase-negative staphylococci	9%	3%	8%	
Enterococci				6%
E. corrodens	3%	6%	3%	
Neisseria sp	73%	69%	14%	6%
M. catarrhalis	3%			
Corynebacterium sp	36%	44%	8%	8%
Anaerobic				
F. necrophorum	27%	56%	58%	22%
Fusobacterium sp	33%	28%	17%	3%
Prevotella sp	79%	91%	57%	
Other anaerobes	9%	3%		3%

Abbreviations: BH: Beta-hemolytical. Sp: species.

Our observations challenge the distinction made by the European Society of Cardiology between quinsy and elective tonsillectomy, namely that antibiotic prophylaxis is only recommended for patients undergoing procedures to treat an established (acute) infection. However, we were unable to evaluate the significance of antibiotic prophylaxis in patients at high risk of infective endocarditis (in both patients undergoing acute and elective tonsillectomy) because the study did not provide an exact quantification of bacteremia (colony-forming units per ml blood). Based on our bacterial findings and the susceptibility patterns, we recommend the use of amoxicillin with clavulanic acid to patients at high risk of infective endocarditis, who undergo tonsillectomy, regardless of indication.

4.1.4 Synergism between *Fusobacterium necrophorum* and other bacteria

In study II, two of 21 (10%) FN isolates from PTA aspirates were found in pure culture, whereas seven (33%) were recovered as a mixture with aerobes, one (5%) with other anaerobes only, and 12 (57%) along with both aerobes and other anaerobes. In the cultures from the tonsillar core at the side of the abscess, 17 of 20 (85%) FN isolates were recovered in polymicrobial mixture with both aerobes and other anaerobes. Hence, there is a great potential for synergism between FN and other bacteria. Such synergism may play a major role in PTA development. Brook and Walker found mutual enhancement between FN and *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacteroides fragilis*, and *Bacteroides asaccharolyticus* [189]. Similarly, Price and McCallum observed synergism between FN and *Bacteroides intermedius* (now called *Prevotella intermedia*) [190]. This latter finding may be a link between the findings in this thesis that FN may be an important pathogen in PTA and the development of antibodies to *P. intermedia* observed by Brook et al [166]. The role of *P. intermedia* in PTA development needs further exploration.

The mechanisms behind the synergy between different bacterial strains may include: lowering of the redox potential by facultative bacteria, thus creating an anaerobic environment for growth of FN (and other anaerobes); the leukotoxin of FN protecting other pathogens from phagocytosis; and FN and other bacteria producing growth factors that stimulate overall bacterial growth [153].

4.2 Virology

In upper respiratory tract infections, viruses (such as rhino-, adeno-, corona-, entero-, influenza-, parainfluenza-, and respiratory syncytial virus) predominate over bacteria [191-192]. In cases with infection primarily localized to the tonsils, the most frequently detected viruses are adeno- and Epstein-Barr virus (EBV) [193-194].

Stenfors and colleagues have conducted a number of *in vitro* studies of patients with IM showing anti-bacterial defenses on the tonsillar mucosa membrane are weakened (decreased coating of bacteria with lactoferrin, lysozyme, and secretory IgA) [195-197] and that there is increased bacterial penetration into the epithelial cells [198]. The impaired immunoglobin production by B-lymphocytes may be due to EBV induced activation of T-suppressor cells [199]. This viral-induced local immunosuppression may facilitate bacterial attachment to the epithelial cells and massive bacterial colonization of the tonsils [197].

An association between viral and bacterial tonsillar infections is further substantiated by a number of studies. Brook and de Leyva found that the tonsillar surfaces of patients with IM contained more bacteria than the same tonsils two month following the IM episode [118]. This reduction in the number of bacteria over time was mostly due to a decrease in the recovery of *P. intermedia, F. nucleatum*, and *S. aureus*. Studies showing efficacy of metronidazole / tinidazole in alleviating the symptoms of tonsillar hypertrophy, and in shortening the duration of fever, in patients with IM provide further support to the role of anaerobes in this disease [200-203].

Apart from an association between EBV and bacterial infection, a few other studies provide additional evidence for an association between viral and bacterial tonsillar infection. Ylikoski and Karjalainen found that 9% of 108 young military men with acute tonsillitis had simultaneous GAS and adenovirus infection [193]. Brook and Gober described 12 children with acute tonsillitis in whom both rapid influenza A virus and GAS tests were positive [204]. Four of these 12 children had a rise in antistreptolysin O and anti-DNase B titers in convalescent sera.

The occurrence of concomitant IM and PTA has been acknowledged for decades [205-206]. Nine studies have looked at this association [I, 65, 205-211]] (Table 4.2.1). The reported incidence ranges from 1.0 to 20%. The average of the incidences based on the literature is 4.1% (CI95; 3.5-4.8%). All studies have been conducted on hospitalized patients (except for one study which does not provide information concerning whether patients were in or out-patients [211]). This may bias the association positively as PTA patients with IM may tend to be more severely clinically affected than those with PTA without concomitant IM. Most studies use an agglutination test for heterophile antibodies for the diagnosis of IM [205-210]. This test is known to lead to false positive and false negative results in the acute disease phase, in a substantial percentage of cases [212].

In study I, we based the diagnosis of IM upon identification of atypical lymphocytes in elevated numbers in the blood [I]. The 26 (3.1%) patients with concomitant IM and PTA displayed significantly lower neutrophil counts (within normal range) than did PTA patients without simultaneous IM ($P<0.001$, t-test). Previous studies have not reported significant differences in clinical appearance or markers of infection between these two groups of patients.

Two case reports describe Herpes simplex virus type 1 infection of the tonsils with complicating PTA [213-214]. The one patient was a severely immunocompromised stem cell transplant patient with chronic myeloid leukemia [214], but the other patient was a 22 year-old woman with an unremarkable medical history [213]. In a study by Tanaka et al, written in Japanese, four of 42 (9.5%) acute tonsillitis patients were thought to have primary Herpes simplex virus infection based on serum antibody profiles [215]. Apart from these studies, the association between Herpes simplex virus and acute tonsillitis / PTA seems anecdotal. With the exception of EBV, the association between viruses and PTA has not in fact been investigated until our group conducted a study on the subject [IV]. This may seem surprising, as viral infections have been shown to predispose to the development of acute bacterial complications in other contexts, such as sinusitis, acute otitis media, and pneumonia [216-218]. Hence, it is plausible that viral infections of the tonsillar mucosa predispose to secondary bacterial infection and abscess formation.

Using PCR-based assays, we examined both tonsils from 80 immunocompetent patients (25 PTA patients and 55 electively tonsillectomized controls), aged 8-30 years, without antibiotic treatment in the month prior to tonsillectomy [IV]. We detected Herpes simplex virus type 1 in one (4%), Adenovirus in five (20%), and EBV in 23 (92%) of 25 PTA patients and similar proportions in the electively tonsillectomized patients (Herpes simplex virus type 1 in four (7%), Adenovirus in six (11%), and EBV in 48 (87%) patients). Herpes simplex virus type 2, Respiratory syncytial virus A and B, and Influenza A and B were not detected in any of the 80 patients. Concordance between contralateral tonsils was poor for Herpes simplex virus type 1 (20%) and Adenovirus (27%), whereas it was high for EBV (94%). EBV load was not different between tonsils from electively tonsillectomized and PTA patients ($P=0.28$, t-test), nor between the abscessed and non-abscessed tonsil of PTA patients ($P=0.43$, t-test).

Our results confirmed prior findings that Herpes simplex virus type 1, Adenovirus, and EBV are frequently present in the tonsils

of patients with recurrent tonsillitis and tonsillar hypertrophy. Our finding of similar proportions of these viruses in PTA patients and electively tonsillectomized patients (with no apparent clinical infection at the time of surgery), suggest that viruses do not play a significant role in the pathogenesis of PTA. As described above, approximately 4% of PTA patients had concomitant IM and as our population size was small, it is possible that Herpes simplex, Adenovirus or other viruses, like EBV, are infrequently involved in PTA development. Of note, none of the 25 PTA patients in our prospective study had atypical lymphocytes in their blood samples. Patients were included throughout the year, although a lower number of PTA patients was inrolled in the spring and winter and a lower number of controls were enrolled in the spring and summer (Table 4.2.2). Hence, the frequency of the RNA viruses studied (Influenza A and B virus and Respiratory syncytial virus) may have been slightly underestimated in both groups. This is unlikely to have influenced our conclusions significantly.

Table 4.2.1 Prevalence of infectious mononucleosis (IM) in patients with peritonsillar abscess (PTA).

Study	Number of patients with concurrent IM	Number of PTA patients	Percentage
Johnsen [205]	4	402	1.0%
Portman [206]	6	30	20.0%
Arkkila [208]	64	928	6.9%
Ryan [209]	8	151	6.0%
Hanna [207]	2	128	1.8%
Shareef [211]	1	66	1.5%
Klug [I]	26	847	3.1%
Ahmad [210]	13	326	4.0%
Windfuhr [65]	22	680	3.2%
Total	**146**	**3,558**	**4.1%**

Table 4.2.2 Seasonal variation for the inclusion of patients with peritonsillar abscess (PTA, n=25) and patients undergoing elective tonsillectomy (controls, n=55) in study IV.

	PTA patients	Controls
Winter	5 (20%)	15 (27%)
Spring	3 (12%)	11 (20%)
Summer	7 (28%)	10 (18%)
Autumn	10 (40%)	19 (35%)

4.3 Conclusions

GAS is a well-established pathogen in PTA. Reviewing the literature, there are several findings supporting a role for GAS in a minority (approximately 20%) of PTA cases. They include relatively high and consistent recovery rates [I, II, 53, 73, 85, 91, 104-111, 121-136, 138], occasional growth in pure culture [II, 73, 91, 107-108, 110-111, 122-123, 125-126], development of anti-GAS antibodies [VIII, 122], more frequent isolation from PTA patients compared to electively tonsillectomised controls [II], and the fact that GAS is a well-documented pathogen in acute tonsillitis [150].
Our findings further suggest a (previously unrecognized) pathogenic role for FN. These findings include high recovery rates from PTA pus aspirates [I, II], significantly higher inflammatory markers in FN-positive patients compared to patients infected with other bacteria [I], higher isolation rates in PTA patients compared to electively tonsillectomised controls [II], the development of anti-FN antibodies [VIII], and a documented association to acute tonsillitis [156, 161-164].
The findings suggestive of pathogenic significance for both GAS and FN are indirect and limited by the fact that the pathogenesis of PTA is unclarified. The studies are based on culture methods which allows detection of multiple viable bacteria in one go. However, newer and more extensive methods (e.g. PCR and DNA-based microarray) may provide important, additional information regarding the significant pathogens. These and other approaches to identify pathogenic significance (e.g. *in vitro* studies) are desirable to confirm and validate the present findings. Furthermore, additional factors (e.g. smoking) are very likely important for PTA development and the current studies don´t have the power for multiple stratifications. Another major limitation for the establishment of FN as pathogen in PTA is the fact that studies have been carried out at our institution only. To some extent, this limitation also applies to GAS: the development of anti-streptococcal antibodies has only been reported by two groups (ours included and we had only two GAS-positive patients with two serum samples for analysis). Furthermore, only our group has documented the relative abundance of GAS in PTA patients compared to controls. The evidence for a pathogenic role of *Prevotella intermedia, Fusobacterium nucleatum*, large colony forming Group C / G streptococci, and *Arcanobacterium haemolyticum* in PTA development is tenuous.
In study II, we found Fusobacterium species (non-necrophorum) and *S. aureus* significantly less frequent and in significantly lighter growth in cultures from PTA patients compared to controls [II]. These findings may be due to overgrowth by the true pathogens during the acute infection or a preceding imbalance of the tonsillar flora. Roos et al found that a relative lack of alfa-Streptococci increased the risk of future events of GAS-positive acute tonsillitis in patients with recurrent tonsillitis [219-220]. Similar mechanisms may be important for the development of PTA.
We found high concordance rates between tonsillar surfaces (aerobes 85% and anaerobes 80%) and cores (aerobes 85% and anaerobes 84%) in patients with PTA. The high concordance rates suggest that PTA patients have bilateral tonsillar infection and that growth of FN is not secondary to abscess development, but FN can act as primary pathogen. Only 56% of FN found in PTA pus aspirates were also detected in tonsillar surface swabs (from PTA side). This finding suggests that FN may not be reliably diagnosed using cultures from surface swabs in patients with acute tonsillitis.
Viridans group streptococci and FN were the most frequent bacteria in blood cultures taken during quincy tonsillectomy, whereas viridans group streptococci, *Staphylococcus aureus*, and *Streptococcus pneumoniae* were the most commonly recovered bacteria during elective tonsillectomy. We found that the frequency and severety of bacteremia was at least as high during elective tonsillectomy as during quinsy tonsillectomy, which challenges the distinction between quinsy and elective tonsillectomy made by the European Society of Cardiology concerning the use of antibiotic prophylaxis in patients at high risk of infective endocarditis. Based on our findings, we recommend amoxicillin with clavulanic acid to patients at high risk of infective endocarditis, who undergo tonsillectomy, regardless of indication.
In vitro studies show mutual enhancement of growth between FN and other bacteria. In PTA aspirates, FN is commonly found in a polymicrobial mixture and synergy between bacteria may play a major role in PTA development. However, more studies are required to support this assumption.

From previous studies it is well-documented that EBV is involved in the development of approximately 4% of PTA´s. Using PCR-based assays, we were unable to substantiate a possible role of EBV or other virus in PTA devlopment.

5. Risk factors for peritonsillar abscess development

5.1 Tobacco smoking

It is well known that children exposed to passive tobacco smoke are at an increased risk of acute otitis media [221], otitis media with effusion [222], and respiratory illness in general [223]. Willatt reported a higher risk of sore throat episodes in children of smoking mothers [224]. Hinton et al found an increased incidence of acute tonsillitis, requiring antibiotic treatment and subsequent tonsillectomy, in children exposed to cigarette smoke on daily basis compared to non-exposed children [225]. However, in a large case-control study, Capper and Canter found no association between children awaiting tonsillectomy because of recurrent tonsillitis and parental smoking [226]. Only few researchers have explored a possible association between smoking and acute tonsillitis in adults. Murthy and Lang found a (statistically non-significant) higher proportion of active smokers (37%) among 109 adults undergoing tonsillectomy because of recurrent acute tonsillitis compared to controls (25%) [227]. In a three week prospective study of 472 recruits, German et al found no association between smoking habits and respiratory tract infections [228]. Considerably larger studies are needed to explore if an association exists between smoking and acute tonsillitis.

Using cohort A, we conducted a study concerning the association between cigarette smoking and PTA [V]. Smoking status was obtained from 679 (80%) patients. As described in detail below, PTA incidence is highly age related. For that reason, it is crucial to stratify patients according to age groups in order to make solid conclusions regarding a possible association between PTA and smoking. Gender stratification was less important in the study as we had relatively equal proportions of males and females (53% males). For all age groups, we found a higher prevalence of smoking among PTA patients compared to the Danish population in general (Table 5.1.1). For the high incidence age groups (15 - 39 years), odds ratios were in the range 2.1-2.8. Previous studies have also found higher prevalences of smokers among PTA patients than the general population [10, 47, 158-160]. However, only Dilkes et al [229] and Lehnerdt et al [67] age-stratified their patients, although they did not calculate odds ratios. Hence, our study is the first to make robust calculations on the magnitude of the association between smoking and PTA. We found that smokers had an approximately 150% (corresponding to an odds ratio of 2.5) increased risk of PTA compared to non-smokers. This applied to both genders. Controlling for age and gender, and disregarding potential biases and confounders, 16% of PTA cases could potentially be avoided if everybody stopped smoking.

The increased risk of PTA seems unrelated to the dialy number of smoked cigarettes, as PTA patients who were smokers, smoked on average less (13.5 cigarettes per day) than smokers in the Danish population (16.5 cigarettes per day). This applied to all age groups. Furthermore, the percentage of heavy smokers (>20 cigarettes per day) in our sample population (11%) was very similar to that in the Danish population (10%). As the odds ratios seem unrelated to age and age probably could serve as a surrogate variable for the number of pack years, the increased risk of PTA most likely sets in relatively quickly after smoking initiation, rather than accumulating over years of smoking.

Table 5.1.1. Prevalence of smokers among patients with peritonsillar abscess (PTA), parapharyngeal abscess (PPA), and in the Danish population in general.

	Prevalence of smokers			Prevalence of smokers		
Age (years)	PTA	Danish pop	OR (95% CI)	PPA	Danish pop	OR (95% CI)
15-19	32.5%	14.6%	2.8 (2.0-4.0)	100%	14.6%	-
20-29	42.2%	26.1%	2.1 (1.5-2.8)	60%	26.1%	4.3 (0.5-2.6)
30-39	49.4%	27.6%	2.6 (1.6-4.1)	50%	27.6%	2.6 (0.5-14)
40-49	67.4%	30.7%	4.7 (2.5-9.5)	60%	30.7%	3.4 (0.8-17)
50-	41.7%	28.5%	1.9 (1.0-3.5)	29%	28.5%	1.1 (0.3-3.3)
All	36.4%	26.8%		45%	26.8%	

Abbreviations: pop: population

FN-associated diseases predominately affect teenagers and young adults [156, 230] which coincides in terms of life stages with when cigarette smoking is commonly initiated. Therefore, we suspected that smoking might increase the risk of FN-positive PTA in particular. Hence, another objective of our study was to correlate smoking habits to bacterial findings in PTA pus specimens. However, we found no association between FN, nor any of the other microbiological findings in our patient population, and smoking. FN-positive PTA is of particular importance in teenagers and young adults (see chapter 5.3). However, in an additional analysis regarding the smoking status of PTA patients aged 15 to 25 years, there was no significant association between smoking status and the incidence of FN-positive PTA (Table 5.1.2) (P=0.63, Fischer´s exact test). Thus, tobacco smoking does not seem to promote PTA via a particular pathogenic organism. In contrast, Hidaka et al, only studying cultures from 65 PTA patients, found an association between smoking and growth of *Streptococcus milleri*, as well as a lack of anaerobic growth [134].

Furthermore, we studied 63 patients with PPA (see chapter 6) [VI]. Smoking status was obtained from 42 (67%) patients. Nineteen (45%) patients admitted to daily smoking. Smoking was more prevalent among patients with PPA compared to the Danish population in general, for all age groups (Table 5.1.1). The age stratified odds ratios for PPA patients were similar to those for the PTA patients, but were not statistical significantly different from 1. Moreover, 65% of PPA patients were males and given that the prevalence of smoking is higher in males (Table 5.1.3), the non-gender adjusted odds ratios are overestimations. Although the data set is the second largest published on patients with PPA, it is too small to make an additional substratification (by gender). Of further note, patients with PPA included in the study were admitted from 2001 to 2011, while the data used for smoking prevalence in the Danish population was collected from 2001 to 2006. Because the prevalence of smokers has slowly, but steadily, been

decreasing in Denmark, this difference in the data collection may also bias the odds ratios positively. Hence, no solid conclusions can be made concerning a possible association between PPA and smoking, but a trend, similar to the well-documented increased risk of PTA among smokers, is present.

Table 5.1.2. PTA patients aged 15 to 25 years admitted to the Ear-Nose-Throat (ENT) Department, AUH, from 2001 to 2006 stratified by smoking status, and the finding of Fusobacterium necrophorum (FN) in PTA pus aspirates.

FN status	Smokers	Non-smokers
FN-positive	43 (33%)	66 (30%)
FN-negative	88 (67%)	153 (70%)

.

Table 5.1.3. Prevalence of male and female smokers in the Danish population in the period 2001-2006.

Age (years)	Males	Females
15-19	15.3%	13.9%
20-29	28.5%	23.7%
30-39	28.9%	25.9%
40-49	32.6%	28.1%
50-	31.7%	25.0%

Note: Data from *The annual Smoking Habit Survey* (ASHS).

5.2 Male gender

Males outnumber females in 42 of 48 studies on PTA (88%) (one study had equal representation) where patient gender was reported (Table 5.2) [I, 8-11, 45, 47, 50, 54-55, 62, 66, 68, 71, 73-77, 85, 90-91, 94, 104-105, 107-109, 124-126, 128-129, 131-133, 136, 138, 207, 231-240]. In total, 4,756 males (58%, CI95 57.2%-59.4%) and 3,395 (42%, CI95 40.6%-42.7%) females were included in the studies. This overwhelming male predominance stands in clear opposition to an even greater female predominance in primary health care contacts secondary to acute tonsillitis [VII, 241]. In a large-scale validation of the Centor and the McIsaac score systems, Fine reported on 206,870 patients aged two years or older (of which 142,081 patients were aged 14 years or older) [241]. Thirty seven percent of all acute pharyngitis cases were male and in the adult population males constituted only 33%. In Denmark, female patients constituted 57% (173,168/302,605) of all consultations in general practices (in Aarhus County from 2001 to 2006) during which a RADT was performed (a fair surrogate for sore throat consultations) [VII]. Females outnumbered males for all age groups except children aged 0-9 years. The reasons for these marked gender discrepancies are largely unexplored. Hence, except for children (in whom there is a female predominance in PTA) male patients seem to be at higher risk of progression from acute tonsillitis to PTA than female subjects. Moreover, there seems to be a gender-related inverse relationship between the incidence of sore throat and PTA.

Table 5.2. Number and percent of males in studies on patients with peritonsillar abscess (PTA).

Study	Number of males	Total number of PTA patients	Male percentage
Grahne [237]	365	725	50%
Bateman [45]	65	120	54%
Beeden [47]	69	111	62%
Bonding [50]	177	317	56%
Templer [9]	59	118	50%
Nielsen [238]	42	76	55%
Holt [233]	15	41	37%
Herbild [234]	103	166	62%
Brook [104]	12	16	75%
Richardson [239]	55	115	48%
Schechter [10]	45	74	61%
Jokinen [105]	29	41	71%
Stegehuis [124]	52	83	63%
Kronenberg [235]	201	290	69%
Haeggström [125]	7	10	70%
Spires [8]	41	62	66%
Jokipii [107]	29	42	69%
Ophir [11]	65	124	52%
Stringer [54]	31	52	60%
Sørensen [240]	289	536	54%
Brook [108]	24	34	71%
Snow [126]	56	91	62%
Wolf [55]	105	160	66%
Herzon [62]	74	130	57%
Muir [109]	65	109	60%
Lilja [128]	34	51	67%
Matsuda [129]	541	724	75%
Cherukuri [236]	111	221	50%
Sakae [73]	20	30	67%
Al Yaghchi [90]	16	46	35%
Risberg [231]	100	198	51%
Sunnergren [138]	54	89	61%
Megalamani [131]	32	60	53%
Gavriel [132]	143	281	51%
Segal [133]	55	126	44%
Hanna [207]	69	128	54%
Klug [I]	448	847	53%
Hsiao [85]	31	56	55%
Albertz [136]	58	112	52%
Chau [94]	28	41	68%
Sowerby [232]	25	46	54%
Uhler [66]	282	460	61%
Ryan [77]	120	200	60%
Tachibana [74]	178	240	74%
Afolabi [76]	13	25	52%
Chung [75]	121	172	70%
Demeslay [71]	83	127	65%
Mazur [91]	64	111	58%
Shaul [68]	56	117	48%
Total	**4,756**	**8,151**	**58%**

The male predominance in PTA can perhaps in part be explained by a higher smoking frequency in men compared to women (Table 5.1.3). It is possible that females consult the health care system at an earlier stage and are therefore treated appropriately, thus avoiding complications more than males. The gender differences could also be related to differences in the immune system. Moreover, males may be more susceptible to FN infections. However, 23.0% of the males admitted to the ENT Department, AUH, in the period 2001 to 2006 were infected with FN compared to 23.1% of females (P=0.80, Fisher´s exact test). Similarly, no significant difference in the recovery rates of GAS was found between males (18.5%) and females (15.3%) (P=0.23, Fisher´s exact test).

There is currently no evidence to suggest that the relative frequencies of individual pathogens are different between the two genders.
In study V, we calculated that, after adjusting for differences in smoking habits (between males and females) and population composition, males still had a 9.5% higher incidence of PTA compared to females. An increased risk among males has also been found in studies of infection in other organs [242-243].

5.3 Age

The incidence rate of PTA is highly age dependent. Using data from the ENT department, AUH (admitted and outpatients), Randers Hospital (another ENT department in Aarhus County), and private ENT practices in Aarhus County, I calculated age- and gender-stratified mean annual incidence rates during the years 2001 to 2006 (see Table 1 in article VII). The dataset used gives a complete picture of PTA patients within Aarhus County (catchment population of 650,000). The incidence of PTA increased from childhood to reach the highest incidence in adolescence (patients aged 15-19 years) (167 cases / 100,000 per year) and declined afterwards gradually until old age (Figure 1 in VII). Girls predominated over boys until the age of 14 years. The mean annual incidence rate for girls aged 13-14 years was significantly higher (93.4 cases / 100,000 per year) compared to boys of the same age (38.0 cases / 100,000 per year) (P=0.001, Chi-square test). Subsequently, men were more frequently affected than women and the incidence rates were significantly higher for men aged 20-29 years and 40-49 years compared to women of the same age (P<0.001 and P=0.041, respectively, Chi-square test).
Risberg et al reported an annual incidence of 124 PTA cases / 100,000 persons aged 14 to 21 years [231]. Similarly, Little et al found an age-related annual PTA incidence between 6.4 and 24.7 cases / 100,000 population for persons aged 0 to 14 years and 15 to 44 years, respectively [244].
The reason for this high degree of age dependence is unknown and unexplored. We found that, among patients admitted to the ENT Department at AUH, FN-positive PTA patients were significantly younger (median age 18 years) than PTA patients in general (21 years) (P<0.001, Kruskal-Wallis test) [I]. One reason may be an increased susceptibility to infection with FN during the teenage years. FN primarily affects patients aged 15-24 years, while GAS-positive PTA is less age dependent (see Figure 2 in article VII). Hence, the relative incidence of PTA patients infected with GAS and FN was largely age dependent (Figure 5.3) and significantly different in favour of GAS for children aged 0-9 years and adults aged 30-39 years (P<0.001 and P=0.017, respectively, binomial probability test) and in favour of FN for teenagers and young adults aged 15-19 and 20-24 years (both P<0.001, binomial probability test). The mechanisms behind this potentially increased susceptibility to tonsillar pathogens in general, and FN in particular in teenagers and young adults, are unknown. A factor that can explain a (minor) part of the very rapidly increasing incidence rate from childhood to teenage life is initiation of cigarette smoking (see chapter 5.1). However, smoking cannot explain the gradual decrease in PTA incidence after the age of 20 as the prevalence of smokers does not decrease with older age (Tables 5.1.1 and 5.1.3). Haegelskjaer Kristensen and Prag found that the incidence of LS, which is also a FN-associated complication of acute tonsillitis, peaks among patients aged 15-24 years [24].

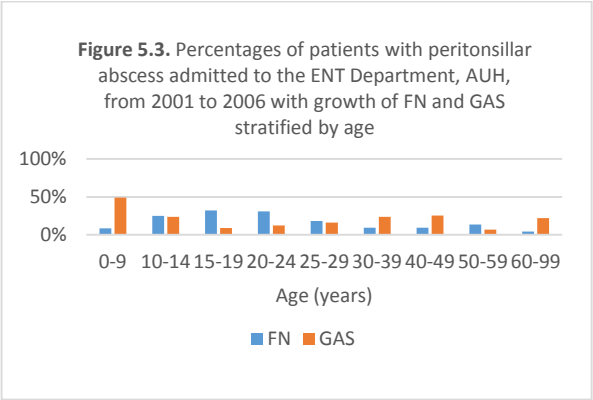

Figure 5.3. Percentages of patients with peritonsillar abscess admitted to the ENT Department, AUH, from 2001 to 2006 with growth of FN and GAS stratified by age

5.4 Season and climate

In Houston, USA, Spires et al reported that 70% of 62 PTA patients presented during the five month period from October to February [8]. In Atlanta, USA, Schechter et al found a trend toward more patients in April, May, and December [10]. Grahne found the smallest number of PTA cases in the period January to March among 725 patients in Finland [237]. In Israel, Marom et al found a trend towards more PTA patients in the winter (116) and spring (118) than in the summer (98) and autumn (95) [72]. However, in another study on 126 Israeli children there was a trend towards more frequent PTA development in the summer and autumn [133]. Also in Israel, Wolf found no clear seasonal pattern in a study of 160 adult PTA patients [55]. In London, United Kingdom, Beeden and Evans found slight peaks in spring and autumn among 111 PTA patients [47] and in Singapore, Ong et al reported a peak incidence towards the end of the year (185 patients) [12]. Matsuda et al found no clear seasonal pattern in 724 Japanese patients [129]. In Denmark, Bonding noticed a rather regular incidence throughout the year [50]. Hence, none of the studies agree on a trend.
We used the complete dataset from Aarhus County during the years 2001 to 2006 to perform a more comprehensive study on seasonal variation [VII]. It was not possible to identify a clear pattern of presentation over the year for PTA and the variations in monthly incidence were statistically insignificant (P=0.437, Chi-square test) (Table 2 in article VII) [VII]. The three months with the lowest number of PTA cases were June (115), December (118), and November (128) and the three months with the highest numbers were July (142), March (144), and January (159). The seasonal variation was less than 8% and also statistically insignificant (P=0.754, Chi-square test). In conclusion, no significant seasonal variation seems to exist in temperate or subtropical climates in Denmark, England, Israel, Japan, or Southern USA. However, when stratifying the 847 patients admitted to the ENT department, AUH from 2001 to 2006 by month/season and microbiology an interesting pattern appears (see Figure 3 and 4 in article VII).
Although the monthly variations over the year were statistically insignificant for both FN and GAS (P=0.856 and P=0.081, respectively, Chi-square test), GAS was significantly more frequently recovered from PTA patients in the winter and spring than in the summer (P=0.002 and P=0.036, respectively, Binomial probability test) [VII]. The distribution of FN was more even over the year, but with higher incidence during the summer than the winter.

This FN-specific seasonal trend did not reach statistical significance (P=0.165, Binomial probability test). Hence, the ratio between GAS and FN was highly dependent upon season (summer vs winter: P<0.001, Chi-square test). No other researchers have explored seasonal variations in PTA microbiology. Kordeluk et al interpreted the contrast between a clear seasonal variation in cases of GAS-positive acute tonsillitis and a lack of seasonal variation in PTA incidence as an argument against the acute tonsillitis theory [115]. However, this seasonal variation in PTA microbiology may explain the lack of coherence between GAS-positive acute tonsillitis and PTA in general - it is counter balanced by an increase in FN infections during the summer. Kordeluk et al, almost certainly, did not culture for the presence of FN among patients with acute tonsillitis and a possible increase of FN-positive acute tonsillitis cases in the summer and autumn would be easy to overlook amongst the large numbers of viral upper respiratory tract infections during this time.

Table 5.4.1 Monthly and seasonal presentation of 522 inpatients with *Fusobacterium necrophorum* (FN) or Group A streptococcus (GAS)-positive peritonsillar abscess (PTA) from 2001 to 2012.

Month	No. of FN-pos PTA patients	No. of GAS-pos PTA patients	No. of FN- and GAS-pos patients	Season	No. of FN-pos PTA patients	No. of GAS-pos PTA patients
December	14	20	3			
January	22	30	4	Winter	58	79
February	22	29	0			
March	24	33	1			
April	23	26	0	Spring	86	76
May	39	17	2			
June	26	14	0			
July	22	7	2	Summer	77	31
August	29	10	0			
September	27	14	1			
October	26	13	1	Autumn	74	41
November	21	14	1			

Abbreviation: pos: positive.

Because the numbers of patients are rather small when stratifying by microbiology and season / month, an additional analysis of all 522 FN- or GAS-positive PTA patients admitted to the ENT Department, AUH, in the period 2001 to 2012 was performed (Table 5.4.1, unpublished data). The monthly and seasonal variations were statistically insignificant among the 295 FN-positive patients (P=0.17 and P=0.14, respectively, Chi-square test), while there were clear monthly and seasonal variations in GAS-positive patients (both P<0.001, Chi-square test). GAS was significantly more frequently recovered in the winter and spring than in the summer (both P<0.001, Binomial probability test) and in the autumn (P<0.001 and P=0.002, respectively). FN was more prevalent during the spring than the winter (P=0.024, Binomial probability test).
Hence, this extended analysis confirms the published observations that the prevalence of GAS-positive PTA varies greatly with seasons, while FN seems to be prevalent throughout the year, but with a trend towards lower incidence during the winter.
When stratifying the patients in study II by the finding of FN and GAS (in the tonsillar core at the side of the abscess) and seasons, a seasonal pattern similar to that found in study VII appears (Table 5.4.2). However, the number of patients in study II is, naturally, too small to make meaningful statistical calculations and draw conclusions regarding seasonal variations.

Table 5.4.2 The total number of patiets with peritonsillar abscess (PTA) and elective tonsillectomized patients (controls) in study II stratified by seasons and the finding of *Fusobacterium Necrophorum* (FN) and Group A streptococci (GAS) in the tonsillar core at the side of the abscess.

	Total number of patients		FN		GAS	
	PTA	Controls	PTA	Controls	PTA	Controls
Winter	9	26	56%	31%	22%	8%
Spring	9	20	67%	15%	22%	0%
Summer	8	10	62%	30%	13%	20%
Autumn	10	24	40%	21%	20%	4%
Total	36	80	56%	24%	19%	6%

PTA incidence rates have been reported in the range of 9 to 41 cases / 100.000 population per year [I, 62, 72, 138, 207, 231-232, 244]. In the United Kingdom [207, 244], Canada [232] and Israel [72], incidences seem lower (9 to 16 cases / 100.000 population per year) than in the USA (30) [62], Sweden (26 to 37) [138, 231], and Denmark (41) [II]. However, in the study by Hanna et al from Northern Ireland [207], only admitted patients were included and in the USA study only patients aged 5 to 59 years were considered [62].
In conclusion, the incidence rate of PTA seems stable across the seasons. However, the specific pathogens associated with PTA development fluctuate with the seasons (at least in Denmark). From the published studies, it is not clear if incidence rates are related to climate.

5.5 Recurrent PTA

In retrospective studies of patients with PTA, 4.8 to 42.5% (mean 12.5%, CI95 11.2%-13.7%) of patients had previously been treated for PTA with aspiration and / or incision (Table 5.5) [9-10, 45, 47-48, 50, 54, 71, 77, 90-91, 94-95, 124, 129-130, 207, 245]. The numbers reflect the frequencies of treatment modalities (tonsillectomy vs ID and aspiration) and, thus, provide little information regarding which treatment is optimal.
In this regard it is interesting to consider what the frequency of PTA recurrence is? Recurrence rates (excluding patients with residual disease) have been reported between 1.8 and 25.3% [8, 11-13, 55, 68, 75, 78, 234-235, 238, 240, 246-247]. The average rate of patients with PTA recurrence(s) based on the 14 published studies to date is 12.2% (CI95 11.2%-13.2%). The true number may be even higher because recurrences can develop years after the initial PTA and the mean follow-up period in the studies (when defined) ranged from 18 month to 17 years [11-12, 55, 68, 235, 246]. Gavriel et al reported a mean time between two PTA episodes of 14 months [130].
Sørensen et al studied PTA patients without recurrent tonsillitis (defined as three or more attacks of tonsillitis annually), severe chronic tonsillitis, or bilateral PTA who were treated with unilateral tonsillectomy. Twenty-four (4.5%) patients developed contralateral PTA (on the side of the remaining tonsil) and in total 33 (6.1%) patients were readmitted for contralateral tonsillectomy [240]. The risk of tonsillar disease requiring removal of the remaining tonsil was 9.3% (30/342) for patients under the age of 30 compared to 0.5% (1/194) for patients older than 30 years

(P<0.0001) [240]. These recurrence rates were possibly underestimates as the follow-up period ranged from one to 11 years, some patients were lost for follow-up (i.e. moved to another region), and not all patients developing disease in the remaining tonsil were necessarily readmitted for hospital care.
Patients with recurrent tonsillitis at the time of initial PTA development seem to have increased risk of PTA recurrence [127, 235, 248]. Kronenberg et al found that recurrent PTA was four times more frequent in patients with previous recurrent tonsillitis (29/72 (40%)) compared with 21 of 218 (9.6%) patients without (P<0.0001) [235]. Similarly, in a study by Savolainen et al, eight of 14 (47%) patients with more than three episodes of tonsillitis had recurrence of PTA compared to 13 of 77 (17%) patients with less than three acute tonsillitis episodes (P<0.05) [127]. In a study of 36 PTA patients, Harrris noted that seven of 12 (58%) patients with a history of either previous recurrent tonsillitis or PTA had PTA recurrence [248].
There is evidence to suggest that recurrence is more frequent in younger than older individuals [234-235, 238, 248]. Nielsen and Greisen found that among 44 patients treated with ID, nine of 27 patients younger than 30 years of age had recurrence compared to one of 17 patients aged 30 years or older (P<0.01) [238]. Similarly, Harris reported that seven of 29 PTA patients under 40 years of age had recurrent disease compared to one of seven patients over the age of 40 years [248]. In a study by Herbild and Bonding, 17% of patients older than 40 years had new episodes of PTA or recurrent tonsillitis compared to 48% of patients younger than 40 years (P<0.01) [234]. Lastly, Kronenberg et al reported that none of the patients over 40 years of age had recurrent PTA or recurrent infections [235].
Taken together, a previous PTA episode increases the risk of recurrent PTA significantly to approximately 12% (compared to a 2-3% lifetime risk of PTA in Denmark). Patients who also suffer from recurrent tonsillitis are at even greater risk of recurrent PTA, especially if younger than 40 years. The risk can be avoided if the patient is (completely) tonsillectomized or reduced markedly if unilateral tonsillectomy is performed in patients older than 30 years. In patients younger than 30 years, the risk of a new PTA can be reduced to approximately 9% if acute unilateral tonsillectomy is performed.
These findings may indicate that scarring or other anatomic changes inflicted by infection within the tonsil or the peritonsillar tissues increases the risk of recurrent tonsillar disease, PTA in particular. However, there is a lack of histological studies supporting this hypothesis and indicating which histological alterations are significant. The increased risk of a second episode of PTA in the contralateral tonsil suggests that histological alterations within the tonsil may be induced by infection or that other predisposing factors (i.e. abnormal local immune response) may be important for PTA development.

Table 5.5. Number and percent of peritonsillar abscess (PTA) patients, who have had previous PTA.

Study	Number of PTA patients with previous PTA	Total number of PTA patients	Percentage of patients with previous PTA
Bateman [45]	37	120	30.8%
Beeden [47]	18	111	16.2%
Brandow [48]	12	156	7.7%
Bonding [50]	35	317	11.0%
Templer [9]	14	119	8.5%
Schechter [10]	4	74	5.4%
Maisel [95]	3	45	6.6%
Stegehuis [124]	4	83	4.8%
Stringer [54]	6	52	11.5%
Chowdhury [245]	3	53	5.7%
Matsuda [129]	48	724	6.6%
Hanna [207]	21	128	16.4%
Gavriel [130]	22	295	7.5%
Al Yaghchi [90]	6	46	13.0%
Chau [94]	11	41	26.8%
Ryan [77]	42	200	20.0%
Demeslay [71]	54	127	42.5%
Mazur [91]	9	111	8.1%
Total	**349**	**2,802**	**12.5%**

5.6 History of acute or recurrent tonsillitis

Nineteen researchers report on the percentage of PTA patients with a history of tonsillar infections [10, 12, 45, 47-48, 50, 54-55, 68, 74-75, 91, 124, 127, 129, 133, 234, 237, 246]. In summary, 10-79% (mean 37% (1198/3205), CI95 35.6-39.0%) of PTA patients had a history of tonsillar diseases. However, no definitions of acute or recurrent tonsillitis were given by any of the authors and some report on history of tonsillar disease in general, while others report on the number of tonsillar infection episodes. Because of the lack of definitions and homogeneity in the referred studies, it is difficult to estimate how common a history of recurrent acute tonsillitis (with five or more episodes within last two years) or acute tonsillitis, is in patients with PTA.
In study II, 11 (31%) patients gave a history of one or more episodes of sore throat with fever and absence from work or school within the last two years. Three (8%) patients had recurrent tonsillitis (defined as five or more episodes within the last two years). In a study of 124 Finnish soldiers with PTA, Jousimies-Somer et al found that the frequency of previous tonsillar / peritonsillar infection was highest (52%) among FN-positive patients and lowest (25%) among GAS-positive patients (P<0.01) [53]. In study II, 18% of patients with a history of one or more episodes of sore throat grew GAS in the tonsillar core at the side of the abscess compared to 64% with growth of FN (P=0.08, Fischer´s exact test). However, FN was almost equally frequent among patients with (64%) and without (52%) a history of sore throat within the last two years (P=0.72, Fisher´s exact test) and so was GAS (18% vs 20%) (P=1.00, Fisher´s exact test).
Currently, there is no solid evidence for an association between PTA and recurrent tonsillitis or previous episodes of acute tonsillitis because there is no solid data on the proportion of teenagers and young adults with well-defined recurrent tonsillitis or sore throat episodes within the last year / two years in the population in general and because the studies lack appropriate definitions and homogeneity. Lastly, the previously found association between FN and previous tonsillar infection in PTA patients [53] was not supported by our study.

5.7 Other potential factors

Periodontal disease

A few researchers have conducted studies that point to an association between periodontal disease and PTA [246, 249-251]. Fried and Forrest noticed that even though the majority of patients were not questioned as to specific dental disease, this was elicited from 11 of 41 (27%) of patients with PTA and seven of 43

(16%) with peritonsillar cellulitis [246]. Studying 14,500 male military conscripts, Meurman et al reported significantly higher incidence of respiratory tract infections during the two weeks before, and the first week after, acute pericoronitis [251]. However, the following prospective study by the Finnish group failed to show significant concomitance of well-known suspected pathogens (GAS in infected tonsil samples and *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* in infected pericoronal pocket samples) [249]. Georgalas et al compared the Community Periodontal Index of Treatment Needs of 158 patients with PTA and 112 patients undergoing elective tonsillectomy for recurrent chronic tonsillitis [250]. They found that patients with PTA had 17 times higher risk of periodontitis compared to patients with recurrent tonsillitis. However, a few considerations have to be stressed. Smoking is strongly implicated in the pathogenesis of periodontal disease [252] and, as discussed above, also significantly associated with PTA. Hence, periodontitis may be a surrogate marker for smoking (or other factors). In contrast, it can be argued that the effect of smoking on PTA is due to enhanced growth of pathogens within nearby periodontal pockets. However, the evidence for an overlap of causative organisms in PTA and periodontitis is very little. None of the potential pathogens in PTA (see chapter 4) are considered pathogens in periodontitis and evidence for involvement of Prevotella species, *Fusobacterium nucleatum*, and Peptostreptococcus species (the most prominent pathogens in periodontitis [253]) in PTA is scarce. Furthermore, the prevalence of periodontitis is markedly higher among patients above the age of 35 (17.4-24.5%) compared to patients at greatest risk of PTA (20-34 years: 10.5% and probably lower for teenagers) [254]. For these reasons, it is doubtfull if periodontitis is a significant factor in PTA development.

Biofilm

A biofilm is a complex, surface-attached organization of single or multiple bacterial species embedded in a self-producing polymeric matrix. Compared to the planktonic form, this matrix improves survival and offers protection from macrophage action, antibiotics, temperature, and pH fluctuations [255]. The presence of biofilms had been detected in tonsillar tissue samples from patients with chronic infection and tonsillar hypertrophy [256-259]. Al-Mazrou and Al-Abdulaziz reported that biofilms were present in significantly more patients with chronic infection (85%) than tonsillar hypertrophy (41%) (P=0.01) [257]. Similarly, Woo et al detected biofilms in 80% of patients with recurrent tonsillitis and 45% of healthy controls (P=0.048) [259]. Hence, there is a growing body of evidence suggesting a role of biofilms in recurrent tonsillitis. However, no studies have been conducted concerning a possible role of biofilms in PTA or acute tonsillitis.

The immune system

PTA development in patients with general immunodeficiency is very rare. In fact, only one such case is described in the literature [214].

PTA development is associated with IM in approximately 4.3% of PTA cases (see chapter 4.2). As described previously, EBV induces impaired local immunological responses and consequent increased bacterial attachment to the epithelial cells and abundant bacterial colonization of the tonsils [197].

Two studies have explored if immunological changes or impairments could be found in PTA patients without concomitant EBV infection [260-261]. Lilja et al found that only a minority of the bacteria recovered from PTA pus, were opsonized by immunoglobulin or complement components [260]. Lilja et al did not include a control group, but refer to a study by Stenfors and Räisänen where immunoglobulin-coated bacteria were generally the rule on tonsillar surfaces [197]. Possible reasons for the relative lack of bacterial opsonization in PTA patients could be rapid phagocytosis following immunoglobulin coating or a blockage of the ducts from Weber´s glands (where large amounts of secretory immunoglobulin is produced). Yet another possible reason could be that the abscess encapsulates the infection and prevents immune cells from being able to attack the bacteria inside. Hence, the lack of opsonization could just be the fact that bacteria were recovered from aspirates rather than the tonsillar surface. However, it cannot be ruled out that the finding is a possible explanation for PTA development.

Human beta-defensins are endogenous peptides with antimicrobial activity against Gram-negative and Gram-positive bacteria, fungi, viruses, and protozoa [262]. Schwaab et al showed that human beta-defensins 1-4 levels were strong in the tonsillar surface epithelium, the crypt-epithelium and also within the abscess, but weak in the lymphatic follicles [261]. However, the interpretation of the study is highly speculative and the exact role of human beta-defensins in PTA and tonsillar diseases in general, are largely unexplored and hypothetic.

Alcohol

In animal models, short-term exposure to alcohol induces damage to the oral mucosa [263]. Long-term exposure induces epithelial atrophy [264], increases the permeability of the mucosa [265], decreases saliva flow with subsequent increase in bacterial concentrations [266], and induces immunosuppressive effects (i.e. T-cell depression and decreased cytotoxicity of natural killer cells) [267]. Hence, chronic alcohol abuse, binge drinking, or just common alcohol intake may be associated with acute tonsillitis or PTA development. No studies have been conducted exploring this possible association between alcohol and tonsillar infections. Nevertheless, alcohol abuse was only noted in the charts of one of 847 PTA patients in study V. However, as with smoking, a sharp rise in PTA incidence is seen in the same life stage, at which alcohol intake and binge drinking is often initiated.

5.8 Conclusions

A number of risk factors for the development of PTA were identified.

In accordance with previous studies, we found an increase in prevalence of smoking among patients with PTA compared to the general Danish population [V]. Stratifying for age and gender, study V was the first to make robust calculations on the magnitude of the association between smoking and PTA. For the high incidence age groups odds ratios were in the range 2.1-2.8 and similar regardless of gender. We suspected that smoking might increase the risk of FN-positive PTA in particular, but we found no association between smoking and FN or any of the other microbiological findings.

Reviewing the literature, 58% of patients included in PTA studies were males. In cohort A, 53% of patients were males. This male

predominance stands in clear opposition to an even greater female predominance in primary health care contacts secondary to acute tonsillitis and there seems to be a gender-related inverse relationship between the incidence of sore throat and PTA. Hence, male patients seem to be at higher risk of progression from acute tonsillitis to PTA than females. Differences in smoking habits (between males and females) and population composition could only explain a minor part of the difference in PTA incidence rates between the two genders, and we calculated that in cohort A, males had a 9.5% higher incidence of PTA compared to females after adjusting for these factors. We found no evidence to suggest that the relative frequencies of individual pathogens are different between the two genders.

We found that the incidence of PTA increased from childhood to peak in adolescence (15-19 years old) and declined afterwards gradually until old age. The reason for the high degree of age dependence is largely unexplored. The relative incidence of PTA patients infected with GAS and FN was largely age dependent with a predominance of FN in patients aged 15-24 years, while GAS-positive PTA was less age dependent. Hence, an explanation for the age-related differences in PTA incidence could be an increased susceptibility to infection with FN during teenage and young adult years. The incidence of PTA in girls was higher than in boys until the age of 14 years. Subsequently, men were more frequently affected than women and the incidence rates of PTA were significantly higher for males aged 20-29 years and 40-49 years compared to females of the same ages.

Based on cohort A and a review of the literature, the incidence rate of PTA seems stable across the seasons. However, we found that the relative recovery of GAS and FN fluctuated with the seasons. GAS was significantly more frequently recovered from PTA patients in the winter and spring than in the summer, while FN recovery was more evenly distributed over the year, but with a trend towards higher incidence during the summer months compared to the winter months. Hence, the ratio between GAS and FN was highly dependent upon season. From the published studies, it is not clear if incidence rates are affected by climate.

Studies document that a previous PTA episode increases the risk of recurrent PTA markedly to approximately 12%. In patients who also suffer from recurrent tonsillitis, the risk of recurrent PTA is even greater, especially if the patient is younger than 40 years. The risk of recurrent PTA can be avoided if a bilateral tonsillectomy is performed. In patients younger than 30 years, the risk of a new PTA can be reduced to approximately 9% if acute unilateral tonsillectomy is performed, while the risk of recurrence is smaller in patients older than 30 years. The reason for the increased risk may be scarring or other anatomic changes of the tonsil inflicted by the infection. However, there is a lack of histological studies supporting this assumption.

In the literature, there is no solid evidence for an association between PTA and recurrent tonsillitis or previous episodes of acute tonsillitis because there is no solid data on the proportion of patients with well-defined recurrent tonsillitis or sore throat episodes in cohorts of PTA patients and in the population in general. It is currently unknown if periodontal disease, immunological impairments, or alcohol consumption are risk factors for PTA development.

6. Parapharyngeal abscess and peritonsillar abscess

Much less frequent than PTA, abscesses may arise peripherally to the pharyngeal constrictor muscle. Such abscesses are referred to as parapharyngeal (PPA) or retropharyngeal abscesses according to whether located laterally or posteriorly to the pharynx. Common symptoms of PPA are sore throat, odynophagia, and fever [17, 268-271]. Signs of PPA include pharyngeal asymmetry, trismus, pharyngeal erythema and oedema, neck swelling, and torticollis [268-271]. In the literature, children (especially young children) seem to have a higher prevalence of PPA compared to adults, but there may be publication bias in favour of studies including children [17-18, 268-274]. Only few researchers report on the gender of PPA patients, but as in PTA there is a trend towards males being overrepresented [269-270, 273-274].

The pathogenesis of PPA may be direct spread of infection from the teeth or pharyngeal mucosa or indirectly via suppurative lymph nodes, secondary to upper airway infection [18, 270]. Controversies exist regarding the management of PPA in terms of surgical approach and antibiotic coverage. Some authors recommend drainage via external incision, while others are in favour of intrapharyngeal incision [19, 275-276]. Recent studies indicate that conservative treatment with intravenous antibiotics alone may be safe and efficient in selected cases, without increasing the risk of widespread infection [268, 270, 274, 277]. Most researchers recommend antibiotic therapy that includes coverage of beta-lactamase-producing bacteria [19, 268, 270, 273]. At the ENT department, AUH, the most frequently used treatment regimen consists of tonsillectomy, internal incision of the abscess, and intravenous penicillin and metronidazole. The addition of tonsillectomy to internal incision is based on the observation that the abscess is frequently located deep to and in close proximity of the tonsil. Thus, the tonsil is removed to facilitate access to the PPA and to optimize abscess drainage.

We performed a retrospective study on all patients admitted to the ENT Department, AUH, from January 2001 to December 2011 with PPA [VI]. In total, 63 patients were included, giving a mean annual incidence of 0.9 cases per 100,000 population. To our knowledge, this is the only published PPA incidence rate.

In our series, all but two (97%) patients complained of sore throat, and all but one (98%) patient had pain on swallowing. Common findings were parapharyngeal swelling on fiber endoscopy (74%), sore neck on palpation (64%), enlarged lymph nodes (57%), and trismus (43%). Furthermore, 56 (89%) patients had signs of pharyngeal (including tonsillar) mucosa infection (erythema, oedema, and / or exudates), three (5%) patients of laryngitis, and one (2%) of epiglottitis. Three (5%) patients had no apparent dental or mucosal infection. Hence, the pharyngeal mucosa was believed to be the site of infectious origin in the vast majority of patients. The fact that 33 (52%) patients had concomitant PTA stressed that the oropharynx was the most common site of primary infection.

PPA patients with and without concomitant PTA shared some similarities, namely preponderance of male gender, median age of 45-46 years, median of four days of symptoms prior to admission, and a great overlap of symptoms and findings. However, PPA patients without PTA seemed more ill as they had higher infection markers (leukocytes: 18.9 vs 15.5 x 10^9 cells/L, P=0.018, t-test; neutrophil counts: 15.9 vs 12.6 x 10^9 cells/L, P=0.015, t-test; CRP: 200 vs 147 mg/L, P=0.08, t-test), were more often admitted to intensive care (40% vs 21%, P=0.30, Fisher´s exact test), intubated or tracheotomised (37% vs 18%, P=0.28, Fisher´s exact

test), and more patients had complication (23% vs 6%, P=0.15, Fisher´s exact test) compared to PPA patients with PTA.
The association between PPA and PTA seems much higher in our study than previously documented [17-19, 268-269, 273]. Only three researchers have previously described a total of nine cases of concomitant PPA and PTA [18-19, 48]. The frequent co-existence of PPA and PTA is not only interesting in terms of the pathogenesis of PPA, but may also give rise to therapeutic considerations as both abscesses ought to be drained for optimal recovery. This favors combined tonsillectomy and intrapharyngeal incision in cases where a PTA is present or suspected.
Cultures were obtained from 61 patients (97%). The most frequently recovered bacteria were GAS, viridians group streptococci, FN, and group C / G streptococci (Table 2 in VI). No significant differences in bacteriological findings between patients with or without concomitant PTA were discovered.
On the contrary to the majority of other studies, only three (5%) patients were under the age of 18 years. PPA patients with concomitant PTA were remarkably older than PTA patients generally are. Only 24% were aged 8 to 30 years compared to 73% of patients with PTA without concurrent PPA admitted to the ENT Department, AUH [I]. This age difference may be the reason for the relatively low recovery rate of FN (5%, see Table 2 in VI) compared to our admitted PTA patients in general (41% of isolates), as this bacterium primarily causes infections in teenagers and young adults [230].
Two male patients (aged 49 and 62 years) with concomitant PPA and necrotizing fasciitis, but without PTA, were included in the study. Both patients underwent internal and external incision of the abscess and extensive debridement of necrotic tissues (external approach). The older patient underwent acute, bilateral tonsillectomy, while the younger patient was previously tonsillectomized (and no remnants were present). The younger patient was tracheotomized and both patients were intubated and admitted to intensive care unit. Additional therapy included broad-spectrum antibiotics (meropenem, clindamycin, and ciprofloxacin), immunoglobulin, and hyperbaric oxygen therapy in both patients. They had relatively long hospital admissions (16 and 18 days, respectively). The younger patient had no known co-morbidities, while the older patient was diagnosed with type 2 diabetes and hypertension. Cultures from the abscess grew mixed oral flora in the younger patient and GAS in the older patient. The older patient suffered from chronic dysphagia and cosmetic defects on his neck, while the younger patient recovered without permanent sequelae.

7. Strengths and limitations of the studies in the thesis

Study I

A major strength of the study is the number of patients (n=847) and cultures (n=760), which makes it the largest study of PTA microbiology in the literature (see Table 4.1.1.1). The patients were well-characterized and the possible factors associated with the microbiological findings (duration of symptoms, smoking status, age, gender, antibiotic treatment) were accounted for (see below).
One limitation of the study is the fact that culture and identification were performed as part of the routine diagnostic procedures. Hence, anaerobes (apart from FN) were seldomly specified and a large proportion (355/760 (47%)) of cultures were labelled "mixed oral flora" (defined as light to moderate growth of viridans group streptococci, Lactobacillus species, coagulase-negative staphylococci, Neisseria species, Prevotella species, and Fusobacterium non-necrophorum alone or in mixture) without further specification. This may have withheld important information concerning bacteria of pathogenic importance. Furthermore, cultures were based on pus aspirates or pus swab samples. Some bacteria, especially anaerobes, may not have been detected due to this latter, suboptimal sampling technique. Hence, the detection rate of bacterial pathogens is very likely to be an underestimate of the real bacterial flora. This assumption is supported by the findings in study II, where FN was recovered in pus from 58% (21/36) of patients compared to 25% (191/760) in this retrospective study. However, the finding that FN-positive patients exhibited significantly higher neutrophil counts and CRP values than FN-negative patients is likely not significantly affected by this.
Another limitation is the fact that both patients with (45%) and without (55%) antibiotic therapy prior to collection of specimens were included in the study, which may alter the microbiologic findings and camouflage the true pathogens. The recovery rates were decreased for both FN (36% vs 44%) and GAS (26% vs 33%) among antibiotically treated patients compared to non-treated patients. Antibiotic treatment was not associated with significant differences in clinical or biochemical findings at admission and, hence, did not seem to affect the finding of differences in inflammatory markers between FN-positive patients and patients infected with other bacteria.
Variables other than the bacterial findings could influence the inflammatory markers. The median age of FN-positive patients was significantly lower than for patients with other bacteria and other potential biases include duration of symptoms, gender, and smoking. The age-corrected differences in CRP and neutrophil count values between FN-positive patients and FN-negative patients were 217 nmol/L (CI95 87-348) and 1.10 x 10^9/L (CI95 0.38-1.83), respectively. These differences were statistically significant (P=0.001, and P=0.003, respectively (multiple linear regression analysis)). Similarly, the duration of symptoms-corrected differences in CRP and neutrophil count values between FN-positive patients and FN-negative patients were statistically significant (P=0.005 and P=0.001, respectively). Correcting for age, duration of symptoms, gender, and smoking, the differences in CRP and neutrophil count remained statistically significant between FN-positive and FN-negative patients (P=0.001 and P=0.009, respectively).
Our findings concerning PTA microbiology are limited to Denmark. Patients and individuals living in other geographic regions may have a different tonsillar flora due to differences in antibiotic prescription habits, climate, or other factors.
The duration of symptoms (median 5 days) leaves plenty of room for shifts in the bacterial flora from acute tonsillitis to PTA. Hence, it is possible that some patients initially had acute tonsillitis with other pathogens (e.g. GAS) and growth of FN is a subsequent overgrowth phenomenon once an abscess was formed. This study does not address this issue, but our findings in study II contradict the hypothesis of shift in pathogens (see below).

Study II

The study constitutes a novel method to address the pathogenic significance of microorganisms associated with PTA. This approach tackles some of the challenges described in chapter 1.2.

With a primary focus on exploring the microbiology of PTA and with a special attention to FN, we prospectively included 36 PTA patients treated with acute, bilateral tonsillectomy and 80 patients (controls) undergoing elective tonsillectomy for recurrent tonsillitis, tonsillar hypertrophy, persistent sore throat syndrome, or combinations of these indications. The controls had no signs or symptoms of clinical infection at the time of surgery. We obtained pus aspirates, tonsillar surface swabs (bilaterally) before removal, both tonsils, blood cultures (see study III), and serum (see study VIII). Thus, we were not only able to compare the culture results from PTA aspirates with our findings in the tonsillar cores and surfaces from the same patients (bilaterally), but also compare to those from the tonsils of clinically non-infected patients (the elective tonsillectomy group).

To make a comparison between the bacterial findings in PTA patients with non-infected controls, comparable materials must be used. We hypothesised that potential pathogens recovered from aspirated pus would also be present in the tonsillar core on the side of the abscess. However, even more potential pathogens were recovered from the tonsillar cores than from aspirated pus, and only few bacteria were isolated from aspirates only (see Table 2 in article II). Based on these findings, we believe that tonsillar core tissue provided an appropriate basis for comparison of the microbiologic flora of infected and clinically non-infected tonsils (electively tonsillectomy group).

A major limitation of the study is the fact that the bacterial isolates from control patients may not represent "normal" tonsillar flora. Ideally, our bacteriologic findings in PTA patients should be compared with the flora of tonsillar tissue from healthy individuals (without a history of prior tonsillar disease). However, such specimens were unobtainable for ethical reasons. Instead, we used tonsils from patients undergoing elective tonsillectomy. Thus, the isolated bacteria may not represent "normal" tonsillar flora. A few studies have been performed comparing tonsillar core tissue from healthy subjects with that from patients suffering from recurrent tonsillitis and tonsillar hypertrophy [101-103]. In a study of eight children with normal and recurrently inflamed tonsils, Brook and Foote found similar organisms, but the concentration (the mean number of each bacterial species per gram of tonsillar tissue was calculated) of all Beta-hemolytical streptococci, all *Bacteroides* species, and all *Peptostreptococcus* species were higher in recurrently inflamed tonsils than in the normal tonsils [101]. Stjernquist-Desatnik et al. found significantly fewer Beta-hemolytical streptococci (in particular GAS) and *Haemophilus influenza* in control patients with sleep apnoea compared to patients with recurrent tonsillitis and tonsillar hypertrophy [103]. However, further studies by Stjernquist-Desatnik and Holst showed no significant differences between the groups with regard to the frequency and quantity of anaerobic and aerobic bacteria [102]. The bacterial findings in the four subgroups of electively tonsillectomised patients in study II were comparable to those recovered from tonsillar cores of healthy control patients in the studies described above. Hence, tonsillar tissue from patients undergoing elective tonsillectomy seems to serve as a reasonable control.

With these limitations in mind, the use of controls and tonsillar cores for comparison paved the road towards separating pathogens from non-pathogens in PTA formation.

It can be argued that multiple (n=84) comparisons were made between bacterial findings in PTA patients and controls. Hence, statistically significant findings may just be random and due to the numerous comparisons. However, comparisons are made for "only" 21 different bacteria. Furthermore, 12 of the 14 statistically significant findings (using $p<0.05$) concerns only three bacteria in which all four calculations (surface PTA/AT side vs surface controls and core PTA/AT side vs core controls) were statistically significant and thus confirming each other. Hence, 84 mutually independent calculations were not performed. Moreover, the study was carried out with the knowledge that GAS is an established pathogen in PTA and our hypothesis that FN may also be a prevalent pathogen. The finding of significantly more frequent recovery of GAS and FN in PTA patients compared to controls does not seem to be random or due to multiple calculations. Calculating the statistics for all recovered bacteria, and not just for GAS and FN, would serve to underscore the results regarding GAS and FN, rather than speaking against them. Lastly, if the Bonferroni correction method is applied (x 84) (which seems far too strict in this situation), FN is still found statistically significantly ($p<0.05$) more frequently in the tonsillar core at the contralateral side of the abscess (called AT side in the article) compared to controls.

The study results can be used to evaluate the hypothesis of a shift in pathogens over the development of PTA (see chapter 1.2 and 7), an aspect that was not described in the article. The almost perfect concordance between recovery of GAS and FN from the tonsillar core at the side of the abscess and aspirated pus (see Table 2 in article II) and between the tonsillar core at the side of the abscess and contralaterally (see Table 3 in acrticle II) argue against this hypothesis. Furthermore, the anti-streptococcal antibody responses in FN-positive PTA patients were very modest (described in chapter 4.1.2.1).

A major strength of the study is the fact that only patients (and controls) without antibiotic treatment during the month preceding surgery were included. Only three previous studies of PTA microbiology have ruled out the possible risk of antibiotic induced alteration of the microbiologic findings, which may camouflage significant pathogens [106, 122, 125].

Only patients (and controls) aged 8 to 30 years were examined and our conclusions are therefore limited to this age group. Especially our conclusion that FN is the most prevalent pathogen in PTA seems to apply primarily to teenagers and young adults. However, the majority of PTA´s arise among patients in this age group [I]. On the other hand, the restricted age span can also be seen as a strength of the study, because the use of a wider age range may had obscured our findings due to age related differences in prevalent pathogens. Moreover, using identical age criteria in patients included in the same geographical area during the same time period reduce the effect of these possible biases.

A more thorough sub-specification of our *Prevotella*, Fusobacterium non-necrophorum, viridans group streptococci, and "other anaerobes" isolates, may have provided interesting and important information. This thesis suggests a prominent role for FN in PTA development. However, other bacteria may play a significant role as well. The addition of PCR methods to our studies, could potentially have provided further information concerning the variety and quantity of the bacteria present. We have shown that the finding of FN can be performed in the daily clinical setting using an inexpensive, selective FN agar plate, which also al-

lows for susceptibility testing. These two major advantages of culturing were why we chose this over PCR- or microarray-based detection techniques.
We used a rather crude semi-quantification, but a more refined quantification (with bacteria per ml of pus or per gram of tonsillar tissue) may have provided valuable information.
Due to the large number, we kept the specimens at minus 80° C until cultures were made. Although studies on the effect of freezing specimens at minus 80° C do not seem to alter the ability to isolate organisms, we cannot rule out that some bacteria sensitive to freezing or present in low numbers may not have been detected. It is a weakness of the study that we did not examine the impact of freezing on recovery. However, the risk of bias seems limited as bacteria were commonly recovered in high numbers and control specimens were treated in the same way.
GAS may be more prevalent in PTA patients than found in this study, because a proportion of patients included in this study tested negative for streptococcal antigens and were therefore not given antibiotics by their general practitioner. Hence, patients infected with bacteria other than GAS, including FN, were more likely to be included in the study due to our inclusion criteria (no antibiotic treatment within the last month) than were GAS-positive patients. However, this possible bias does not affect the comparison between elective tonsillectomized and PTA patients (and our conclusions) concerning FN, but may alter the ratio between GAS and FN in PTA patients. The ratio of the two pathogens in study I may be closer to the true ratio.
All patients were enrolled by Jens-Jacob Henriksen or Tejs Ehlers Klug at three departments during the years of our specialization. Patients were included on days we were at work. Hence, patients are not consecutive which would have been optimal. However, no PTA patients, who were asked to participate, refused inclusion. Hence, the inclusion bias seems modest. Of note, all three departments had the same indications for acute tonsillectomy due to PTA during the entire period. We organized the inclusion of patients in this way because we were concerned that increased variation in sample collection and storage would be introduced if the inclusion of patients was performed by a greater number of doctors. The fact that the materials were collected by only two researchers is a strength of the study as it is likely to have minimized variations in the collection and handling of the specimens.
Lastly, it should be stated that the study was performed in compliance with the rules for recruitement of children and adolescents. Consequently, written acceptance was obtained from both parents of children and adolescents under the age of 18 years.

Study III

The study quantifies and describes bacteremia during quinsy and elective tonsillectomy. Based on these observations an evaluation of the current antibiotic prophylaxis recommendations (in Europe) for patients undergoing tonsillectomy is performed.
This is the first study of bacteremia in PTA patients. Severeal strengths of our study include that we had a non-acutely infected control group of patients undergoing the procedure, none of the patients were treated with antibiotics prior to surgery, and we had cultures from the tonsils for all patients, so comparison between blood and tonsillar cultures could be performed. This allowed us to confirm the abundance of FN in PTA patients and viridans group streptococci in electively tonsillectomized patients.
One major limitation is the fact that the study does not provide an exact quantification of colony-forming units per ml blood. We were therefore unable to evaluate the significance of antibiotic prophylaxis in patients at high risk of infective endocarditis. Furthermore, patients included in the study were children and young adults, whereas the majority of patients with infective endocarditis are older. The predominant bacteria in PTA are related to age (see chapter 5.3) and the tonsillar flora is also different between children and adults with recurrent tonsillitis [114]. Additionally, our recommendations are based on Danish patients and may not hold true for other geographic regions, as the tonsillar flora and patterns of resistance may be different elsewhere.
The study shows that bacteremia during acute tonsillectomy in PTA patients is very frequent, but the study does not provide information as to whether bacteremia occurs in PTA patients at other times than during surgery or in PTA patients treated with aspiration or incision.
The collection of blood was carried out by only two researchers (see chapter 3.2), which served to minimize variations in the collection and handling of the specimens. The drawing of blood to each of the four bottles was stopped when 10 ml was obtained (visually guided by the lines on the bottles), which could easily be done within two minutes. Hence, the blood sample volume may have varied between eight and 12 ml and it was not confirmed in any other way. However, the same (average) volume of blood with the same variation was obtained in PTA patients and controls, and should not bias the comparison. The incubation of blood culture bottles was centralized at AUH and the interval from sampling until incubation was between one and eight hours (the bottles were kept at room temperature until incubation).

Study IV

We speculated that since viruses are frequently involved in acute tonsillitis, and PTA is considered a complication of acute tonsillitis, and given that viral infections are known to predispose to the development of acute bacterial complications in other organs, they may also be involved in the pathogenesis of PTA. However, we found no statistically significant difference in the frequency of the viruses in question (EBV, adenovirus, herpes simplex virus 1 and 2, influenza virus A and B, respiratory syncytial virus A and B) between PTA patients and electively tonsillectomized patients.
The facts that we examined tissue from both tonsils of patients with PTA as well as from control patients and that we performed quantitiative analyses for multiple, carefully selected viruses (based on previous findings in patients with acute tonsillitis) serve as strengths of the study.
Although we examined for the presence of several viruses, the number were nevertheless limited. Other viruses may be significant in PTA development. The population size was small and it is possible that the explored viruses are infrequently involved in PTA development. Moreover, it is possible that a preceding virus infection is cleared by the time-point we were sampling. Another limitation is the fact that tonsils from electively tonsillectomized patients were used instead of tonsils from healthy individuals. Hence, the viral prevalence may be different from healthy tonsils. It is further significant to note that the C_t value was high for herpes simplex virus 1 and adenovirus, indicating that the viral load was likely quite low. For EBV, no differences in viral load between the two groups were detected.

The examined RNA viruses were not detected in any of our samples. It is possible that the viral RNA was not preserved for a variety of reasons, including freezing of the tonsils at minus 80° C. However, all samples were positive for 18S RNA and generally with a strong signal. Furthermore, our findings are in line with other studies. [278-279]. We sampled a very small part of the tonsils, which may underestimate the viral prevalence.
Only patients aged 8 to 30 years were examined and our conclusions are limited to this age group. Lastly, there was a significant difference in the gender distribution of the two groups. However, there is no evidence to suggest that the prevalences of the examined viruses are different between the two genders.

Study V

The study aims to investigate whether smokers are at increased risk of PTA, if the male predominance in PTA can be explained by gender differences in smoking habits, and to elucidate if smoking is associated with certain bacterial findings.
This is the largest study to investigate the association between smoking and PTA. Therefore, the conclusions that smoking is associated with increased risk of PTA and that this association is unrelated to microbiology are quite robust.
Because the incidence of PTA is highly age related, it is crucial to stratify patients according to age groups in order to make solid conclusions regarding a possible association between PTA and smoking. Another strength of the study is that precise population data for Aarhus County and relatively solid and reliable age-stratified data on smoking habits in Denmark for the same time period were obtained. Using these data sets, the study is the first to make robust calculations on the magnitude of the association between smoking and PTA. Also using these data sets, it was possible to calculate if the observed higher incidence of PTA among males compared to females could be explained by differences in smoking habits between the two genders. Furthermore, the percentage of PTA cases, which potentially could be avoided if nobody smoked, was calculated. Admittedly, these calculations are subject to great uncertainty due to multiple potential biases and confounders, but they are the first of their kind and are relevant in the understanding of PTA epidemiology.
As the study is retrospective in nature, conclusions on causality cannot be drawn. It can be argued that smoking could potentially serve as surrogate variable for other factors associated with increased risk of PTA development, such as oral hygiene and alcohol. Future prospective studies would be more suitable regarding conclusions on causality and could better address possible confounders.
Information regarding passive exposure to smoke and previous smoking habits were rarely collected and therefore not considered in our calculations. Not considering these factors may have lead us to underestimate the effect of tobacco on PTA formation. Furthermore, information about current smoking behaviour was obtained by asking the patient either orally or in writing, and not verified otherwise. However, smoking habits in the Danish general population were also only assessed by doing a telephone survey on a representative sample of the population. In 20% of PTA patient records no information concerning smoking status was obtained, which could also bias our data set. As smoking is not associated with guilt or shame regarding upper airway infection development, the effect of this potential bias seems limited. Nevertheless, in the presence of their unknowing parents, some teenagers may have denied smoking, resulting in underestimation of the association between smoking and PTA. This might have been avoided in the surveying of the general population, because the telephone interview used here may have provided more confidentiality.
We had no data on the prevalence of recurrent PTA and it can be argued that patients, who stop smoking after experiencing a PTA may constitute a potential bias. However, in Aarhus, most younger patients (who are the ones at greatest risk of PTA recurrence) are tonsillectomized (and therefore very unlikely to experience recurrence) and it is doubtfull if an episode of PTA is reason to stop smoking for most teenagers and young adults.
Our catchment area was restricted to Aarhus County, which may not be representative of the Danish population as a whole. However, based on population data from Aarhus County, we have accounted for potential differences in age and gender structure. Furthermore, the potential for selection bias is limited as Denmark has a fairly homogenous population.

Study VI

We aimed to characterize patients with PPA, to explore the relationship between PPA and PTA, to identify the pathogens associated with PPA, and to review our management of this severe deep neck infection.
This relatively large (compared to other studies of PPA) study of patients with PPA is the first to report a mean annual incidence rate. Furthermore, the study is the first to document frequent concomitant PPA and PTA formation. This dual pharyngeal abscess development may not be surprising, but is clinically important.
Although the study comprises the second largest group of PPA patients published in international journals, 63 patients are relatively few for stratification. Hence, some of the trends noted in the comparison between PPA patients with and without concomitant PTA did not reach statistical significance (CRP levels, admission to intensive care, intubation/tracheotomy frequency, and complication frequency). Furthermore, our reported complication rates and conclusions concerning the safety and efficacy of tonsillectomy and internal incision must be regarded in this context of relatively few patients.
Similarly to article I and VII, cultures were performed on pus aspirates and pus swab samples. Culture and identification were performed as part of the routine diagnostic procedures. Furthermore, 49% of patients were treated with antibiotics prior to culturing. For these reasons, the majority of culture results were labeled “mixed oral flora” without further specification. A more thorough bacteriological set up, using pus aspirates only, would likely have provided more comprehensive information as to the pathogens involved in the development of PPA. Furthermore, separate pus aspirates from the two abscesses in patients with concomitant PPA and PTA may have provided important information regarding the significant pathogens involved in the spread of infection.
The study is retrospective and the PTA and PPA diagnoses were based on the findings during surgery as they were described in the patient journals. This raises the question if these diagnoses are reliable. Surgeons, who operate patients with PPA at AUH, have experience from multiple previous acute tonsillectomies due to PTA. Hence, they are very aware that a PTA is bounded by the tonsillar capsule and the pharyngeal constrictor muscle because

this muscle serves as the deep landmark for dissection, which must not be perforated or injured when performing a tonsillectomy. Therefore, the location of the abscesses are well-described in regards to the relation to the constrictor muscle in the journals. Hence, the categorisation of abscesses in the study seems very reliable.
Nine of the 30 PPA patients without PTA, were not tonsillectomized during the acute operation. Two of the nine patients were previously tonsillectomized and because there was no tonsillar remnants present, there were no space for a PTA. Hence, in seven patients it cannot be ruled out, that they had an undiagnosed concurrent PTA. However, it seems very unlikely that a PTA was missed in light of optimal circumstances for diagnosis (patients were in general anesthesia, mouth gag was used, and there was easy access to perform an aspiration if the surgeon was in doubt of PTA formation). Furthermore, seven of the nine patients underwent a contrast-enhanced CT scan prior to surgery showing no signs of additional PTA. Lastly, all nine patients recovered without secondary, additional tonsillectomy.

Study VII
The study aims to explore the age- and gender-stratified incidence rates of PTA, the seasonal variation of PTA, and the gender-, age-, and seasonal-stratified microbiology of PTA in order to identify risk factors for PTA development.
It is the first study to explore the associations between microbiology, seasons, age, and gender in patients with PTA. Furthermore, it is the first study to calculate age- and gender-stratified mean annual incidence rates. Similarly to study V, a strength of the study is the use of precise population data for Aarhus County. Because the associations are interconnected, it is a strength that more risk factors are calculated using the same patient cohort. Although the data set is the largest on PTA patients published in international literature, relatively few patients were in each group when stratifying by month and microbiology. Hence, the trend that FN-positive patients were more prevalent in the summer than in the winter did not reach statistical significance and the finding may just be random. Another limitation of the study is the fact that it was performed in a temperate country. Seasonal variations may be different or non-existing in other climates.
Cultures were performed on pus aspirates and pus swab samples (see chapter 3.1). Culture and identification were performed as part of the routine diagnostic procedures. Hence, some bacteria were not specified and others may not have been detected because of the specification methods and occasional suboptimal sampling technique.

Study VIII
We developed an IFA assay and measured anti-FN antibody levels in acute and convalescent sera from 15 PTA patients undergoing acute tonsillectomy and 47 patients admitted for elective tonsillectomy. Although the IFA method used for detection of anti-FN antibodies in the study is a standard method commonly used to detect antibodies to other bacteria, the method has not previously been applied for the detection of anti-FN antibodies. Of note, the IFA assay was highly specific, as indicated by the absence of cross-reactivity to *F. nucleatum*.
A strength of the study is the fact that thorough tonsillar cultures were also performed in the same patients. Based on these extensive cultures, we were able to devide patients into FN-positive and FN-negative. Thus, our findings are in continuation with the findings in study I and II and support our conclusion that FN may be of pathogenic importance in PTA. Moreover, as we also obtained blood cultures in the same patients, we were able to check whether the development of anti-FN antibodies was related to FN-bacteremia.
A major limitation of the study is that the study group was very small (and smaller than initially anticipated due to loss of sera during storage). Nevertheless, we found that anti-FN antibodies developed significantly more frequently in FN-positive PTA patients compared to FN-negative PTA patients and to electively tonsillectomized subjects.
We only included patients aged 8-30 years. Thus, our conclusion that FN is a prominent pathogen in PTA development may not apply to patients outside this age range. Moreover, we did not determine the presence of antibodies against other microorganisms recovered in PTA, with the exception of antibodies against streptococci and *F. nucleatum.* Thus, it is feasible that antibodies against other bacteria could also be present. However, for these other bacteria co-isolated in PTA pus aspirates there is very little evidence for a pathogenic role in PTA development. Furthermore, only one previous study has been performed measuring anti-streptococcal antibodies in PTA patients.

8. Summery of conclusions, clinical implications, and future research

8.1 Summery of conclusions and clinical implications

GAS has been widely recognized as an established pathogen in PTA by clinicians and researchers for decades. Prior to our studies, the findings supporting a role for GAS in PTA development included relatively high and consistent recovery rates (approximately 20%), occasional growth in pure culture, detection of relatively high levels of anti-GAS antibodies in one study, and the fact that GAS is a well-documented pathogen in acute tonsillitis.
Our findings confirm that GAS can act as pathogen in PTA, but further suggest a (previously unrecognized) pathogenic role for FN. The supportive findings include high isolation rates in a larger retrospective study and a smaller prospective study, significantly higher inflammatory markers in FN-positive patients compared to patients infected with other bacteria, higher isolation rates in PTA patients compared to electively tonsillectomised controls, and the development of anti-FN antibodies in FN-positive PTA patients.
Recent studies from other institutions document an association to acute tonsillitis as well, providing supporting evidence that FN is a pathogen in tonsillar infections overall. However, the findings pointing to a role of both GAS and FN are indirect and limited by the fact that the pathogenesis of PTA remains unclear. Furthermore, our studies suggesting that FN is a prevalent and significant pathogen in PTA have been carried out at our institution only. Additional bacteria may be significant in PTA development and synergy between bacteria may be important in the pathogenesis of PTA.
Based on our findings suggesting that FN is a frequent pathogen in PTA, clindamycin is recommended instead of a macrolide in penicillin-allergic patients with PTA. Furthermore, cultures made from PTA aspirates should include a selective FN-agar plate in order to identify growth of this bacterium.
Recent studies document an association between recovery of FN from tonsillar surface swabs and sore throat, suggesting that FN also plays a role in acute tonsillitis. Studying the bacterial flora of

both tonsils in study II, we found almost perfect bacterial concordance between the tonsillar core at the side of the abscess and contralaterally. This finding indicate that FN is not a secondary overgrowth phenomenon once an abscess is formed, but can act as pathogen in severe acute tonsillitis. With the associations described in the studies in this thesis, we recommend clinicans to have increased focus on acute tonsillitis patients aged 15-24 years, who consult health professionals with regards to symptoms and findings pointing to incipient peritonsillar involvement. It is important for physicians to know that these patients are (most often) RADT-negative. Especially teenage, smoking males seem to be at high risk of FN-positive PTA.

Using PCR-based assays, we were unable to substantiate a possible role of virus in PTA development. Previous studies (including study I) implicate EBV infection in approximately 4% of PTA patients.

There are multiple additional factors associated with increased risk of PTA development. These factors include tobacco smoking, male gender, age 15-29 years, and previous PTA.

In contrast to GAS-positive acute tonsillitis, we found that PTA occurs throughout the year without significant seasonal variation. However, the microbiology of PTA fluctuated with seasons: GAS was more prevalent in the winter while FN was prevalent throughout the year. Hence, the variations in GAS-positive PTA resembles the previously documented seasonality of GAS-positive acute tonsillitis and underscores the relationship between acute tonsillitis and PTA.

Based on our blood culture results obtained during elective and acute tonsillectomy, we recommend the use of amoxicillin with clavulanic acid to patients with a high risk of infective endocarditis who undergo tonsillectomy, regardless of indication.

We found that 52% of PPA patients had concomitant PTA. This association between PPA and PTA was much higher than previously reported. Based on this frequent intimate association, we recommend acute tonsillectomy, in addition to internal incision, in PPA patients if the abscess is located in proximity of the tonsil. The frequent co-existance of PPA and PTA suggests that the two entities share common pathogens. However, FN and GAS were only recovered from 5% and 13% of our routine cultures from PPA patients, respectively.

8.2 Future research

We have conducted a number of studies with novel findings:

1. FN is a significant and prevalent pathogen in PTA.
2. Bacteremia during abscess tonsillectomy is no more prevalent than during elective tonsillectomy.
3. The development of anti-FN antibodies in FN-positive PTA patients.

We have used novel approaches as principles to suggest pathogenic significance of candidate microorganisms:

1. Comparative microbiology between PTA patients and “normal tonsils”.
2. Measurements indicating larger inflammatory response compared to clinically equivalent infection.

The validity (both internal and external) of our findings would be strengthened substantially if our findings were to be confirmed by other centers.

In a repetition of our comparative study between the microbiology of acutely infected tonsils from PTA patients and “normal tonsils”, it would be preferable to use healthy subjects as controls. However, it is unethical to remove the tonsils from healthy individuals without clinical indication for surgery. When looking at the other categories of patients with indication for tonsillectomy (apart from the ones used in cohort B), it seems very difficult to obtain “normal tonsils”: patients with obstructive sleep apnea syndrome have hypertrophic tonsils, the tonsils of patients suspected to have tonsillar malignancy or carcinoma with unknown primary cannot be collected for ethical reasons, patients with PFAPA (periodic fever, aphtous stomatitis, pharyngitis, and adenitis) are too young and may have altered tonsillar immunology and microbiology, and very few patients with psoriasis undergo tonsillectomy and may also harbor other pathogens than healthy individuals. A voluminous biopsy from the tonsillar core could serve as an appropriate alternative to whole tonsil specimen. This approach has not previously been used, but may be feasible although recruitment and ethical issues may constitute serious obstacles. Another alternative to “normal tonsillar” tissue for comparison is tonsillar surface swab specimens obtained from “normal tonsils”. However, in culture studies, such specimens give lower bacterial recovery rates (especially anaerobes) than tonsillar core tissue specimens.

Other approaches to explore significant pathogens in PTA are also important in order to establish FN (and GAS) as significant pathogen(s) in PTA. One way to do this could be FN genome sequencing, whereby strains recovered from patients with invasive infection (e.g. PTA and LS) could be compared to strains from the tonsils of healthy subjects in order to investigate differences in virulence factors and identify more pathogenic strains.

Only one PTA study using microarray methodology has been conducted and it is unclear whether microbiome studies are helpful in identifying other potential pathogens in PTA [280]. However, it seems important to conduct more complete microbiome studies to understand the complex diversity of PTA microbiology and interactions better. It is plausible that cultures only give partial information regarding significant pathogens in PTA and that newer methods (e.g. PCR, microarray) provide a more detailed (and appropriate) picture of PTA microbiology. Supplementary studies considering mutual synergy of the bacteria associated with PTA (similar to those of FN described in chapter 4.1.4) seem important in the pursuit of significant pathogens.

To my knowledge, no animals have tonsillar tissue corresponding to human anatomy and no animals develop PTA spontaneously. Hence, the establishment of an appropriate animal model seems difficult.

We found that PTA development is highly related to age and gender, but our studies gave no indication as to why. Studies of local and systemic immune responses may be important understanding the mechanisms behind these associations at a molecular level. Studies of the local immune response may also be important in describing the mechanisms leading to either acute tonsillitis or progression to abscess formation (PTA pathogenesis). Immune cytochemical assays applied to tonsillar surface swab specimens or, better, immunehistochemical assays performed on tonsillar tissue biopsies are possible methods for such studies. Microdialysis is another interesting technique, which can be used to measure interleukins or other immunological molecules. It has not yet been

used in tonsillar studies, however practical and ethical obstacles may hinder the applicability.

Previous PTA and recurrent acute tonsillitis are likely important risk factors for PTA development. Histological studies may provide better understanding of the background for these risk factors and the pathogenesis of PTA in general. For instance, histological studies could identify whether tissue scarring or (partial) blockage of the ducts of the Weber glands may increase the risk of PTA. A study combining both histological and immunological approaches would be optimal.

Our findings suggest that FN is a prevalent pathogen in PTA and recent studies report an association between FN and acute tonsillitis. In study II, we found that the tonsillar core bacteriology (and especillay FN) was almost identical beween the two sides in PTA patients, which indicate that the growth of FN is not subsequent to abscess development, but that FN may also be significant in severe, non-abscessed acute tonsillitis. Hence, it seems obvious to suggest that FN can be a primary pathogen in acute tonsillitis and that timely antibiotic treatment directed against FN may reduce the symptoms and the risk of complications, especially PTA and LS. Future studies of patients with FN-positive acute tonsillitis focusing on the optimal methods (clinical characteristics, culture, PCR, or other) for diagnosis and whether antibiotics (and which) can reduce symptoms and avoid complications are warranted. It would be optimal to conduct a prospective, placebo-controlled, double-blinded trial randomizing patients with acute tonsillitis (from whom bilateral tonsillar surface swbs are obtained for culture and PCR analysis) for different antibiotics (penicillin, amoxicillin, and metronidazole) for 10 days and monitoring symptoms, findings, adverse reactions, and complications. In an impressive study of 14,610 patients treated for acute tonsillitis in 616 general practices, Little et al found that symptoms and clinical findings were unable to predict those who developed complications, including PTA [280]. FN may be the missing link between acute tonsillitis and PTA. A large-scale study of patients with acute tonsillitis including a quick test for detection of FN (similar to the RADT) and inflammatory markers (based on the findings in study I) in addition to symptoms, findings, gender, and age, may identify patients at high risk of PTA. However, a FN-quick test is not available yet.

Our somewhat disappointing bacteriologic findings in patients with PPA and the lack of well-defined pathogens in the literature, warrants prospective studies with a focus to detect all potential pathogens. The considerations concerning approaches to identifying significant pathogens described for PTA also apply to PPA. The low incidence of PPA constitutes a great challenge for microbiological PPA studies.

9. Summary

9.1 English

PTA is a collection of pus located between the tonsillar capsule and the pharyngeal constrictor muscle. It is considered a complication of acute tonsillitis and is the most prevalent deep neck infection (approximately 2000 cases annually in Denmark) and cause of acute admission to Danish ENT departments. Teenagers and young adults are most commonly affected and males may predominate over females. However, no studies of age- and gender-stratified incidence rates have previously been published. Furthermore, smoking may be associated with increased risk of peritonsillar abscess (PTA) development, although the magnitude of the association has not been estimated. Complications are relatively rare. They include parapharyngeal abscess (PPA), upper airway obstruction, Lemierre´s syndrome, necrotizing fasciitis, mediastinitis, erosion of the internal carotid artery, brain abcess, and streptococcal toxic shock syndrome.

The treatment consists of abscess drainage and antimicrobial therapy. There are three accepted methods of surgical intervension: needle aspration, incision and drainage (ID), and acute tonsillectomy (á chaud). Internationally, there is a strong trend towards less invasive surgical approach to PTA treatment with avoidance of acute tonsillectomy, needle aspiration instead of ID, and in some cases even antibiotic treatment without surgical drainage. The preferred antibiotic regimen varies greatly between countries and centers.

Group A streptococcus (GAS) is the only established pathogen in PTA. However, GAS is only recovered from approximately 20% of PTA patients. The pathogens in the remaining 80% are unknown. Culturing of PTA pus aspirates often yields a polymicrobial mixture of aerobes and anaerobes. As the tonsils of healthy individuals are already heavily and diversely colonized, the identification of significant pathogens is challenging. In addition, when studying PTA microbiology, one must consider diagnostic precision, collection, handling, and transportation of appropriate specimens, choice of methodology for detection and quantification of microorganisms, current or recent antibiotic treatment of patients, potential shift in significant pathogens during the course of infection, and factors associated with increased risk of PTA development.

The trend towards de-escalated surgical intervention and increasing reliance on antibiotic treatment, require studies defining the significant pathogens in PTA in order to determine optimal antibiotic regimens. Complications secondary to PTA may be avoided or better controlled with improved knowledge concerning the significant pathogens in PTA. Furthermore, identification of pathogens other than GAS, may lead the way for earlier bacterial diagnosis and timely intervention before abscess formation in sore throat patients. The identification and quantification of risk factors for PTA development constitutes another approach to reduce the incidence of PTA.

As clinicians, we noticed that FN was recovered from PTA patients with increasing frequency and that patients infected with FN seemed to be more severely affected than patients infected with other bacteria. Furthermore, we occationally observed concomitant PPA in addition to a PTA, which made us hypothesize that PPA and PTA is often closely related and may share significant pathogens.

Hence, our aims were:

1. To explore the microbiology of PTA with a special attention to *Fusobacterium necrophorum* (FN).
2. To elucidate whether smoking, age, gender, and seasons are risk factors for the development of PTA.
3. To characterize patients with PPA, explore the relationship between PPA and PTA, identify the pathogens associated with PPA, and review our management of PPA.

In a retrospective study on all 847 PTA patients admitted to the ENT department at AUH from 2001 to 2006, we found that FN was the most prevalent (23%) bacterial strain in pus specimens. FN-positive patients displayed significantly higher infection markers (CRP and neutrophil counts) than patients infected with other

bacteria (P=0.01 and P<0.001, respectively). In a subsequent prospective and comparative study on 36 PTA patients and 80 patients undergoing elective tonsillectomy (controls), we recovered FN from 58% of PTA aspirates. Furthermore, FN was detected significantly more frequently in the tonsillar cores of PTA patients (56%) compared to the tonsillar cores of the controls (24%) (P=0.001). We also analysed sera taken acutely and at least two weeks after surgery for the presence of anti-FN antibodies. We found increasing levels (at least two-fold) of anti-FN antibodies in eight of 11 FN-positive (in the tonsillar cultures) PTA patients, which was significantly more frequent compared to none of four FN-negative PTA patients and nine of 47 electively tonsillectomized controls (P=0.026 and P<0.001, respectively).
Blood cultures obtained during acute tonsillectomy mirrored the bacterial findings in the tonsillar specimens with 22% of patients having bacteremia with FN. However, bacteremia during elective tonsillectomy was at least as prevalent as bacteremia during quinsy tonsillectomy, which challenges the distinction made by the European Society of Cardiology between quinsy and elective tonsillectomy, namely that antibiotic prophylaxis is only recommended to patients undergoing procedures to treat an established infection (i.e. PTA).
Using PCR analysis for the presence of herpes simplex 1 and 2, adenovirus, influenza A and B, Epstein-Barr Virus, and respiratory syncytial virus A and B, we explored a possible role of viruses in PTA. However, our results did not indicate that any of these viruses are involved in the development of PTA. Privious studies have documented an association between EBV and PTA in approximately 4% of PTA cases.
In addition to the involvement of GAS, the following findings suggest a pathogenic role for FN in PTA:
1. Repeated high isolation rates of FN in PTA pus aspirates.
2. Higher isolation rates in PTA patients compared to electively tonsillectomised controls.
3. Development of anti-FN antibodies in FN-positive patients with PTA.
4. Significantly higher inflammatory markers in FN-positive patients compared to PTA patients infected with other bacteria.

We studied the smoking habits among the same 847 PTA patients admitted to the ENT department, Aarhus University Hospital from 2001 to 2006. We found that smoking was associated with increased risk of PTA for both genders and across all age groups. The increased risk of PTA among smokers was not related to specific bacteria. Conclusions on causality cannot be drawn from this retrospective study, but the pathophysiology behind the increased risk of PTA in smokers may be related to, previously shown, alterations in the tonsillar, bacterial flora or the local and systemical inflammatory and immunological milieu.
Studying all 1,620 patients with PTA in Aarhus County from 2001 to 2006 and using population data for Aarhus County for the same six years, age- and gender-stratified mean annual incidence rates of PTA were calculated. The incidence of PTA was highly related to age and gender. The seasonal variation of PTA was insignificant. However, the microbiology of PTA fluctuated with seasons: GAS-positive PTA cases were significantly more prevalent in the winter and spring compared to the summer, while FN-positive PTA patients exhibited a more even distribution over the year, but with a trend towards higher prevalence in the summer than in the winter.

In a series of 63 patients with PPA, we found that 33 (52%) patients had concomitant PTA. This association between PPA and PTA was much higher than previously documented. We therefore suggest that combined tonsillectomy and intrapharyngeal incision in cases where PTA is present or suspected. The results of our routine cultures could not support a frequent role of FN in PPA. Based on our findings suggesting that FN is a frequent pathogen in PTA, we recommend clindamycin instead of a macrolide in penicillin-allergic patients with PTA. Furthermore, cultures made from PTA aspirates should include a selective FN-agar plate in order to identify growth of this bacterium.
Recent studies of sore throat patients document an association between recovery of FN and acute tonsillitis. Studying the bacterial flora of both tonsils in study II, we found almost perfect concordance between the bacterial findings of the tonsillar core at the side of the abscess and contralaterally. This finding suggests that FN is not a subsequent overgrowth phenomenon after abscess development, but that FN can act as pathogen in severe acute tonsillitis. Future studies of patients with FN-positive acute tonsillitis focusing on the optimal methods (clinical characteristics, culture, polymerase chain reaction, or other) for diagnosis and whether antibiotics (and which) can reduce symptoms and avoid complications are warranted. Until further studies are undertaken, we recommend clinicians to have increased focus on acute tonsillitis patients aged 15-24 years with regards to symptoms and findings suggestive of incipient peritonsillar involvement.
We have conducted a number of studies with novel findings:
1. FN is a significant and prevalent pathogen in PTA.
2. Bacteremia during abscess tonsillectomy is no more prevalent than during elective tonsillectomy.
3. The development of anti-FN antibodies in FN-positive PTA patients.
We have used novel approaches as principles to suggest pathogenic significance of candidate microorganisms:
1. Comparative microbiology between PTA patients and “normal tonsils”.
2. Measurements indicating larger inflammatory response compared to clinically equivalent infection.

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