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Aetiology and clinical findings

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JON PALLON graduated as a medical doctor from Lund University in 2008 and currently works as a general practitioner at Skärvet primary health care centre in Växjö, Sweden.

A sore throat is one of the most common reasons people visit their primary health care centre, and more than half of these visits lead to an antibiotic prescription. A better understanding of which patients will benefit from treatment might reduce the prescription rate. This thesis explores aetiological and clinical findings in patients with throat infections in primary health care through three prospective observational studies and one registry-based study.

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Pharyngotonsillitis in primary health care

Aetiology and clinical findings

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Jon Pallon

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UNIVERSITY

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Abstract

Pharyngotonsillitis, or acute sore throat, is a common reason for attending primary health care and a common reason for antibiotic prescription. Group A Streptococcus (GAS) has long been considered the most important pathogen in pharyngotonsillitis, but a wide array of other bacteria and viruses have also been associated with this condition. However, few studies have used modern approaches for aetiological detection to evaluate the clinical symptoms associated with these other microorganisms. This thesis aims to learn more about which viruses and bacteria are present in patients seeking primary health care for acute sore throat and how these microorganisms are associated with the clinical course, complications and subsequent re-consultation for sore throat.

The thesis is based on four observational studies in Swedish primary health care – three prospective cohort studies and one retrospective registry-based study. The prospective studies were performed with similar designs in two cohorts of 348 young adults and 111 children, respectively, and included both symptomatic patients attending primary health care for acute sore throat and healthy controls. All subjects were sampled and screened with PCR and culture for 20–29 different viruses and bacteria and followed up by diaries or a review of electronic medical records. In the registry-based study, all 14 024 patients in Region Kronoberg who were diagnosed with pharyngo¬tonsillitis between 2012 and 2016 and subjected to aetiological testing with a rapid antigen detection test for GAS or with a throat culture were selected to analyse the association between aetiology, antibiotic prescription and re-consultation for pharyngotonsillitis or a complication.

The prospective studies showed that GAS was the most common finding in both children and young adults, and Streptococcus dysgalactiae subsp. equisimilis (SDSE) and Fusobacterium necrophorum were rare in children. Viruses were less prevalent than expected, especially in children. In children, the detection rate of viruses and bacteria was high also in healthy controls and did not differ significantly from the patients. Clinical signs and symptoms of viruses and bacteria overlapped extensively in both children and adults, so neither single nor combined symptoms were able to predict GAS or other aetiologies with a high probability. Cough and coryza have high negative predictive values for GAS but cannot readily be used to predict viruses. The Centor score was more predictive of any bacterial finding than of GAS specifically. The rapid antigen detection test (RADT) had an overall a high sensitivity and specificity for GAS but showed the best performance in patients with a Centor score of 3–4. In the follow-up of the prospective studies, young adult patients with GAS had a higher rate of re-consultation for a sore throat within a month than patients with other aetiologies, although not in a longer perspective of 2 years. In the registry-based study, antibiotic prescription was associated with a lower rate of return visits for pharyngotonsillitis in patients with a positive RADT for GAS. However, antibiotics were not associated with a lower incidence of purulent complications regardless of the aetiological finding.

In conclusion, our findings suggest that GAS remains the most important pathogen in pharyngotonsillitis, both in children and adults. SDSE was rare in children and uncommon in young adults and did not distinguish itself as a significant cause of acute pharyngotonsillitis, recurrent infections, or complications. F. necrophorum was rare in children but commonly detected in young adults. Moreover, it was associated with a higher incidence of peritonsillitis in the registry-based study than were GAS and SDSE. The large prevalence of respiratory viruses and bacteria in healthy children makes it challenging to judge the diagnostic relevance of an aetiological finding in a patient. Clinical signs and symptoms of viruses and bacteria overlapped too much in both children and adults, so neither single nor combined symptoms helped determine aetiology. However, cough and coryza might be helpful to rule out GAS. The results of the registry-based study suggest that antibiotics offer some protection against re-consultation for a sore throat in patients with a positive RADT. In contrast, antibiotics did not seem to protect against purulent complications regardless of aetiology.

Key words pharyngotonsillitis, Streptococcus pyogenes, primary health care, Fusobacterium necrophorum

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To Lovisa, Selma and Liv

"I neither know nor think that I know"
Plato

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Abstract

Pharyngotonsillitis, or acute sore throat, is a common reason for attending primary health care and a common reason for antibiotic prescription. Group A Streptococcus (GAS) has long been considered the most important pathogen in pharyngotonsillitis, but a wide array of other bacteria and viruses have also been associated with this condition. However, few studies have used modern approaches for aetiological detection to evaluate the clinical symptoms associated with these other microorganisms.

This thesis aims to learn more about which viruses and bacteria are present in patients seeking primary health care for acute sore throat and how these microorganisms are associated with the clinical course, complications and subsequent re-consultation for sore throat.

The thesis is based on four observational studies in Swedish primary health care – three prospective cohort studies and one retrospective registry-based study. The prospective studies were performed with similar designs in two cohorts of 348 young adults and 111 children, respectively, and included both symptomatic patients attending primary health care for acute sore throat and healthy controls. All subjects were sampled and screened with PCR and culture for 20–29 different viruses and bacteria and followed up by diaries or a review of electronic medical records. In the registry-based study, all 14 024 patients in Region Kronoberg who were diagnosed with pharyngotonsillitis between 2012 and 2016 and subjected to aetiological testing with a rapid antigen detection test for GAS or with a throat culture were selected to analyse the association between aetiology, antibiotic prescription and re-consultation for pharyngotonsillitis or a complication.

The prospective studies showed that GAS was the most common finding in both children and young adults, and *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) and *Fusobacterium necrophorum* were rare in children. Viruses were less prevalent than expected, especially in children. In children, the detection rate of viruses and bacteria was high also in healthy controls and did not differ significantly from the patients.

Clinical signs and symptoms of viruses and bacteria overlapped extensively in both children and adults, so neither single nor combined symptoms were able to predict GAS or other aetiologies with a high probability. Cough and coryza have high negative predictive values for GAS but cannot readily be used to predict viruses. The Centor score was more predictive of any bacterial finding than of GAS specifically. The rapid antigen detection test (RADT) had an overall a high

sensitivity and specificity for GAS but showed the best performance in patients with a Centor score of 3–4.

In the follow-up of the prospective studies, young adult patients with GAS had a higher rate of re-consultation for a sore throat within a month than patients with other aetiologies, although not in a longer perspective of 2 years.

In the registry-based study, antibiotic prescription was associated with a lower rate of return visits for pharyngotonsillitis in patients with a positive RADT for GAS. However, antibiotics were not associated with a lower incidence of purulent complications regardless of the aetiological finding.

In conclusion, our findings suggest that GAS remains the most important pathogen in pharyngotonsillitis, both in children and adults. SDSE was rare in children and uncommon in young adults and did not distinguish itself as a significant cause of acute pharyngotonsillitis, recurrent infections, or complications. *F. necrophorum* was rare in children but commonly detected in young adults. Moreover, it was associated with a higher incidence of peritonsillitis in the registry-based study than were GAS and SDSE.

The large prevalence of respiratory viruses and bacteria in healthy children makes it challenging to judge the diagnostic relevance of an aetiological finding in a patient.

Clinical signs and symptoms of viruses and bacteria overlapped too much in both children and adults, so neither single nor combined symptoms helped determine aetiology. However, cough and coryza might be helpful to rule out GAS.

The results of the registry-based study suggest that antibiotics offer some protection against re-consultation for a sore throat in patients with a positive RADT. In contrast, antibiotics did not seem to protect against purulent complications regardless of aetiology.

Populärvetenskaplig sammanfattning

Faryngotonsillit, eller halsfluss, är en vanlig orsak till att patienter söker primärvård och är även en vanlig orsak till antibiotikaförskrivning. Bakterien Grupp A-streptokocker (GAS) har sedan länge varit den viktigaste mikroorganismen vid halsfluss, inte minst på grund av dess koppling till följdsjukdomar som exempelvis reumatisk hjärtsjukdom, men det finns många andra bakterier och virus som också förknippats med halsfluss. Dock saknas det studier som använder moderna metoder för påvisande av dessa andra bakterier och virus, och som undersöker vilka kliniska symptom de ger upphov till.

Syftet med denna avhandling var att skaffa mer kunskap om vilka bakterier och virus som kan påvisas hos patienter som söker primärvård för halsfluss, och vad de ger upphov till för symtom och kliniskt förlopp, inklusive följdsjukdomar och nya besök för halsfluss.

Avhandlingen baseras på fyra observationsstudier i svensk primärvård, av vilka tre följde patienter framåt över tid och en var en tillbakablickande registerstudie. De tre framåtblickande studierna genomfördes med liknande design i två grupper av patienter, dels 348 unga vuxna, dels 111 barn, och innefattade både sjuka patienter som sökte primärvård för halsfluss, och symtomfria kontrollpersoner. Samtliga studiepersoner genomgick provtagning och screenades avseende 20–29 olika virus och bakterier med PCR-teknik och bakterieodling. I tillägg registrerades uppgifter om kliniska symtom och fynd. Uppföljning skedde genom dagböcker eller journalgranskning. I registerstudien valdes alla 14 024 patienter ut som diagnostiserats med halsfluss i Region Kronoberg under åren 2012–16 och som genomgått rutinmässig testning för bakterier med snabbtest för GAS eller med svalgodling. Därefter undersöktes sambandet mellan påvisad bakterie, antibiotikaförskrivning och nya besök för halsfluss eller följdsjukdomar.

De framåtblickande studierna visade att GAS var det vanligaste fyndet hos både barn och vuxna, och att bakterierna *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) och *Fusobacterium necrophorum* var sällsynta hos barn. Hos barn sågs ett stort bärarskap av både virus och bakterier hos friska individer, och andelen friska barn med ett påvisat virus eller bakterie skilde sig inte statistiskt säkerställt från de sjuka patienterna.

De kliniska symtomen hos patienterna var överlag väldigt lika, oavsett vilket virus eller bakterie som kunde påvisas, och skilde sig inte tillräckligt mycket åt för att kunna användas diagnostiskt, vare sig enskilt eller i kombination. Snuva och hosta utesluter GAS med stor sannolikhet, men kan inte användas för att påvisa

virus. Centor-kriterierna var bättre på att påvisa bakterier överlag, än GAS specifikt. Snabbtestet för GAS var överlag bra på att upptäcka patienter med GAS, och ännu träffsäkrare när det gällde att utesluta GAS. Allra träffsäkrast var snabbtestet hos de patienter som hade högst Centor-poäng (3–4 poäng av 4 möjliga).

I uppföljningen av de framåtblickande studierna gjorde patienter med GAS fler återbesök för halsfluss inom en månad, jämfört med patienter med virus eller andra bakterier. Efter två år sågs dock inga gruppskillnader i andelen patienter som hade sökt minst en gång igen för halsfluss.

I registerstudien var antibiotikaförskrivning förknippat med en lägre andel återbesök för halsfluss inom en månad hos patienter med ett positivt snabbtest för GAS, jämfört med de som inte fick antibiotika. Däremot var antibiotikaförskrivning inte förknippat med en lägre andel följdsjukdomar, oavsett resultat på snabbtestet.

Våra fynd tyder på att GAS fortsatt måste betraktas som den viktigaste mikroorganismen vid halsfluss, både hos barn och vuxna. SDSE utmärkte sig inte som extra betydelsefull när det gäller återinsjuknande i halsfluss eller i följdsjukdomar. *F. necrophorum* var ovanlig hos barn men påvisades ofta hos unga vuxna; i registerstudien förknippades den även i högre utsträckning med utvecklandet av halsböld än vad GAS och SDSE gjorde.

Den höga förekomsten av luftvägsvirus och bakterier hos friska barn gör det svårt att bedöma relevansen av ett påvisat virus eller bakterie hos en patient, eftersom även barn med symtom kan antas ha ett sådant bärarskap jämte sin infektion.

Kliniska symtom och fynd överlappade alltför mycket mellan patienter med olika fynd av virus och bakterier för att vara diagnostiskt meningsfulla. Däremot kan hosta och snuva utesluta GAS med hög sannolikhet.

Registerstudien antyder att antibiotika skyddar mot återbesök för halsfluss på kort sikt hos patienter med positivt snabbtest för GAS. Däremot verkar inte antibiotika skydda mot följdsjukdomar, oavsett vad snabbtestet visar.

Abbreviations

ADB	Anti-deoxyribonuclease
ASO	Anti-streptolysin O
CI	Confidence interval
CRP	C-reactive protein
EBV	Epstein-Barr Virus
EMR	Electronic medical record
EPV	Aetiological predictive value
GAS	Group A Streptococci, in this thesis equal to *Streptococcus pyogenes*
GCS	Group C Streptococci
GGS	Group G Streptococci
ICD	International Statistical Classification of Diseases
NPV	Negative predictive value
PCR	Polymerase chain reaction
PHC	Primary health care
PHCC	Primary health care centre
PPV	Positive predictive value
RADT	Rapid antigen detection test
RSV	Respiratory syncytial virus
SDSE	*Streptococcus dysgalactiae* subsp. *equisimilis*

Glossary and definitions

Beta-haemolytic bacteria	Bacteria that haemolyse (rupture) red blood cells on blood agar plates.
Colony size	Bacteria that grow on solid media form colonies, visible to the human eye. Historically, colony size (together with carbohydrate surface antigens and haemolysis) was used to differentiate streptococcal species.
Group A streptococci	Streptococci that express the carbohydrate surface antigen A and react with Lancefield group A typing serum. The vast majority correspond to the species *Streptococcus pyogenes*.
Group C streptococci	Streptococci that express the carbohydrate surface antigen C and react with Lancefield group C typing serum. As with group G, most human isolates correspond to the species *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE).
Group G streptococci	Streptococci that express the carbohydrate surface antigen G and react with Lancefield group G typing serum. As with group C, most human isolates correspond to the species *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE).
Lancefield classification	In the early 20th century, Rebecca C. Lancefield discovered that beta-haemolytic streptococcal bacteria could be classified serologically based on the carbohydrate composition of bacterial antigens found on their cell wall. She proved that there exist phenotypically distinct groups of streptococci.
Pharyngotonsillitis	Inflammation of the pharynx and tonsils, most often, but not necessarily, caused by an infection. In general, tonsillitis (inflammation of the tonsils) and pharyngitis (inflammation of the pharynx) are used interchangeably with this term.

Pharynx	The part of the throat that lies between the mouth and the food pipe (oesophagus).
Sore throat	The patients' subjective experience of discomfort or pain in the throat, which can imply both irritation and inflammation of the pharynx. Unfortunately, this imprecise term is also widely used by medical professionals interchangeably with pharyngitis. In this thesis, the term pharyngotonsillitis refers to a sore throat with infectious aetiology diagnosed by a physician, whereas a sore throat refers to the patient's subjective experience of symptoms.

Thesis at a glance

Study	Aim	Methods	Results	Conclusions
I	To investigate if the proportion of return visits for a sore throat or complications after an episode of pharyngotonsillitis is associated with microbial aetiology.	Prospective observational study of 220 adults with pharyngotonsillitis and 128 controls in primary health care. Follow-up with electronic medical file review.	Patients with GAS returned more often for a sore throat within 30 days than patients with non-GAS aetiology. Complications were overall uncommon.	GAS was the most important pathogen. *F. necrophorum* was not a major cause of return visits for a sore throat or complications.
II	To assess how well signs and symptoms in pharyngotonsillitis predict aetiology and return visits for a complication or a sore throat.	Prospective controlled observational study of 220 adults with pharyngotonsillitis and 128 controls in primary health care (the same cohort as in Study I).	Cough and coryza were more common in patients with viral aetiology than in patients with bacteria. A lack of cough was predictive of GAS.	Signs and symptoms were insufficient to rule in GAS or other pathogens. Cough and coryza were both helpful to rule out GAS.
III	To assess the association between different microbial aetiologies and clinical findings in children with pharyngotonsillitis.	Prospective controlled observational study of 77 children with acute sore throat and 34 controls in primary health care. Follow-up with electronic medical file review.	A pathogen was detected in 86% of patients and in 71% of controls. GAS was most common in both groups. Clinical findings were not helpful for distinguishing pathogens.	Bacteria and viruses were common in both patients and controls, which makes it hard to interpret the relevance of an aetiological finding in a symptomatic child.
IV	To investigate if antibiotic prescription to patients with pharyngotonsillitis is associated with the incidence of new visits for pharyngotonsillitis, complications or tonsillectomy.	Retrospective registry-based study of 14 024 patients with a diagnosed pharyngotonsillitis in and aetiological testing primary health care.	Antibiotics were associated with fewer return visits for pharyngotonsillitis within 30 days in patients with a positive rapid antigen detection test for GAS.	Antibiotics appeared to protect against new visits for pharyngotonsillitis in patients with GAS, but not against complications regardless of aetiology.

Original studies

This thesis is based on the following studies referred to in the text by their Roman numerals:

I. Pallon J, Sundqvist M, Hedin K. A 2-year follow-up study of patients with pharyngotonsillitis. BMC Infect Dis. 2018;18(1):3.

II. Pallon J, Rööst M, Sundqvist M, Hedin K. The aetiology of pharyngotonsillitis in primary health care: a prospective observational study. BMC Infect Dis. 2021;21(1):971.

III. Pallon J, Sundqvist M, Rööst M, Danielsson P, Neumark T, Skovbjerg S, Svedin J, Hedin K. Presence of microorganisms in children with pharyngotonsillitis and healthy controls: a prospective study in primary healthcare. Infection. 2021;49(4):715-724.

IV. Pallon J, Sundqvist M, Rööst M, Hedin K. Association between bacterial finding, antibiotic treatment and clinical course in patients with pharyngotonsillitis: a registry-based study in primary healthcare in Sweden. BMC Infect Dis. 2021;21(1):779.

Introduction

Group A streptococcus (GAS) has long been the most important pathogen in acute sore throat due to its ability to cause rheumatic fever – a severe complication that often leads to rheumatic heart disease and sometimes death [1]. With the advent of penicillin, throat infections could finally be managed, lowering the incidence of rheumatic fever. Thankfully, despite 100 years of use, GAS continues to be fully susceptible to penicillins with no recorded cases of resistance [2, 3]. In addition to GAS, several other bacteria and viruses have been associated with pharyngotonsillitis [4]. However, GAS remains the only pathogen with a proven benefit of antibiotic treatment [5].

Complications of an acute episode of pharyngotonsillitis are rare, and rheumatic fever is almost absent from most industrialised countries [1]. Instead, purulent complications such as peritonsillar abscess, otitis media and sinusitis characterise most cases [6]. Antibiotics are thought to give some protection against these purulent complications [6], but the number of patients needed to treat to prevent one complication is too high to justify treatment [5]. Moreover, many complications occur without a previous episode of pharyngotonsillitis [7].

Instead of preventing complications, many guidelines agree that antibiotic treatment is primarily indicated for relieving symptoms but only in patients with GAS or in high-risk patients with a severe illness [5, 8, 9]. The ideal prescription rate in primary health care (PHC), where most of these visits occur, has been estimated to be 13% [10, 11]. In reality, however, more than half of the patients with pharyngotonsillitis are prescribed antibiotics [10, 12, 13].

We need a better way to identify the patients with pharyngotonsillitis who will genuinely benefit from antibiotics and the patients who are at risk of severe complications. Unnecessary antibiotic prescribing has many negative consequences, including an increased risk of side effects [14], a disturbance of the normal bacterial flora of the gut [15] and the development of bacterial resistance [16].

Background

Pharyngotonsillitis

Definitions

The terms pharyngotonsillitis, tonsillopharyngitis, pharyngitis, tonsillitis, throat infection and sore throat all describe an acute episode of inflammation of the pharynx (throat), the palatine tonsils, or both. This inflammation is usually, but not necessarily, caused by an infection (see Aetiology). While the above terms are often used interchangeably by medical professionals, by definition, a sore throat is the patient's subjective sensation of symptoms, whereas the other terms require an objective verification of an inflammatory process, at the minimum a visible redness of the mucous membranes. In contrast to acute, self-limiting episodes, a sore throat can also be recurrent or chronic. In this thesis, pharyngotonsillitis refers to an acute episode of sore throat with infectious aetiology diagnosed by a physician, whereas a sore throat refers to the patient's subjective experience of symptoms.

Epidemiology

A sore throat is a common symptom in the general population: in a community-based survey in England, the average person had 1.6 episodes a year, with the highest incidence in children 0–4 years old and in females, independent of age [19]. In a Scottish survey, 31% of the respondents had experienced a severe sore throat in the previous year [20]. However, most episodes of sore throat are benign, and only 8–13% lead to contact with health care [19, 21]. The tendency to contact health care is strongly associated with the severity of symptoms [19, 21], the duration of symptoms, young age and fever but not with gender [19]. Although most episodes are managed with self-care, acute sore throat still accounts for 11–17% of all visits for an infection to Swedish PHC [12, 22]. In the United States, acute sore throat accounts for 1–2% of all ambulatory care visits to PHC [23, 24] and 6–8% of children visits [25].

Infectious aetiology

Most cases of acute sore throat are caused by an infectious agent (Table 1). Respiratory viruses and GAS comprise the majority of pathogens, but the microbial panorama varies with age, setting and season.

Respiratory viruses

In both children and adults, respiratory viruses such as adenoviruses, rhinoviruses, enteroviruses, influenza virus B, parainfluenza viruses, coronaviruses, and respiratory syncytial virus (RSV) are considered the most common infectious aetiology [4, 26–29].

Other viruses

Epstein-Barr virus (EBV), a herpesvirus mainly transmitted via saliva, is responsible for most cases of infectious mononucleosis, and about 95% of adults worldwide are infected. Most infections occur in early childhood with few symptoms, whereas the clinical syndrome of mononucleosis typically results from primary infection in the second or third decade of life [30]. Cytomegalovirus (CMV), also a herpesvirus, can cause a clinical condition similar to mononucleosis [30]. Herpes simplex viruses (HSV) can also cause a sore throat, typically with redness and painful vesicular lesions involving the lips, gums, or throat [31]. As with EBV, primary infection with CMV and HSV is highest in adolescents and young adults [30]. In addition to herpes viruses, rubella virus, measles virus, and several other viruses have been associated with acute pharyngotonsillitis [4].

Streptococcus pyogenes

S. pyogenes (beta-haemolytic Lancefield's group A streptococcus, GAS) is the most common bacterial aetiology of pharyngotonsillitis [4, 5] and the most pathogenic species in the genus Streptococcus [32]. Apart from throat infections, it can cause skin and soft tissue infections, ranging from mild to moderately severe infections of the skin (impetigo and erysipelas) and subcutaneous tissue (cellulitis) to deeper and potentially life-threatening infections of muscle fascia (necrotising fasciitis) and muscle (myositis and myonecrosis), or spread systemically in the body, causing sepsis [33]. *S. pyogenes* possesses several virulence factors, of which M-protein antigen is the major one (although not unique to *S. pyogenes*). Although *S. pyogenes* is often used synonymously with GAS, other streptococci can also express group A antigen [32]. In this thesis, *S. pyogenes* is described as GAS.

According to meta-analyses, the prevalence of GAS in pharyngotonsillitis in clinical settings (i.e., patients who self-present to a health care provider, also denoted as passive recruitment) is 14% in adults and 37% in children [34, 35]. This pattern seems similar in OECD countries and non-OECD countries [34, 35]. However, few population-based studies have documented the incidence of GAS

pharyngitis in less developed countries, and the true incidence might be 5–10 times greater than in developed countries [1].

A difficulty with GAS is that it often colonises asymptomatic individuals, especially children, which makes it hard to interpret the meaning of a laboratory finding of GAS (see Asymptomatic carriage).

Streptococcus dysgalactiae subspecies *equisimilis*

Historically, beta-haemolytic large-colony streptococci were identified and described by their different carbohydrate antigens. In this taxonomy, Lancefield's group C and Lancefield's group G streptococci were considered separate groups. Since then, genetic investigations have identified these strains as similar and proposed that they should be considered one subspecies – *S. dysgalactiae* ssp. *equisimilis* (SDSE) [32]. Although this species can sometimes express other antigens, such as A and L, for the sake of simplicity, group C and G streptococci and SDSE are considered as the same species in this thesis. SDSE is most closely related to *S. pyogenes*, with a 72% genome sequence similarity. They also share many virulence factors, including M protein, streptolysins, streptokinase and exotoxins [36].

Although SDSE is now recognised as an important bacterial pathogen [37], its pathogenic role in pharyngotonsillitis has been debated for decades and continue to be. Whereas some argue that SDSE causes infection with similar signs and symptoms as GAS [38–43], others have found no support for it as a pathogen (e.g., it is often recovered from asymptomatic individuals) or found that it causes milder symptoms than GAS [44–46]. There is even support that colonisation with SDSE can be protective against GAS infection [47]. The prevalence of SDSE seems to rise with age; it is uncommon in young children [44] but recovered in 7–11% of symptomatic adolescents and adults [29, 39, 42].

Fusobacterium necrophorum

The anaerobic rod *F. necrophorum* is perhaps best known for its association with the severe Lemierre's syndrome [48, 49] but is also thought to contribute to the development of peritonsillar abscesses [50, 51]. Recently, *F. necrophorum* has been suggested as a possible pathogen in acute pharyngotonsillitis as it can be recovered from up to 19% of patients with a sore throat in PHC – only second to GAS [29, 42, 45, 52, 53]. However, as *F. necrophorum* is also frequently recovered from healthy controls, some argue that its role as a causative agent in pharyngotonsillitis is still unproven [45, 53, 54]. *F. necrophorum* is most prevalent in patients 13–40 years old and is seldom found in younger children [52, 55, 56].

Other bacteria

Among the less common causes of pharyngotonsillitis are *Arcanobacterium haemolyticum*, an anaerobic bacillus that accounts for 1–2.5% of sore throats,

mainly in adolescents 15–18 years old [57], *Corynebacterium diphtheriae* [4], *Yersinia pestis* [4], *Yersinia enterocolitica* [4], *Francisella tularensis* [4], mixed anaerobes [4], and the atypical bacteria *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* [4, 26, 58]. *M. pneumoniae* appears to cause pharyngotonsillitis in itself, whereas *C. pneumoniae* seems to be mainly a co-pathogen [4, 26]. In rare cases, the filamentous bacteria actinomycetes can cause pharyngeal symptoms [59].

Sexually transmitted infections

HIV is an uncommon cause of pharyngitis in the general population but more prevalent in risk groups. Approximately half of the infected persons experience acute pharyngitis, fever and swollen lymph glands [60]. *Neisseria gonorrhoeae* can cause gonococcal pharyngitis [4], and *Treponema pallidum* causes pharyngitis in half of the patients with secondary syphilis [61]. *Chlamydia trachomatis* has been suggested to cause symptomatic throat infections, but the evidence to support this is scarce [62]. However, with most sexually transmitted infections, there will be additional symptoms to guide the clinician towards a correct diagnosis.

Fungi and parasites

The painful swallowing associated with oropharyngeal infection with *Candida albicans* (thrush) might be mistaken for bacterial or viral pharyngotonsillitis. Such fungal infections are generally associated with certain diseases or medical conditions and usually indicate a dysfunctional immune system [63]. Toxoplasmosis, caused by ingestion of the protozoan parasite *Toxoplasma gondii*, affects a third of the world population and, in some cases, can cause a sore throat [64].

Table 1
Microorganisms associated with pharyngotonsillitis [1, 4, 5, 26–32, 34–64].

Bacteria	*Treponema pallidum*	Respiratory syncytial virus
Streptococcus pyogenes	*Francisella tularensis*	Epstein-Barr virus
Streptococcus dysgalactiae ssp. *equisimilis*	Actinomycetes	Cytomegalovirus
Arcanobacterium haemolyticum		HIV
Fusobacterium necrophorum	**Viruses**	Herpes simplex virus
Mixed anaerobes	Rhinovirus	
Mycoplasma pneumoniae	Adenovirus	**Fungi**
Chlamydia pneumoniae	Coronaviruses	Candida
Neisseria gonorrhoeae	Enteroviruses	
Corynebacterium diphtheriae	Influenza A virus	**Parasites**
Yersinia pestis	Influenza B virus	*Toxoplasma gondii*
Yersinia enterocolitica	Parainfluenza viruses	

Non-infectious aetiology

Many cases of a sore throat are of non-infectious aetiology, although the exact proportion is hard to quantify. These aetiologies can be roughly be divided into two categories: physicochemical factors and environmental factors [65].

Among the physicochemical factors associated with a sore throat are cigarette smoking (including passive smoking), snoring, trauma (e.g., tracheal intubation), shouting, overused vocal cords (e.g., with teachers) [65–67], medications (e.g., ACE inhibitors and chemotherapy), autoimmune diseases (e.g., Kawasaki disease, Beçhet syndrome and Periodic fever with aphthous stomatitis, pharyngitis and adenitis), gastroesophageal reflux [68], allergic rhinitis and sinusitis [69] and diseases that predispose to infection (e.g., thyroiditis) [70].

Several environmental factors can cause a sore throat: ambient air pollution (ozone, nitrogen oxides, traffic fumes and fine dust), indoor air pollution (poor ventilation, air-conditioning components, particulates, and mould), occupational and hazard-associated irritants (industrial particulates, chemicals, fumes, odours, gasses, and endotoxin), temperature and humidity (cold or dry air) [65].

Asymptomatic carriage

As described above, several microbial agents are thought to be able to cause pharyngotonsillitis. However, many of these pathogens are also frequently found in asymptomatic individuals [5], which causes significant interpretational problems for clinicians and researchers. Whereas bacterial infections can be sub-clinical (i.e., with mild or no symptoms), carriage refers to mere colonisation without any host response to the microorganism [71].

GAS is the most common bacterium in pharyngotonsillitis, and its poorly understood carrier state [72] is therefore of particular importance [26, 34, 71]. Carriage is defined as a persistent colonisation state without any immune response as measured by an increase in serological antibody titres (see Diagnosis) [71]. In studies that rely on both throat swabs and serological markers, only 50–60% of children with GAS growth in throat samples had a serologically confirmed GAS infection [34]. Although there are several pitfalls to serological diagnosis [71], this still implies that in studies where throat culture alone is used for diagnosis many patients with GAS probably have viral pharyngotonsillitis with a concomitant GAS carriage. The prevalence of GAS in asymptomatic individuals varies with age and is higher in children (11–12%) than in adults (2.0%) [34, 35].

SDSE and *F. necrophorum* are both frequently recovered from asymptomatic individuals, which strengthens the view that they are – at least at times – innocent bystanders rather than causative pathogens [44, 45, 54]. However, as is evident from GAS, the existence of a carrier state does not contradict an active infection in other instances.

Detection of viral material in asymptomatic individuals can represent carriage, prolonged post-infectious shedding, a sub-clinical (low-virulent) infection or the incubatory period preceding symptomatic infection. As with bacteria, the detection rates of several respiratory viruses with PCR are also high in asymptomatic individuals, at least in children, making it hard to interpret a finding [73].

Clinical features

Despite its confined anatomical location, infectious pharyngotonsillitis is a heterogeneous condition with multiple aetiologies and associated with a broad range of local and systemic symptoms. In rare cases, the infection can spread beyond the pharyngeal space and cause complications that give rise to yet another range of symptoms (see Complications below).

As respiratory viruses and GAS are the most common pathogens of pharyngotonsillitis, many attempts have been made to characterise the clinical features of these infections to discriminate between them without aetiological tests. Traditionally, cough, nasal congestion, coryza, conjunctivitis, oral ulcers, hoarseness, diarrhoea, absence of fever and viral exanthema have been considered typical viral features. In contrast, a sudden onset of sore throat, fever, tonsillopharyngeal or uvular oedema, tonsillar exudates, cervical lymphadenitis, a lack of cough, a lack of coryza, headache, abdominal pain, nausea, scarlatiniform rash and strawberry tongue have been associated with GAS [4, 74, 75]. Unfortunately, none of these symptoms are specific for GAS, and the features of viral and GAS pharyngotonsillitis overlap too broadly to reliably discriminate between the two [26, 74, 76, 77].

Aside from GAS and respiratory viruses, individuals with pharyngotonsillitis where SDSE has been detected can present with symptoms that resemble or are indistinguishable from those of GAS [5, 41, 43, 78]. The same is true for *F. necrophorum* [29, 42, 53], although the latter has also been associated with a cough [29]. *A. haemolyticum* differs from GAS as it causes a rash that resembles a rash caused by scarlet fever in about half of the cases [57], and *C. diphtheriae* causes the characteristic adherent grey membranes of diphtheria (from the Greek word for leather) [79], although this disease is now rare in countries with childhood immunisation programmes. Pharyngotonsillitis caused by *M. pneumoniae* and *C. pneumoniae* is sometimes accompanied by acute bronchitis or, more seldom, pneumonia [80].

Mononucleosis is a clinical syndrome characterised by fever, malaise, pharyngitis and swollen lymph glands and is most often caused by EBV. CMV can cause a similar condition but with less pronounced symptoms from the throat [30]. Although rare, HIV can also present similarly [60].

Complications

Pharyngotonsillitis is associated with acute purulent complications, such as sinusitis, media otitis, lymphadenitis and peritonsillar abscess, and, in patients with GAS infection, post-infectious immunological complications such as rheumatic fever and glomerulonephritis [5, 6, 81]. Purulent complications are rare in a clinical PHC setting and are estimated to occur in 1.4% of adult patients presenting with acute sore throat [81]. However, most cases of peritonsillitis are not preceded by a diagnosed pharyngotonsillitis [7]. Rheumatic fever and glomerulonephritis used to be a severe threat but are rare in industrialised countries [5]. Globally, the burden of GAS disease is still high, due both to throat infections and skin infections, and rheumatic fever and subsequent rheumatic heart disease are highly prevalent in some parts of the world where they continue to be a significant cause of morbidity and mortality [1].

Few large studies have investigated complications after an episode of pharyngotonsillitis with detected SDSE, and the case reports that describe complications following such infection are sparse and of low evidence [5].

F. necrophorum is the primary pathogen causing the rare but potentially deadly Lemierre's syndrome, typically characterised by suppurative thrombophlebitis of the internal jugular vein and a subsequent metastatic spread of septic emboli throughout the body [49]. Furthermore, *F. necrophorum* has also been strongly associated with peritonsillar abscesses [51].

Both GAS and SDSE have been recovered from the bloodstream in invasive disease. Although these infections are rare, there have been reports of an increasing incidence of invasive SDSE infections during the past decades, in some regions surpassing those of GAS and group B streptococci [82]. Invasive disease is most often a consequence of skin or soft tissue infections but can also be preceded by pharyngotonsillitis [83]. However, the incidence is too low (0.002% of all episodes of pharyngotonsillitis) to justify antibiotic treatment of pharyngotonsillitis for this reason, and antibiotics might lack a protective effect [84].

Recurrent and chronic pharyngotonsillitis

Most episodes of pharyngotonsillitis resolve spontaneously, with or without antibiotics, but in some patients, the infection becomes recurrent, with three or more episodes a year, or even chronic when the infections withstand antibiotic treatment [85, 86]. It is unknown why antibiotic treatment fails, but among the suggested explanations are biofilm formation of certain bacteria (e.g., *S. aureus* and *H. influenzae* [87]), a polymicrobial aerobic and anaerobic flora in the tonsillar crypts [88], and infection with *F. necrophorum* [89]. Traditionally, surgical removal of the tonsils (tonsillectomy) has been thought to solve the problem, but in reality, tonsillectomy only prevents tonsillitis, not pharyngitis, so factors other than the tonsils are at work in the pathogenesis of recurrent disease. Moreover, a meta-

analysis of the few randomised trials that have been performed showed no clear long-term benefit of tonsillectomy [86]. *F. necrophorum* can be detected in many patients scheduled for tonsillectomy due to recurrent throat infections but also in many asymptomatic patients several months after tonsillectomy, suggesting either that *F. necrophorum* can only cause infection under certain circumstances, such as in the presence of tonsillar tissue or a co-pathogen, or that it is a mere bystander [90]. Several guidelines recommend the shift to macrolides, clindamycin or cephalosporins in recurrent pharyngotonsillitis; however, no trial has investigated the effectiveness of such treatment [85].

Pharyngotonsillitis in children

As previously stated, respiratory viruses are thought to cause most pharyngotonsillitis cases in children [4, 26, 27].

GAS is the most common bacterial aetiology in children and accounts for a higher proportion of pharyngotonsillitis episodes than in adults [4]. Its prevalence varies with age and is often low during the first 3 years of life and then increases with age [74]. Therefore, many guidelines recommend not testing patients <3 years old for GAS unless they have risk factors for adverse events [74, 91]. However, the number of studies to support this recommendation is low. There are also conflicting findings: in a meta-analysis, the prevalence of GAS in children 0–5 years old was estimated to be 24% (compared to 37% in children 5–18 years old) [35], and other studies have found that GAS pharyngitis is relatively common (29%) in children 2–3 years old [92] and may present already in the first year of life [93].

Aside from infections, the carriage rate of GAS in children is also much higher than in adults, with up to half of all detections of GAS representing carriage rather than a true infection, which makes it difficult to value the significance of a finding (see Asymptomatic carriage) [34, 93].

Finally, SDSE and *F. necrophorum* are uncommon in children but become more prevalent in adolescence [44, 52, 55, 56].

Diagnosis

Although the diagnosis of infectious pharyngotonsillitis is usually evident from clinical signs and medical history, determining the causative aetiological agent is much more challenging and sometimes impossible for the clinician. In practice, however, most guidelines only focus on identifying or ruling out GAS due to its connection with immunological complications and the lack of evidence for treating patients with other aetiologies [5, 74, 91, 94].

Clinical scoring systems

As individual signs and symptoms in pharyngotonsillitis are not sufficient to discriminate between different aetiologies, several attempts have been made to group signs and symptoms into clinical scoring systems to increase the diagnostic accuracy. Unfortunately, although the accuracy of a model usually increases with the number of variables included, an overly comprehensive score might discourage physicians from using it. Therefore, Joachim et al.'s 9-item score might be the most accurate score for GAS [95, 96], whereas the 4-item Centor score, which predicts GAS growth in throat culture, is presently the most widely used scoring system. The Centor score is based on data from a hospital emergency room in 1981 and gives 1 point each for fever, swollen lymph glands, tonsillar exudates and absence of a cough, where 4 points give a 56% probability of GAS [97]. Although the Centor score has been validated on patients in PHC, it should be used with caution in low-prevalence settings (such as PHC) [75, 76]. Centor et al. have recently argued that the score also predicts other bacteria such as SDSE and *F. necrophorum* [42].

A modified and validated version of the Centor score – the McIsaac score – considers age and adds 1 point to patients 3–14 years old due to the higher prevalence of GAS in young people [98].

As an alternative to the Centor score, the 5-item score FeverPAIN is used in Great Britain and is said to predict GAS and other streptococci [78, 94].

Even with the scores mentioned above, the predictive values are modest at best, especially in settings with a low prevalence of GAS. Hence, several guidelines recommend using the scores primarily to select which patients should proceed to a rapid antigen detection test (RADT) (see below) [5, 74, 91].

Rapid antigen detection test (RADT) for group A streptococci

In contrast to cultures, RADTs are point-of-care tests that give the clinician a result within minutes. In modern RADTs, a swab is rolled against the tonsils and transferred to a liquid medium. The medium is then applied to a lateral flow test, which uses lateral flow to transport the sample slowly to an area with antibody-labelled particles. If antigen from GAS is present (viable or non-viable), a visible mark appears.

The sensitivity and specificity, compared to culture, is usually reported by the manufacturers to be high. In contrast, meta-analyses report a sensitivity of 86–91% [99, 100] – i.e., manufacturers tend to exaggerate the sensitivity in package inserts (a sensitivity of 86% means that the RADT misses 1/7 of patients with GAS) [101]. A negative test result can also be caused by an incorrect sampling technique [71].

The routine use of RADTs differs between countries and are not recommended by guidelines in Great Britain, Belgium, France and the Netherlands [8]. In Sweden, RADTs are recommended in patients with a Centor score of 3–4 who could benefit from antibiotics [91].

Throat culture

In a throat culture, bacteria from throat samples grow on solid media for bacterial identification and has long been considered the reference standard for GAS detection in pharyngotonsillitis. Therefore, throat cultures are the basis of most studies on this topic and are also widely used in clinical settings. Moreover, isolates obtained through throat culture can be used to determine the susceptibility of the bacteria to different antibiotics, both in study settings and in clinical practice. Despite these advantages, most guidelines do not recommend routine cultures [5, 74, 91]. Although the reference standard, a reliable test result depends on correct sampling technique, adequate transportation conditions and relevant laboratory techniques. However, the method is not fail-safe as studies have shown that some patients have serological signs of infection despite a negative throat culture [71].

The term throat culture usually refers to detecting large-colony beta-haemolytic streptococci, generally grown on agar medium supplemented with sheep blood [102]. Other bacteria, such as arcanobacteria and fusobacteria, can also be grown in culture but with specific nutrient and atmosphere demands [103]. Additionally, *F. necrophorum* is slow-growing [103].

The major disadvantage with throat cultures for clinicians is that it takes at least one day to obtain a negative result and up to five days to grow *F. necrophorum*. That is, both diagnosis and the decision to prescribe antibiotics must rely on other factors.

Anti-streptolysin O (ASO) and anti-deoxyribonuclease (ADB) antibodies

ASO and ADB are antibodies created by the immune system in response to GAS infection. An increase in antibody titres is considered the reference standard for determining an actual GAS infection when used with a throat culture [34]. The use of GAS strain identification, such as *emm* typing, could heighten the diagnostic accuracy even further [71]. The antibodies circulate in the blood and can be obtained by a blood serum sample. Therefore, they are serological markers [34, 71, 104]. Unfortunately, because it takes 1–4 weeks for these antibodies to form, they are unhelpful in diagnosing acute pharyngotonsillitis [71]. However, they help diagnose rheumatic fever and post-streptococcal glomerulonephritis and can also be used in research and GAS infection outbreaks. ADB is considered more specific to GAS infection, whereas ASO can also be formed in response to SDSE [71]. On the other hand, up to 20% of patients with a GAS infection never mount an ASO response, and the sensitivity of ADB is also thought to be imperfect [104]. Some patients with a GAS carriage can exhibit a prolonged elevation of titres for years, leading to a false positive of a point estimate, and other patients might increase their titres from low levels to levels that are still under the upper limit of normal. Therefore, the most accurate diagnosis of GAS infection requires ≥2 sequential serum samples about a month apart to evaluate the kinetics of the immune response [71].

Polymerase chain reaction (PCR)

PCR is a molecular method that can take a tiny amount of genetic material (i.e., DNA or RNA) and amplify it into billions of copies for analysis [105]. It has wide usage in many scientific areas, among them diagnostics of infectious diseases. PCR can be used to look for a single species in a sample or detect several pathogens in a multiplexed fashion. The PCR technique has evolved rapidly over the last decades and now offers a wide range of opportunities. It has become faster, more reliable and cheaper [105]. To date, PCR has no role in routine diagnostics in pharyngotonsillitis. In research, PCR and multiplexed PCR are very suitable methods to detect both viruses and bacteria.

C-reactive protein (CRP)

CRP is an acute-phase protein that increases during inflammation to trigger the complement pathway of the immune system [106]. Once understood to protect the body against bacterial infections and named for its interaction with the (C)-polysaccharide of *Streptococcus pneumoniae*, it is now known to be a marker of many different infections as well as non-infectious inflammatory conditions [106]. CRP is easily measured with a blood sample, and numerous point-of-care tests exist in clinical practice to aid the physician with an immediate result. However, CRP has never been proven helpful in diagnosing pharyngotonsillitis – e.g., in one study of 149 patients, there were no significant differences in CRP levels between different aetiologies [107] – and guidelines do not recommend using CRP [5, 74, 91, 94]. Despite this, CRP is widely used in Swedish PHC, and more than one in four patients with pharyngotonsillitis are tested [12, 22].

Aetiological predictive value (EPV)

Given the high carriage rate of GAS in children, it can be demanding to interpret a positive finding of a culture or an RADT. If the carriage rate is known, a mathematical formula can calculate the EPV that tells the clinician how probable it is that GAS is the cause of the infection rather than an innocent bystander [108]. The formula shows that the lower the carriage rate in the population, the higher the probability of a true infection if GAS is detected. The method is further described in Methods.

Antibiotic treatment

Whom to treat

Most guidelines are based on the following observations: pharyngotonsillitis is a self-limiting infection that resolves within a week without antibiotic treatment; the benefits of treatment are small; antibiotics have adverse effects; and complications are too rare to justify treatment [5, 74, 91, 94]. Moreover, many guidelines agree

that GAS is the only pathogen that warrants antibiotic treatment [5, 74, 91]. However, in the United Kingdom, other bacteria may be treated as well, and the recommendation is to use the Centor Score or Fever-PAIN to identify patients with bacterial aetiology who are more likely to benefit from treatment [94]. No guideline recommends aetiological testing for *F. necrophorum*.

In Sweden, the Medical Products Agency and The Swedish strategic programme against antibiotic resistance (Strama) make the following recommendations: use the Centor score; use RADTs for GAS only in patients with a score of 3–4 who could benefit from antibiotic treatment; treat only patients with a positive result; and refer very ill patients to a hospital regardless of suspected aetiology [91].

Benefits of treatment

A meta-analysis of trials investigating the effects of antibiotic treatment in patients with an acute sore throat, regardless of cause, showed that antibiotics shorten the duration of symptoms by a modest 16 hours over 7 days [6]. The effect was more pronounced in patients with GAS, where antibiotics led to a lower proportion of patients experiencing pain on day 7 compared with placebo (relative risk 0.29; 95% CI 0.12–0.70). Moreover, the absolute effect of antibiotics on suppurative complications was very low and did not justify treatment. Rheumatic fever is almost absent from industrialised countries, but the results indicate that antibiotics reduced the risk by more than two-thirds in high prevalence settings. Unfortunately, only three included studies were performed after 2000 due to a lack of recent studies with placebo groups [6].

A randomised controlled trial in 2003 included in the above meta-analysis indicated that symptoms resolved 2.5 days earlier in patients with GAS and a Centor score of 3–4 if they were treated with antibiotics, compared with no treatment [9]. Moreover, the authors suggested that antibiotics were possibly effective in patients with non-group A streptococci and a Centor score of 3–4.

Harms of treatment

Among the many negative aspects of antibiotic prescribing are side effects, such as rashes and allergy [14], ecological effects on the bacterial microbiota [15], increased bacterial resistance [16] [16](see sub-section Antibiotic resistance below), the cost [109], and changed behaviour in patients due to “medicalisation” [5].

Choice of antibiotic

Penicillin V is the treatment of choice for GAS pharyngotonsillitis in Europe and North America due to its efficacy, safety, narrow spectrum of activity, and low cost, albeit with some differences in the recommended dosage and total exposure [5, 8, 74, 110]. Amoxicillin might be an alternative in children because of its less adverse taste but should be used with caution in adolescents since amoxicillin often elicits a rash in patients with mononucleosis. For individuals with penicillin allergy,

cephalexin, cefadroxil, clindamycin, azithromycin and clarithromycin are recommended in North America [74]; clarithromycin or erythromycin is recommended in Great Britain [94]; and clindamycin is recommended in Sweden [91]. These discrepancies between guidelines cannot be fully explained by regional variations in antibiotic resistance but point to differences in how the evidence is selected and interpreted [8].

Antibiotic resistance

Antibiotic resistance refers to a decreased effectiveness of antibiotics in treating bacterial infections. Like humans, bacteria evolve Darwinianly, adapting to their environment through genetic mutations and natural selection [111]. If the environment exerts a selection pressure through antibiotic use, those bacteria whose mutations entail bacterial resistance will be favoured and become more common over time. The greater the antibiotic pressure, the faster the selection and clonal expansion of these mutated strains. Bacteria can also transfer genetic material horizontally – i.e., a mutation originating in one species can spread to other species as well [16, 111].

Although antimicrobial resistance is spreading faster because of excessive antibiotic use in healthcare and agriculture [16], resistance has existed for millions of years. Long before the era of antibiotic drugs, bacteria encountered toxic substances in nature produced by other microorganisms with the potential to kill them and had to develop protective mechanisms to survive [111]. As most of our antibiotic drugs are naturally produced by microorganisms (e.g., fungi), it is easy to understand how bacteria have an inherent capability to fight these drugs.

Antibiotic overuse in primary health care

Despite a reduction in antibiotic prescriptions in many countries, PHC is still responsible for most antibiotics prescribed to people [112]. Many of these prescriptions are for respiratory infections, which make up more than half of all consultations for an infection in PHC, urinary tract infections, and skin and soft tissue infections [12]. Therefore, antibiotic prescription in PHC contributes to the high levels of antibiotic resistance in the community and increased use of second-line antibiotics [112].

Of all the respiratory infections in PHC, pharyngotonsillitis constitutes about 15–20% [12, 22]. Despite the now widespread knowledge about the modest effect of antibiotic treatment of pharyngotonsillitis and the calculated ideal prescription rate of 13% [10, 11], still more than half of these patients in industrialised countries receive a prescription: In Sweden, about 60% of patients with pharyngotonsillitis

are prescribed antibiotics [12, 22], in England 59% [10], in the Netherlands 55–58% [13], and in the USA 53% [25].

Resistance to different classes of antibiotics

According to most guidelines, penicillin V is the drug of choice for treating pharyngotonsillitis caused by GAS [5, 74, 91, 94]. Therefore, it is reassuring, albeit somewhat puzzling, that both GAS and SDSE have a 100% susceptibility to penicillin V, a level that has not changed over the last 100 years [2, 113]. Moreover, beta-haemolytic streptococci are fully susceptible to other beta-lactam antibiotics such as amoxicillin and cephalosporins [2]. Possible explanations might be that beta-haemolytic streptococci are unable to express beta-lactamase, that beta-lactamase is toxic to streptococci, that genetic transfer is difficult, that the right circumstances have not yet occurred, or that expressing low-affinity penicillin-binding proteins – a common mechanism of penicillin resistance – would be lethal to these bacteria [3]. Despite this in vitro susceptibility, there are reports of treatment failures in vivo, which might be explained by factors other than resistance. Examples of such factors are GAS carriage with a concomitant viral infection, too low a dosage of penicillin, too few days of treatment, poor compliance to treatment, co-existence of betalactamase-producing bacteria, the eradication of favourable *Streptococcus salivarius* (thereby increasing the risk of re-infection), epithelial internalisation of GAS, and production of a biofilm [2].

Recently, there was a worrying report of two near-identical clinical isolates of *S. pyogenes* with a decreased in vitro susceptibility to the beta-lactam antibiotics ampicillin and cefotaxime but not to penicillin G as a result of a missense mutation in the gene coding for penicillin-binding protein 2X (*pbp2x gene*) [18]. This report led another research group to investigate an extensive library of *S. pyogenes* in search of mutations in this gene; the research group found 37 nonsynonymous mutations in 137 strains from all over the world. Many of these strains also had a decreased in vitro susceptibility to six different beta-lactam antibiotics, including penicillin G, albeit to a lesser degree than in the other study. However, the researchers conclude that these geographically widespread mutations do not indicate clinical resistance but might be the first step in such development, which warrants ongoing surveillance of GAS susceptibility to beta-lactam antibiotics [17].

In addition to beta-lactam antibiotics, macrolides and lincosamides (e.g., erythromycin and clindamycin) are used to treat streptococcal infections. There are numerous reports of resistance to these antibiotic classes, sometimes exceeding 20%; however, because different methods are used for susceptibility testing, and reference breakpoints change over time, it is difficult to compare findings from different locations and periods [82].

In Sweden, most strains of GAS are susceptible to clindamycin. In 2019, only 3.0% of 536 blood isolates from invasive GAS infections showed resistance. This percentage has been relatively stable over the last 7 years [114]. In the USA, a recent

study found a 15% resistance to clindamycin in GAS isolates from children with pharyngitis, and in China, 96% of GAS isolates from patients with scarlet fever were resistant to clindamycin [115]. In Norway, the resistance to clindamycin and erythromycin in GAS is low; however, in SDSE and group B streptococci there has been a steady increase in resistance to these antibiotics over the last 15 years [82]. Between 1997 and 2001 in Finland, GAS resistance to erythromycin varied between 7.4% and 17%, and regional resistance correlated with the regional use of macrolides [116].

The Swedish strategic programme against antibiotic resistance (Strama)

Founded in 1995, Strama encourages rational use of antibiotics in PHC and hospitals to reduce antibiotic resistance [117]. Strama acts as an advisory body to Folkhälsomyndigheten (the Public Health Agency of Sweden), communicates local prescription data to clinicians nationwide through regional groups, and publish national treatment recommendations. Strama also works to prevent and control infections through collaborations with regional groups.

Factors influencing antibiotic prescribing

Although the incidence of sore throat is high in the community (and more so among women and young children), the tendency to consult a doctor is associated with the severity of symptoms, the duration of symptoms, young age and fever [19, 21]. Qualitative research has shown that the main reasons for patients to contact their PHC doctor are for pain relief, perceived severe symptoms and non-resolving symptoms [19]. Surprisingly, none of these factors predict antibiotic prescription to patients who see a doctor; instead, a prescription is most strongly associated with male gender and self-perceived anxiety [19].

There have also been reports of perceived patient pressure as a driver of antibiotic prescription [118]. In addition, qualitative studies of other factors affecting antibiotic prescription have shown a complex interplay of several factors [19], among these perceived clinical need [118], clinical uncertainty [119, 120] and the doctor's desire to maintain a good relationship with the patient or parent [121].

Knowledge gaps

Although pharyngotonsillitis is highly researched, much focus has been on identifying and treating GAS due to its association with rheumatic fever. However, most cases of pharyngotonsillitis are caused by pathogens other than GAS, and many of these studies on GAS are more than half a century old. As immunological complications are now rare in the industrialised world and treatment is mainly warranted for symptom relief, the focus needs to be shifted towards aetiologies other than GAS and to purulent rather than immunological complications. Of particular interest to this thesis were the following knowledge gaps:

- Few sore throat studies in children identify a broad spectrum of viruses and bacteria using modern PCR techniques, both in symptomatic patients and healthy controls and during different seasons.
- Few studies explore the symptoms and clinical courses associated with a broad range of microorganisms, including the incidence of complications, tonsillectomy, and recurrent episodes of pharyngotonsillitis, and evaluate these symptoms' diagnostic and predictive value.
- Although both SDSE and *F. necrophorum* have been proposed as potential pathogens in pharyngotonsillitis, there is still no consensus on this matter. Moreover, no trial has been published that evaluates the effect of antibiotic treatment of patients with these bacteria as a primary outcome.

Aims

General aims

This thesis has three overall aims: to learn more about which viruses and bacteria can be recovered from patients with pharyngotonsillitis; to learn more about the symptoms and clinical courses associated with these microorganisms; and to learn more about how antibiotic treatments affect the clinical courses.

Specific aims

- To evaluate whether the microbial aetiology of young adults with pharyngotonsillitis is associated with the incidence of new visits for a sore throat, a complication or tonsillectomy over 2 years.
- To use PCR and culture to investigate which viruses and bacteria can be recovered in throat samples from children <15 years old presenting with acute sore throat in PHC and healthy controls.
- To study the symptoms and clinical courses of pharyngotonsillitis associated with different viruses and bacteria in children and adults and evaluate whether they differ enough to be clinically helpful in diagnosis.
- To use both prospective observational cohorts and retrospective registry data to assess whether antibiotic treatment of patients with pharyngotonsillitis affects the incidence of new visits for a sore throat and complications.

Methods

Design and setting

This thesis is based on four observational studies in PHC in southern Sweden: three prospective cohort studies with patients and controls and one registry-based study with patients. Although the cohort studies were well suited to collect microbial samples and detailed clinical data, they were not sufficiently large to measure the incidence of rare complications. The much larger registry-based study, on the other hand, lacked data on clinical symptoms and medical history.

Studies I and II used a prospective controlled cohort of adults recruited from 5 PHCC in Region Kronoberg during 2 winters (2010–12). Study III used a prospective cohort of children recruited from 4 PHCC in the regions of Kronoberg, Kalmar, and Skåne during 3 full years (2014–17). The participants in Studies I–III were subsequently followed up through medical file review.

Study IV used retrospective registry data from 5 full years (2012–16) from 31 of 34 PHC centres in Region Kronoberg.

The electronic medical records (EMR) system used in Region Kronoberg during all study periods is a comprehensive system used in both PHC and hospitals (Cambio Cosmic, Cambio Health care Systems, Linköping, Sweden).

Study populations

Prospective recruitment

Studies I and II built on a cohort of 220 patients and 128 controls previously recruited from ambulatory care by Hedin et al. [29]. Participants were recruited from patients 15–45 years old who presented to the phone triage nurse at their PHCC with suspected acute pharyngotonsillitis and were considered in need of a physician's visit according to national guidelines [91]. Apart from study-related procedures, patients received care as usual. In addition, 128 non-infected controls were recruited from patients who visited their PHCC for other reasons. However, as 2 controls had been incorrectly registered, they were removed from the dataset in Study II, leaving 126 controls.

Study III had a similar design but recruited 77 patients and 34 controls 0–14 years old. The inclusion criteria were acute sore throat as a primary complaint (or clinical signs of pharyngotonsillitis in the youngest) and a duration ≤7 days; exclusion criteria were an imminent complication (peritonsillitis, sinusitis, acute otitis media or lymphadenitis colli) or obstructive airway disease. The controls were recruited from non-infected children who visited the PHCC for other reasons. Apart from study-related procedures, patients received care as usual.

Registry data

In Study IV, we selected all 20 858 patients who were diagnosed with pharyngotonsillitis in PHC or a hospital clinic in Region Kronoberg between 2012 and 2016. From this population, 14 024 patients who had been tested with an RADT or a throat culture in PHC were selected based on certain criteria to form two cohorts (for details, see sub-section Data collection below).

Data collection

Aetiological data

The prospective cohorts

As previously described [29], patients and controls in Studies I and II were sampled from the throat, nasopharynx and blood and screened for 20 bacteria and viruses. Routine culture was used for beta-haemolytic streptococci (Lancefield group A, C, and G), a selective anaerobic culture plate was used for *F. necrophorum* (incubated anaerobically for 5 days), multiplexed PCR was used for 2 intracellular bacteria (*M. pneumoniae* and *C. pneumoniae*) and 13 viruses (adenovirus, bocavirus, coronaviruses (NL63, OC43, HKU1, and 229E), enterovirus, influenza A and B virus, metapneumovirus, parainfluenza virus, rhinovirus, and respiratory syncytial virus), and serology was used for Epstein-Barr virus (Table 2).

In Study III, throat samples were collected with a nylon-flocked swab (ESwab®, Copan Diagnostics Inc., Murrieta, CA) and screened for 29 bacteria and viruses. Routine culture was used for beta-haemolytic streptococci (Lancefield group A, B, C, and G), *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, Gram-negative rods and *A. haemolyticum*, real-time PCR was used for *F. necrophorum*, and multiplexed real-time PCR was used for 5 bacteria (*S. pneumoniae*, *H. influenzae*, *B. pertussis*, *C. pneumoniae* and *M. pneumoniae*) and 15 viruses (adenovirus, bocavirus, coronaviruses (NL63, OC43, HKU1, and 229E), enterovirus, influenza A and B virus, metapneumovirus, parainfluenza virus 1–3, rhinovirus and respiratory syncytial virus) [123].

Performing an RADT for GAS (described below) was not part of the prospective cohort studies, but the physicians were allowed to use these tests in their routine care of the patient.

Table 2
Aetiological tests for viruses and bacteria in the four studies.

	Study I + II			Study III		Study IV	
	Culture	PCR	Serology	Culture	PCR	Culture	RADT
Bacteria							
Group A streptococci	X			X		X	X
Group B streptococci				X			
Group C streptococci	X			X		X	
Group G streptococci	X			X		X	
F. necrophorum	X				X	X	
S. pneumoniae				X	X		
H. influenzae				X	X		
M. catarrhalis				X			
S. aureus				X			
Gram-negative rods				X			
A. haemolyticum				X			
B. pertussis					X		
M. pneumoniae					X		
C. pneumoniae					X		
Viruses							
Adenovirus		X			X		
Bocavirus		X			X		
Coronavirus NL63		X			X		
Coronavirus OC43		X			X		
Coronavirus HKU1		X			X		
Coronavirus 229E		X			X		
Enterovirus		X			X		
Epstein-Barr virus			X				
Influenza A virus		X			X		
Influenza B virus		X			X		
Metapneumovirus		X			X		
Parainfluenza virus 1		X			X		
Parainfluenza virus 2		X			X		
Parainfluenza virus 3		X			X		
Rhinovirus		X			X		
Respiratory syncytial virus		X			X		

PCR = polymerase chain reaction; RADT = rapid antigen detection test.
PCR refers to real-time multiplexed PCR, except for *F. necrophorum* in Study II.
Culture refers to standard procedures for beta-haemolytic streptococci, *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, Gram-negative rods and *A. haemolyticum*, and a selective anaerobic culture plate for *F. necrophorum*.

The registry-based study

Routine throat cultures in Region Kronoberg to recover large colony beta-haemolytic streptococci used standard procedures [29]. Starting in 2013, the laboratory also offered an extended throat culture that added an anaerobic plate to recover *F. necrophorum* [29]. In late 2013, Matrix-assisted laser desorption/ionisation with time-of-flight mass spectrometer (MALDI-TOF) [124] was introduced for the species identification of streptococci, and the reporting of streptococci transitioned from Lancefield classification to species identification. GAS would henceforth be reported as *S. pyogenes*, and most of group C and G streptococci were reported as SDSE. In the registry-based study, findings of group C and G streptococci were reported as SDSE. RADTs for GAS are described in the next section.

Rapid antigen detection test for group A streptococci

RADTs for GAS are routinely used at most PHCCs in Sweden, with physicians and laboratory staff trained in the sampling technique. During the whole study periods of all four studies (2011–17), the RADT kit used in Region Kronoberg was QuickVue Dipstick Strep A® (Quidel Corporation, San Diego, CA, USA), a lateral-flow immunoassay using antibody-labelled particles that detects viable and nonviable organisms [125].

Clinical data

Signs and symptoms

In the two prospective cohorts (Studies I–III), clinical data such as tonsillar coating, swollen lymph glands, petechiae, cough, coryza, duration of illness, and fever were collected at inclusion from examination, medical history and temperature measurements.

In the registry-based study (Study IV), clinical data from the EMR system were not available.

Symptom diaries

In the prospective cohort of children (Study III), the parents were asked to keep a structured diary for 10 days and record symptoms, analgesics use, antibiotics use, and morning temperature. Each day they also assessed whether their child was still unwell and if their child missed preschool or school due to their illness. The diary was to be returned by mail; as a reminder, the parents of each patient were contacted by phone 2 weeks after inclusion.

Follow-up

In the two prospective cohorts (Studies I–III), patients and controls were followed up by a review of EMRs from PHC and hospital clinics. In the registry-based study (Study IV), patients were followed prospectively in the database.

The follow-up period varied between 3 months (Studies II–IV) and 2 years (Study I). The EMRs and the registry were reviewed for new visits for pharyngotonsillitis, a complication (peritonsillitis, sinusitis, acute otitis media or lymphadenitis colli), and tonsillectomy, and the EMRs were also reviewed for signs and symptoms.

Registry data

Retrospective data were extracted from the EMR system and the laboratory information system (ADBAKT, Autonik, Nyköping, Sweden) between 2012 and 2016 as described below.

First, 20 858 patients diagnosed with pharyngotonsillitis in conjunction with a physician's visit to a PHC or a hospital clinic were identified by diagnosis code. All visits to a physician require the physician to register a code according to the 10th revision of the *International Statistical Classification of Diseases and Related Health Problems* (ICD-10) or its Swedish PHC edition (KSH97-P). Our definition of pharyngotonsillitis was either J02x or J03x.

Second, the subset of 14 024 patients with at least one eligible visit to a PHC with aetiological testing was selected. Aetiological testing was defined as an RADT for GAS performed on the day of the visit or a throat culture performed within 7 days of the visit. The following exclusion criteria for a visit were used: 1) pharyngotonsillitis or a complication (peritonsillitis, media otitis, sinusitis, lymphadenitis or sepsis) the last 30 days; 2) antibiotic prescription the last 30 days; 3) missing data of the last 30 days; 4) a complication on the same day as the visit; and 5) prescription of an antibiotic type not indicated for a sore throat (i.e., phenoxymethylpenicillin (penicillin V), cefadroxil, clindamycin, amoxicillin, erythromycin, and azithromycin).

Third, from these patients a cohort was formed with all patients tested with an RADT. For each patient, the first eligible visit was denoted as the index visit.

Fourth, using the same patients as in step 2, we formed a new explanatory cohort, consisting of all patients who had a culture performed. As before, the first eligible visit with a culture was denoted as the index visit. As most of these patients had an RADT before the culture, they were included in both cohorts, with common index visits.

For all patients, data were extracted regarding age and sex, RADTs from PHC, throat cultures and antibiotic prescriptions from PHC and hospital clinics, and they were linked by visit date and Swedish personal identification number.

The outcomes were defined as a new visit for pharyngotonsillitis or a complication (defined as above) in a PHC or a hospital clinic within 30 days and 60 days from the index visit and tonsillectomy within 90 days.

Statistics

Software

Data were cleaned and analysed using Excel 2019 (Microsoft, Redmond, WA, USA), SPSS 23.0 and 25.0 (IBM, Armonk, NY, USA) and MedCalc (MedCalc Software Ltd., Ostend, Belgium).

Descriptive statistics

Frequencies and proportions were reported as numbers and percentages. Variance and central tendency of continuous variables were reported as mean and standard deviation, or, if non-normal distribution or small sample sizes, as median and interquartile range (IQR).

Positive predictive values were defined as true positives/all positives, and negative predictive values were defined as true negatives/all negatives.

Sensitivity was defined as true positives/(true positives + false negatives), and specificity was defined as true negatives/(true negatives + false positives).

Group comparisons

Categorical data

For comparison of categorical data, Pearson χ^2, Fisher's exact test or Mantel-Haenzel trend test was used for independent groups, and McNemar's test was used for paired data.

Continuous data

For comparison of three or more variables with non-normal distribution, Kruskal-Wallis H test was used, reported with the H statistic, degrees of freedom and p-value.

Regression models

To predict aetiology from sign and symptoms, multiple logistic regression was used, reported with both crude and adjusted odds ratios with 95% confidence intervals and p-values. The number of variables in the model was limited in accordance with the rule of thumb that states that the maximum number of variables should be the number of participants divided by 10–15.

P-values

For all the studies, a p-value <0.05 was considered significant, and p-values were reported rounded to one significant digit [126, 127].

Confidence intervals

Throughout the studies 95% was set as the confidence level. Confidence intervals for sensitivity and specificity were calculated with the binomial (Clopper-Pearson) "exact" method. Confidence intervals for predictive values were calculated as standard logit confidence intervals according to Mercaldo et al. [128].

Power calculations

In the prospective cohort for Studies I and II, the sample size was set to 150 patients and 150 controls so that no participant would represent a percentage >1 (personal communication with Hedin et al. [29]). Moreover, based on a small pilot study by one of the authors (MS), where *F. necrophorum* was detected in 11% of patients and 5% of controls (unpublished data), the chosen sample size was calculated so as to detect a 10% difference in the presence of *F. necrophorum* between patients and controls, given a power of 0.8 and an α value of 0.05 [29].

In Study III, no power calculation was performed. Instead, based on earlier reports [26, 35], the inclusion of 100 patients and 100 controls was estimated to be sufficient to describe the epidemiological situation.

In Study IV, no power calculation was performed as there would be no sampling. Instead, all available data were collected from 5 full years, starting one year before the introduction of extended anaerobic routine cultures for *F. necrophorum* in Region Kronoberg.

Aetiological predictive value (EPV)

Throat pathogens can be detected in healthy people with no signs of infection due to bacterial carriage, low-virulent infections or prolonged shedding of viral material. Therefore, one can assume that this background prevalence of pathogens is also

present to some extent in infected people – e.g., an asymptomatic carriage of GAS in a person infected by a virus. The difficulty is then how to interpret a positive finding of a pathogen in an infected person – i.e., how to determine whether a finding represents a true infection or a mere non-infectious presence.

EPV is a statistical method that accounts for such asymptomatic presence and provides a measure of the uncertainty with 95% confidence intervals [108]. The requisites are estimates of the prevalence of the pathogen in both symptomatic and healthy persons, the sensitivity of the test to detect it, and theta (i.e., the ratio of the background prevalence between symptomatic and healthy persons). Based on previous work [108], we assumed a 90% sensitivity of a throat culture to detect bacteria and a theta value of 0.9.

Ethical considerations

Informed consent

All participants in Studies I and II and parents or legal guardians in Study III gave written informed consent and could withdraw at any time. In Study III, verbal consent or assent in the youngest was obtained from the child.

Research with children

Although it would be unethical not to conduct research with children, it is important to recognise that children might not understand, partly or wholly, the implications of participation in a study. Information should be given in a language that matches the child's maturity, and the researcher should always seek consent or – if not possible – assent from the child before proceeding. Informed consent should also be obtained from the parents or legal guardians. Any study with children also needs to build methods to support the child when needed [129].

In Study III, both patients and controls were sampled with a throat swab – a harmless procedure but highly unpleasant to most people and often eliciting a vomiting reflex. Although throat samples are routinely used in patients with a sore throat, the controls would not otherwise have been swabbed. However, the transient discomfort in both patients and controls must be weighed against the potential benefits of learning more about throat infections in children.

Medical file review

Traditionally, medical file review has been a common source of data for biomedical research, but the potential gain from such studies must be balanced against subject

protection, including privacy and indirect re-identification [130].

In Studies I–III, all participants or parents gave written informed consent to medical file review. In the review process, the reviewer had access to all PHC and hospital clinics records, including records prior to inclusion. However, only records that seemed relevant to the study, based on heading, diagnosis code, or specific tests, were read through during review, and no records were printed or shared in other ways.

Big Data

Data sets generated from thousands of people can generally be thought of as Big Data, with its ethical implications. When it comes to registry data of patients in the health care system, the information is even more sensitive, and Mittelstadt and Floridi point to five key areas of concern: informed consent, privacy, ownership of the data, objectivity, and "Big Data Divides" created between those who have or lack the resources to analyse Big Data [131].

Study IV is no exception regarding these areas of concern, and the included patients were never given the possibility to consent. However, measures were taken (anonymisation and data protection) to assure privacy of all participants.

Ethics approval

Studies I and II were approved by the regional ethics review board in Linköping, Sweden (2010/267-31), with two amendments (2013/286-32 and 2015/146-32) as was Study IV (2016/529-31). Study III was approved by the regional ethics review board in Lund, Sweden (2014/314) with one amendment (2016/157).

Results

Presence of bacteria and viruses

Sampling of patients attending for acute sore throat

Children

Study III compared the findings of respiratory viruses and bacteria in throat samples from 77 children 0–14 years old attending PHC for acute sore throat with 34 healthy control children.

In total, 19 of the 29 targeted pathogens were detected in the study, of which at least one was detected in 66 (86%) patients and 24 (71%) controls ($p = 0.06$). Bacteria were found in 53 (69%) patients and in 20 (59%) controls ($p = 0.3$), and viruses in 28 (36%) patients and 9 (26%) controls ($p = 0.3$).

GAS was the most prevalent pathogen in both patients (49%) and controls (32%) ($p = 0.1$) (Table 3 and Table 4). In patients, the most common findings after GAS were *H. influenzae*, *S. aureus*, rhinovirus and influenza B virus. In controls, rhinovirus (21%) was the most common finding after GAS. Group C or G streptococci were found in 1 patient and 4 (12%) controls, and *F. necrophorum* was found in 1 patient and 1 control. None of the 19 pathogens differed in prevalence between patients and controls with statistical significance (data partly shown in Table 3). A combination of 2 or 3 pathogens was found in 23 (30%) patients and 9 (26%) controls. The most common combination among patients was GAS and influenza B virus (n = 4). Still, GAS was mostly found as a sole pathogen (in 71% and 55% of patients and controls with GAS, respectively).

Table 3
The five most commonly detected pathogens by PCR or throat culture in 77 children 0–14 years old attending for acute sore throat in primary health care, and their prevalences in 34 healthy children (controls).

Detected pathogen, n (%)	Patients (n = 77)	Controls (n = 34)	*p*	Test
Group A streptococci	38 (49)	11 (32)	0.1	X^2
H. influenzae	9 (12)	2 (6)	0.5	Fisher's
S. aureus	7 (9)	3 (9)	1	Fisher's
Rhinovirus	7 (9)	7 (21)	0.1	X^2
Influenza B virus	6 (8)	–	0.2	Fisher's

Young adults

Studies I and II were based on microbial and clinical data previously collected by Hedin et al. from patients 15–45 years old attending PHC for acute sore throat and healthy controls [29].

In summary, 155/220 (71%) patients and 26/128 (20%) controls had at least one of the 20 targeted pathogens ($p < 0.001$). Bacteria were found in 103 (47%) patients and in 17 (13%) controls ($p < 0.001$), and viruses in 70 (32%) patients 11 (8.6%) controls ($p < 0.001$) (Table 4).

GAS was the most common finding in patients (30%), followed by *F. necrophorum* (15%), influenza B virus (7.3%) and rhinovirus (6.4%). In controls, Group G streptococcus was the most common finding (9/128; 7.0%), followed by *F. necrophorum* (3.1%), GAS (2.3%) and rhinovirus (2.3%).

Table 4
The most commonly detected pathogens in 77 children 0–14 years old, and 220 adults 15–45 years old [29], attending for acute sore throat in primary health care, and their prevalences in healthy controls.

	Detected pathogen, n (%)			
	Children 0–14 years old		Adults 15–45 years old[1]	
	Patients (n = 77)	Controls (n = 34)	Patients (n = 220)	Controls (n = 128)
Any bacteria[2]	53 (69)	20 (59)	103 (47)	17 (13)
Any virus	28 (36)	9 (26)	70 (32)	11 (8.6)
Group A streptococci	38 (49)	11 (32)	66 (30)	3 (2.3)
H. influenzae[3]	9 (12)	2 (6)	N/A	N/A
S. aureus[3]	7 (9)	3 (9)	N/A	N/A
F. necrophorum	1 (1.3)	1 (2.9)	33 (15)	4 (3.1)
Influenza B virus	6 (8)	–	16 (7.3)	–
Rhinovirus	7 (9)	7 (21)	14 (6.4)	3 (2.3)

[1] Complete aetiological data from the adults were previously published by Hedin et al. [29].

[2] The children were screened for group A, B, C, and G streptococci, *S. pneumoniae*, *H. influenzae*, *S. aureus*, *F. necrophorum*, *M. catarrhalis*, Gram-negative rods, *A. haemolyticum*, *B. pertussis*, *M. pneumoniae*, and *C. pneumoniae* and with a multiplexed PCR panel for respiratory viruses (see Table 2). The adults were screened for group A, C, and G streptococci, *F. necrophorum*, *M. pneumoniae*, and *C. pneumoniae,* with serology for Epstein-Barr virus and a multiplexed PCR panel for respiratory viruses much similar to that used in the children [29].

[3] The adults were not tested for *H. influenzae* and *S. aureus*. N/A = not applicable.

Registry data of patients diagnosed with pharyngotonsillitis

In Study IV, we identified 14 024 patients of all ages with a diagnosed pharyngotonsillitis in PHC who had performed aetiological testing with an RADT or a throat culture, or both.

Patients with a rapid antigen detection test for group A streptococci

In the cohort of 13 781 patients who performed an RADT for GAS on the same day as the index visit, 9 170 (67%) had a positive result. Among children 0–14 years old in this cohort, the proportion was 4 056/5 080 (80%).

Patients with a regular or extended throat culture

In the cohort of 1 370 patients who performed a throat culture within 7 days of the index visit, 54% had a regular culture, of which 31% had bacterial growth, and 46% patients had an extended culture, of which 37% had bacterial growth (Table 5). Almost half of the cultures (47%) were performed in patients 15–29 years old. Most patients (74%) who were cultured also were tested with an RADT of which 19% were positive for GAS.

GAS was detected in 15% of all cultures and SDSE in 14% ($p = 0.6$), although only as concomitant findings in 2 patients. SDSE was most prevalent in patients aged 15–29 (63% of all findings of SDSE), with a detection rate of 18% in this age group. Among children 0–14 years old, GAS was detected in 27% of the patients and SDSE in 10%.

F. necrophorum was detected in 15% of patients with extended cultures: in 80% as the sole finding, in 4% concomitant with GAS, and in 16% concomitant with SDSE. The prevalence of *F. necrophorum* was similar to that of GAS and SDSE in extended cultures (12% and 14%, respectively) ($p = 0.21$). None of the extended cultures had a concomitant growth of all three bacteria. A majority (79%) of patients with *F. necrophorum* were 15–29 years old, and the detection rate in extended cultures in this age group was 23%. Among children 0–14 years old, *F. necrophorum* was only detected in 2 (2.2%) patients with extended cultures.

Table 5
Results from 1 370 routine regular and extended throat cultures performed within 7 days from the index visit for pharyngotonsillitis in primary health care.

Throat culture finding, n (%)	Regular culture (n = 745)	Extended culture[1] (n = 625)
Any bacteria[2]	231 (31)	234 (37)
S. pyogenes	128 (17)	73 (12)
SDSE	104 (14)	86 (14)
F. necrophorum	N/A	95 (15)
No pathogen	514 (69)	391 (63)

N/A = not applicable; SDSE = *S. dysgalactiae* ssp. *equisimilis* (formerly described as group C or G streptococci).

[1] Extended cultures include an anaerobic plate for the recovery of *F. necrophorum* in addition to the routine plate for beta-haemolytic streptococci.

[2] As some patients had concomitant growth of 2 bacterial species, the total number of positive cultures is less than the sum of the individual findings.

Clinical findings

The following section is based on clinical data from the prospective cohort of 77 children 0–14 years old attending for acute sore throat (Study III) and from the prospective cohort of 220 young adults 15–45 years old attending for acute sore throat (Study II). In addition, the section presents the prevalence of different signs and symptoms in relation to aetiology (as determined by PCR, throat culture, and serology).

Signs and symptoms in relation to aetiology

Children

The prevalence of clinical symptoms in relation to the mutually exclusive and exhaustive aetiological groups "only bacteria" (in which GAS was found in 29/30 patients; 97%), "only viruses", "viruses + bacteria" and "no pathogen" are shown in Figure 1a. The median number of days with a sore throat before inclusion was 3 (IQR 2–5) in all patients, and there were no differences across the aetiological groups ($p = 0.3$). Fever ≥38.5 °C at the visit was rare in all groups. Cough and coryza were observed in a higher proportion of patients with "only viruses" than in the other groups; however, none of the symptoms in Figure 1a differed with a statistical significance in prevalence between the four aetiological groups or between "only viruses" and "only bacteria" (data not shown). A renewed analysis, excluding *H. influenzae* and *S. aureus* from the bacterial groups, did not change this finding (data not shown). Coryza was, however, more common in patients with "only viruses" than in patients with "only GAS" ($p = 0.04$).

Most patients (61%) had a Centor score of 0–2, and 39% of the patients had 3. No patient had a score of 4, attributed mainly to the few patients with fever at the visit. The distribution of Centor scores for different aetiological groups is presented in Figure 2a.

Young adults

The prevalence of clinical symptoms in relation to aetiology is partly shown in Figure 1b. As with the children, the aetiologies were divided into the mutually exclusive and exhaustive groups "only bacteria" (in which GAS was found in 55/85 patients; 65%), "only viruses", "viruses + bacteria", and "no pathogen".

The number of days with a sore throat before inclusion differed between groups ($p = 0.004$), with a median of 4 days (IQR 3–7) for all patients and the highest number among patients with "no pathogen" (median 6 days, IQR 3–10). A cough was more common in patients with "only viruses" (67%) compared to patients with "only bacteria" (21%) ($p < 0.001$), as was coryza (67% vs. 24%; $p < 0.001$) and

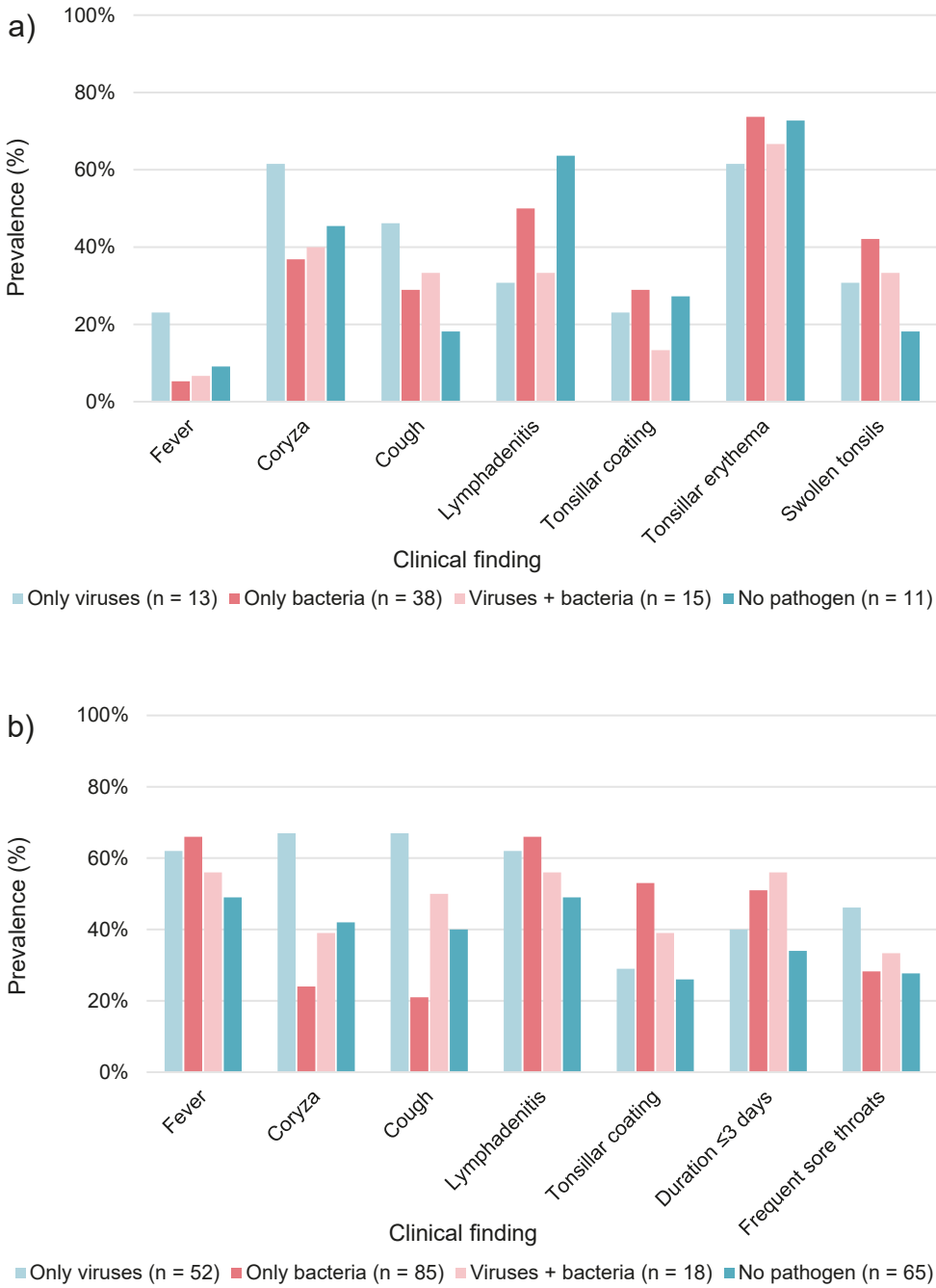

Figure 1
Prevalence of signs and symptoms for different aetiologies (as determined by PCR, throat culture or serology) in patients attending with acute sore throat in primary health care: a) 77 patients 0–14 years old and b) 220 patients 15–45 years old. Fever was defined as a temperature ≥38.5 °C at the visit. The groups are mutually exclusive.

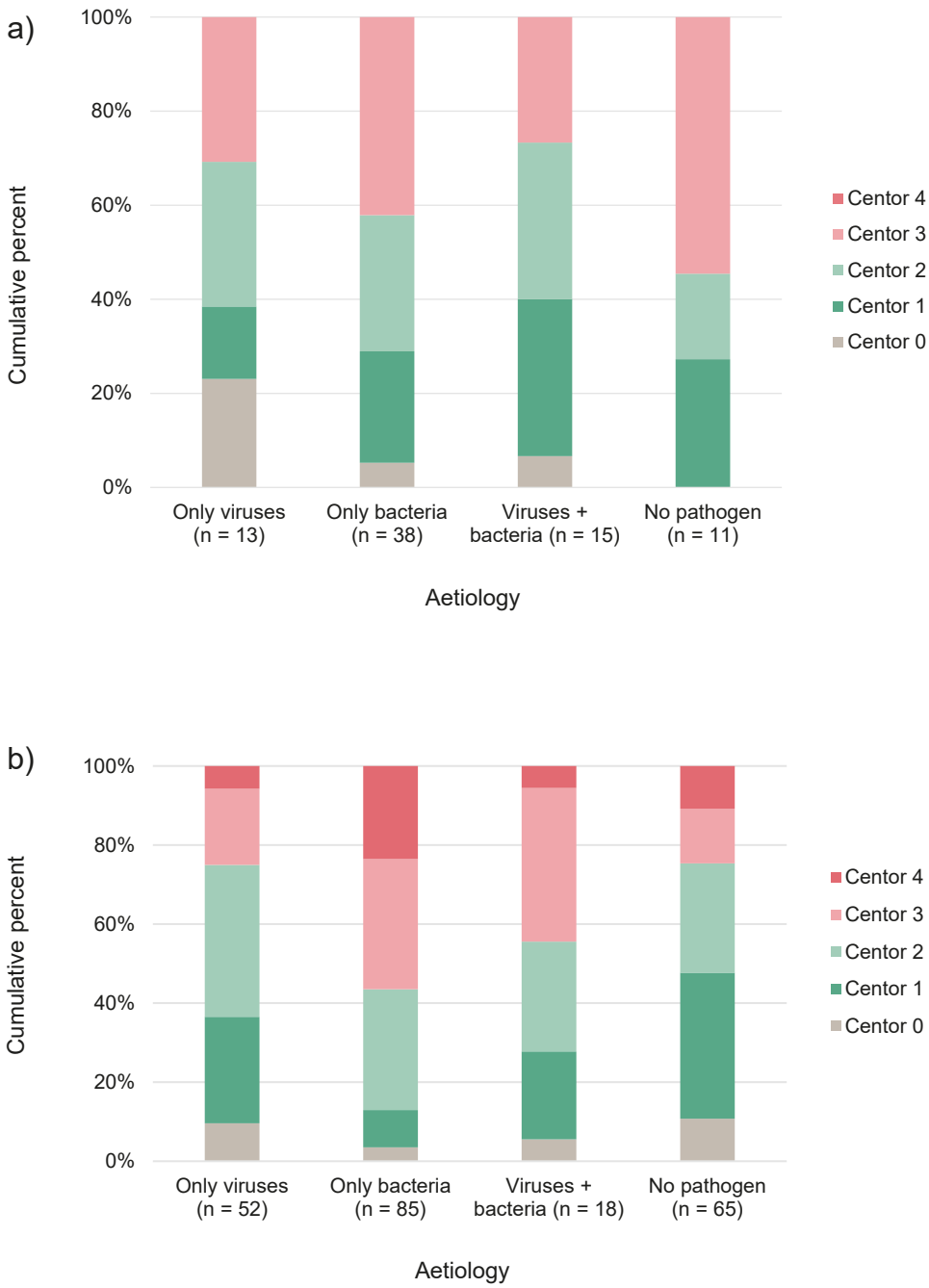

Figure 2
Distribution of Centor scores for different aetiologies (as determined by PCR, throat culture or serology) in patients attending with acute sore throat in primary health care: a) 77 patients 0–14 years old and b) 220 patients 15–45 years old. The groups are mutually exclusive.

a history of frequent sore throats (46% vs. 28%; $p = 0.03$). In contrast, tonsillar coating was more common in patients with "only bacteria" (53%) than in patients with "only viruses" (29%) ($p = 0.006$). The prevalence of lymphadenitis, fever, and duration of symptoms ≤3 days were similar between these two groups (data not shown).

Most patients (61%) had a Centor score of 0–2, and 39% of the patients had 3–4. The highest proportion with a score of 3–4 was observed among patients with GAS (64%). Moreover, a score of 3–4 was more common in patients with "only bacteria" (56%) than in patients with "only viruses" (25%) ($p < 0.001$). The distribution of Centor scores for different aetiological groups are presented in Figure 2b, and for group A, C, and G streptococci in Figure 3.

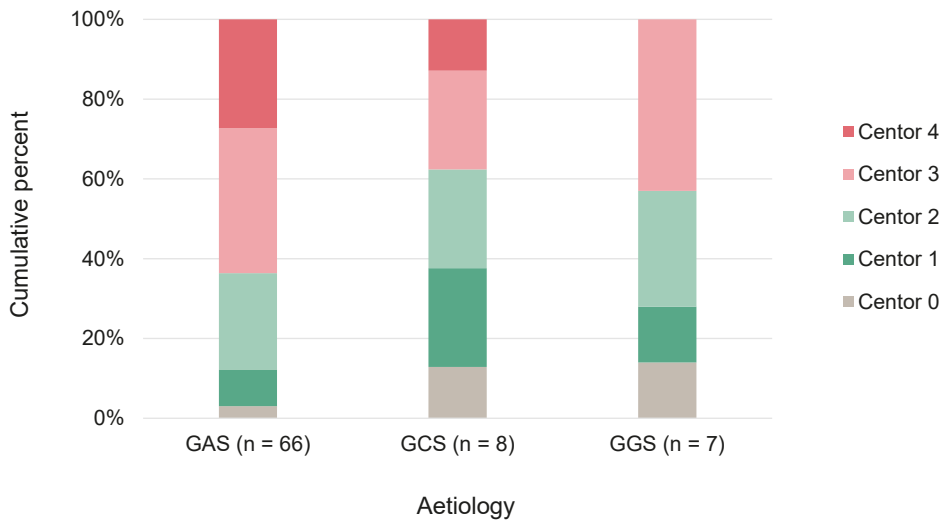

Figure 3
Distribution of Centor scores in 81 patients 15–45 years attending with acute sore throat in primary health care and with findings of group A (GAS), C (GCS), and G (GGS) streptococci in throat culture. Although GCS and GGS are traditionally regarded as separate entities in accordance with the C or G surface antigen they express, it is now known that most cases of GCS and GGS in humans belong to the same species: *S. dysgalactiae* ssp. *equisimilis* (SDSE).

Clinical course in children as registered in symptom diaries

We received complete diaries from 71% of the patients. Of these, 95% reported a resolution of their sore throat within 10 days, although 9% reported recurrent symptoms after the initial resolution. The fastest resolution was reported in patients with GAS treated with antibiotics (median = 3 days; IQR 1.5–3.5), and the slowest resolution was reported in patients with GAS not treated with antibiotics (median = 4.5 days; IQR 2.3–8.8). However, this difference was not statistically significant ($p = 0.1$).

Diagnosis and prediction

The previous section described the frequency of clinical symptoms for different known aetiologies in Studies II and III. In this section, things are turned around to focus on how well these symptoms can predict aetiology. Such a prediction is possible when we know the total prevalence of different pathogens and their associated symptoms.

Signs and symptoms

Children

Overall, single symptoms had only low to moderate PPVs for different aetiologies. The PPV of swollen tonsils for detecting GAS was 67% (95% CI 51–80%), and the corresponding value for lymphadenitis was 53% (95% CI 41–64%). Coryza was most common in patients with "only viruses", but the low prevalence of viruses in this cohort resulted in a PPV of coryza for detecting "only viruses" of only 24% (95% CI 16–35%). Most patients with GAS did not have a cough, but neither did many other patients, so the PPV of lack of cough for detecting GAS was a mere 55% (95% CI 47–62%).

Young adults

In Study II, predictive values were calculated for the aetiologies "any bacteria" and GAS. Overall, single symptoms had only low to moderate PPVs for both groups but were better at predicting "any bacteria" than GAS specifically (Figure 4a). The NPVs for GAS were the highest for "absence of coryza" (87%; 95% CI 79–92%) and "absence of a cough" (86%; 95% CI 79–92%), implying that a presence of a cough or coryza would rule out most cases of GAS.

In a multiple regression model, tonsillar coating and absence of cough were associated with both GAS and "any bacteria", whereas lymphadenitis and fever were not (data not shown).

Centor score

The Centor scoring system predicts GAS in throat culture and gives 1 point each for fever, absence of cough, lymphadenitis and tonsillar coating (purulent exudates). Thus, there are many possible combinations of symptoms for each score level, except for the highest level.

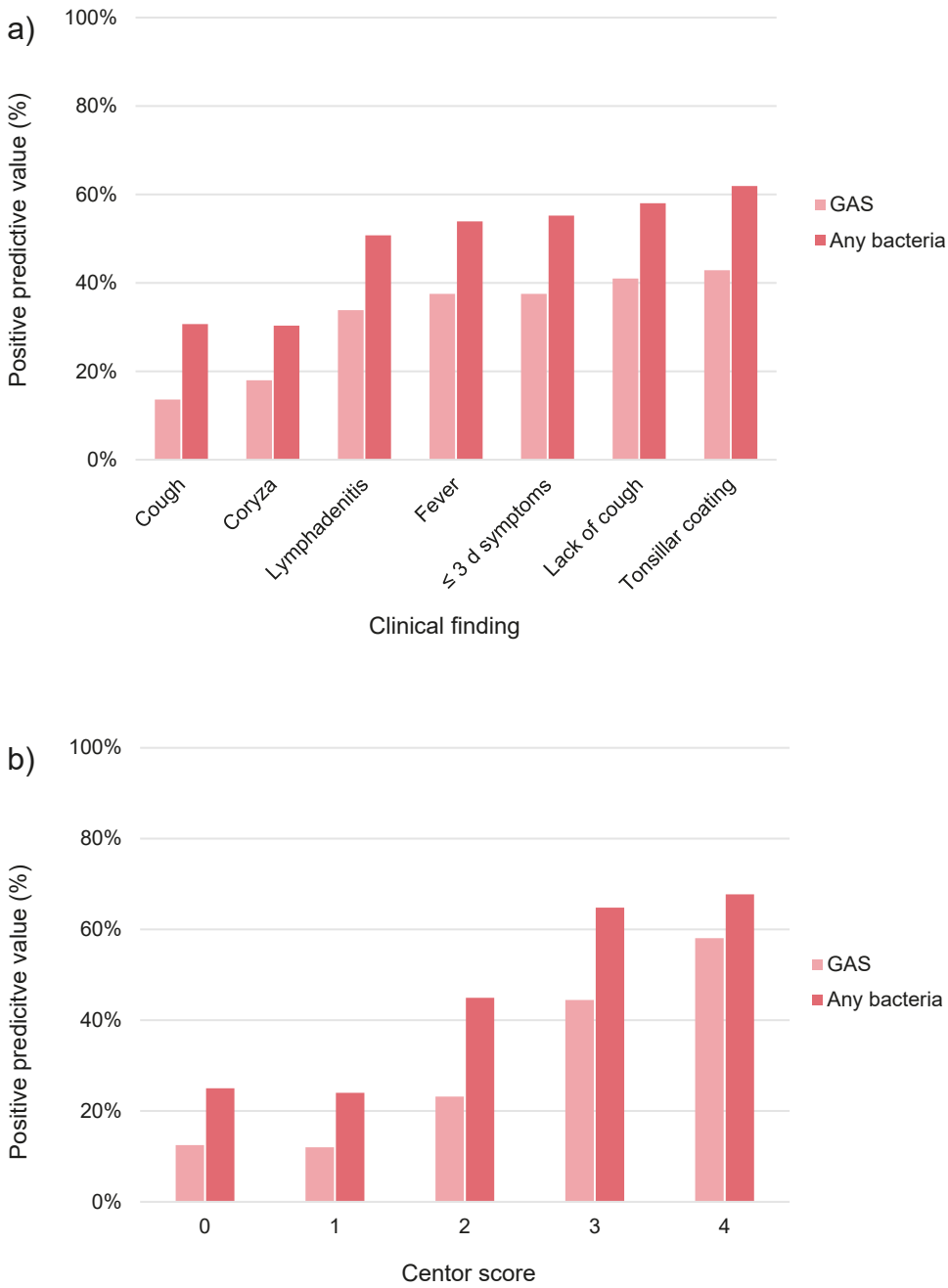

Figure 4
Positive predictive values (expressed as percentages) of clinical signs and symptoms (Figure a) and Centor score (Figure b) in predicting group A streptococci (GAS) and any bacteria (including GAS) in throat culture in 220 patients 15–45 years old attending primary health care for acute sore throat.

Children

Almost half of the patients with GAS (45%) had a score of 3, but so did 31% of patients with "only viruses" (p = 0.5) and 39% of all patients, so the PPV of a Centor score of 3 for detecting GAS was a modest 57% (95% CI 43–70%). Conversely, as low Centor scores were common also among patients with GAS, the corresponding NPV for GAS (i.e., the probability that a patient with absence of a Centor score of 3–4 (i.e., a score of 0–2) has an aetiology other than GAS) – was only 55% (95% CI 46–64%).

Young adults

The positive PPVs of different Centor scores for the aetiologies are shown in Figure 4b and Figure 5. Although viruses were more probable at low scores, the probability of a bacterial finding increased with each step. The PPV for GAS, which made up most bacterial findings, increased with each Centor score and reached 58% (95% CI 42–73%) at a score of 4. However, the PPVs for "any bacteria" were even higher, with a PPV of 68% (95% CI 51–81%) at a score of 4. A regression analysis with a score of 0 as reference also showed a positive association between odds ratios for both GAS and "any bacteria" and an increasing score (data not shown).

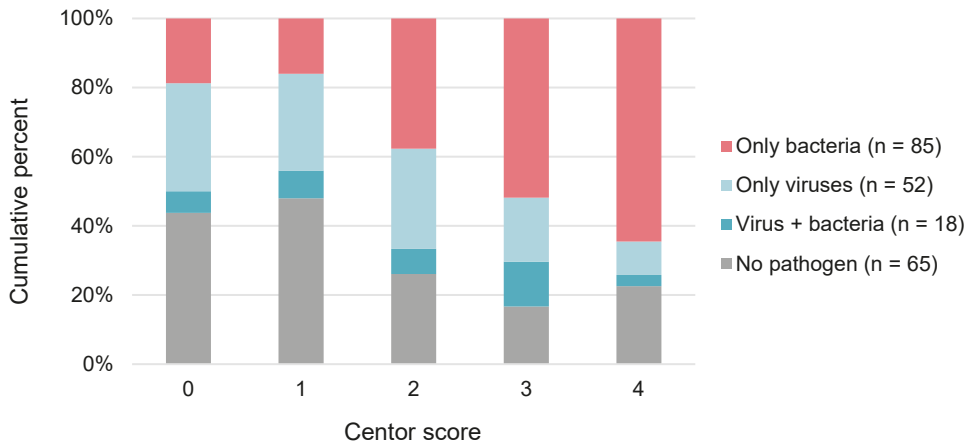

Figure 5
Distribution of aetiologies at different Centor scores in 220 patients 15–45 years old attending primary health care with acute sore throat.

Rapid antigen detection test for group A streptococci

Despite the RADT not being a mandatory part of the two prospective cohort studies (Studies II and III), most patients in both cohorts were tested.

Children

In total, 69% of the patients were tested with an RADT, and 60% of these tests were positive. Using throat culture as reference, the sensitivity of the RADT was 89%, the specificity was 69%, the positive predictive value was 75%, and the negative predictive value was 86% (Table 6). RADTs were more frequently used in patients with a Centor score of 3–4 (93%) than patients with Centor 0–2 (53%). In patients with a Centor score of 3–4, the RADT was positive in 64%, and in patients with a score of 0–2, the test was positive in 56% ($p = 0.5$).

Table 6
Relationship between results from rapid antigen detection tests (RADT) for group A streptococci (GAS) and throat cultures for GAS in 77 patients 0–14 years old attending with acute sore throat in primary health care.

	Positive culture	Negative culture	Total
RADT for GAS +	24	8	32
RADT for GAS −	3	18	21
Total	27	26	53

Sensitivity: 24/27 = 89% (95% CI 71–98%); specificity: 18/26 = 69% (95% CI 48–86%); positive predictive value: 24/32 = 75% (95% CI 62–84%); negative predictive value: 18/21 = 86% (95% CI 67–95%).

Young adults

In total, 94% of the patients were tested with an RADT, and 33% of these tests were positive. Using throat culture as reference, the sensitivity of the RADT was 89%, the specificity was 92%, the positive predictive value was 84%, and the negative predictive value was 95% (Table 7). In patients with a Centor score of 3–4, the RADT was positive in 53%, and in patients with a score of 0–2, the test was positive in 20% ($p < 0.001$). The distribution of true and false positive and negative RADTs is presented in Figure 6.

Table 7
Relationship between results from rapid antigen detection tests (RADT) for group A streptococci (GAS) and throat cultures for GAS in 220 patients 15–45 years old attending with acute sore throat in primary health care.

	Positive culture	Negative culture	Total
RADT for GAS +	57	11	68
RADT for GAS −	7	132	139
Total	64	143	207

Sensitivity: 57/64 = 89% (95% CI 79–95%); specificity: 132/143 = 92% (95% CI 87–96%); positive predictive value: 57/68 = 84% (95% CI 74–90%); negative predictive value: 132/139 = 95% (95% CI 90–97%).

The positive predictive value of a positive RADT increased with each level of Centor score (Figure 7) as patients with low scores had a lower prevalence of GAS and, therefore, a larger proportion of false positives. For example, although the PPV for GAS in patients with a positive RADT and a Centor score of 1 was a modest 60% (95% CI 24–88%), the PPV for GAS of a positive RADT and a score of 3–4 was 93% (95% CI 81–97%). The sensitivity of the RADT also increased with each

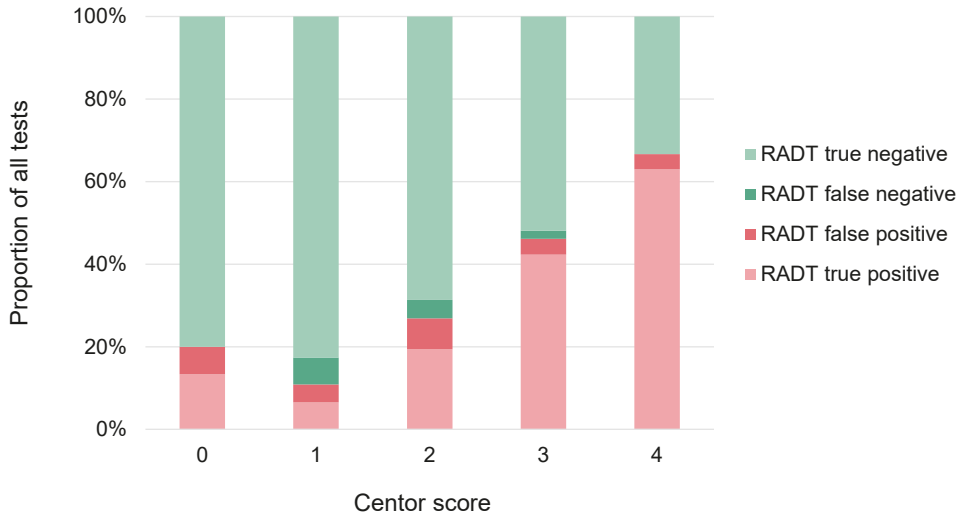

Figure 6
Distribution of true and false positive and negative RADTs for GAS in relation to Centor score, compared with throat culture, in 207 patients 15–45 years old attending with acute sore throat in primary health care.
GAS = group A streptococci; RADT = Rapid antigen detection test.

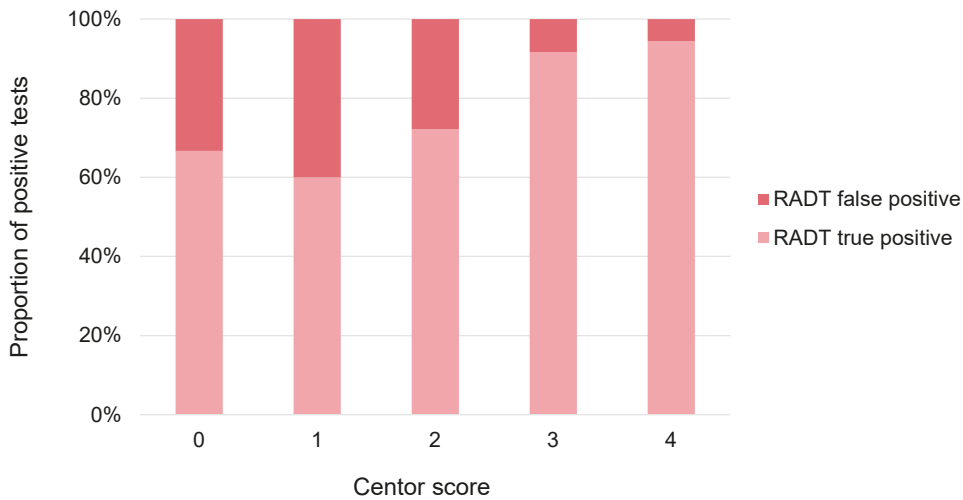

Figure 7
PPV of a positive RADT for GAS at different Centor scores. The graph displays the distribution of true and false positive RADTs for GAS in relation to Centor score, compared with throat culture, in 207 patients 15–45 years old attending with acute sore throat in primary health care. PPV = true positives / (true positives + false positives).
GAS = group A streptococci; PPV = positive predictive value; RADT = Rapid antigen detection test.

level of Centor score, varying between 50% and 100%. In contrast, the negative predictive value was high at all Centor scores (93–100%).

Aetiology in young adults with a negative test result

The distributions of aetiologies at different Centor scores in the 139 patients with a negative RADT are presented in Figure 8. The 37 patients with a Centor score of 3–4 but a negative RADT had the following aetiologies: "any bacteria" (38%), "only viruses" (32%), and "no pathogen" (30%). The 14 patients with bacteria had *F. necrophorum* (n = 9), group G streptococci (n = 3), group C streptococci (n = 2), and GAS (n = 1).

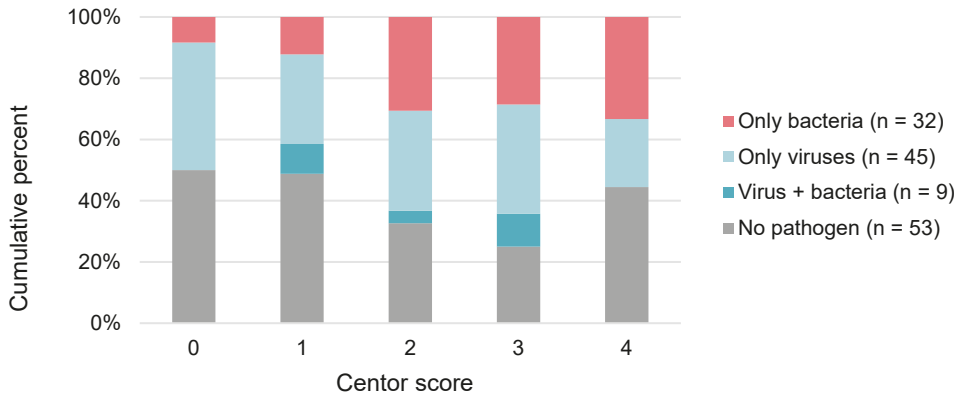

Figure 8
Distribution of aetiologies at different Centor scores in 139 patients 15–45 years old who attended primary health care with acute sore throat and had a negative rapid antigen detection test (RADT) for group A streptococci (GAS).

Aetiological predictive values (EPV)

Even in a situation where an aetiological test with high specificity (i.e., a low rate of false positives) has detected a pathogen in a symptomatic patient, there is still a probability that this pathogen only represents a non-infectious carriage and that some other pathogen is, in fact, responsible for the symptoms (see Introduction).

Children

As GAS was highly prevalent in throat cultures of both patients (49%) and healthy children (32%), the resulting positive EPV for GAS was a moderate 54%, with a huge confidence interval (95% CI 0–92%). This EPV implies that GAS was the causative pathogen in only about half of the patients testing positive for GAS and that some other virus or bacterium may have been responsible for the symptoms. In patients with a Centor score of 3–4, the corresponding value was slightly higher, 67%, but still with a great uncertainty (95% CI 0–97%).

Young adults

A 30% prevalence of GAS among patients and 2.4% among controls resulted in a positive EPV for GAS of 95% (95% CI 81–100%), implying that most detected cases of GAS truly represented an infection.

The 47% prevalence of "any bacteria" among patients and 13% in controls resulted in a positive EPV for "any bacteria" of 84% (95% CI 62–95%).

Follow-up

In the prospective cohorts (Studies I–III), patients and controls were followed up by reviewing electronic medical records. In the retrospective registry-based study (Study IV), patients were followed in the registry from the index visit and forward. An overview of the follow-up periods and clinical outcomes for the four studies are presented in Table 8.

New visits for pharyngotonsillitis

The prospective cohort of children

In the cohort of children 0–14 years old, 12/77 (16%) patients made a new visit for a sore throat within 3 months from inclusion, after a median of 25 days (IQR 18–53). Four of these patients had worsened or non-resolving symptoms, and they all had non-treated GAS at inclusion. The other 8 patients had a new episode of sore throat, and 5 of these had GAS at inclusion, 3 of whom received antibiotics. None of the controls consulted for a sore throat during the follow-up period.

The prospective cohort of young adults

In the cohort of young adults, 207 (94%) patients and 108 (86%) controls could be followed for 2 years. Of these, 21 (10%) patients and 4 (3.7%) controls made a new visit for a sore throat within 30 days from inclusion ($p = 0.045$). Of these 21 patients, 4 had non-resolving symptoms, and 17 had a new episode.

Patients with GAS as the sole microbial finding had a higher proportion of new visits within 30 days (20%) than patients with no GAS (6.4%) ($p = 0.02$). This difference remained when including the patients where GAS was found concomitant with other pathogens (18%) ($p = 0.01$).

None of the 10 patients with *F. necrophorum* as the sole microbial finding re-consulted for a sore throat within 30 days, in contrast to 11% of the patients with no *F. necrophorum* ($p = 0.08$). Including the patients where *F. necrophorum* was found concomitant with other pathogens, 7% re-consulted within 30 days ($p = 0.7$, compared with no *F. necrophorum*).

Patients with a Centor score of 4 re-consulted most frequently (19%), in contrast to patients with a score of 0, where none re-consulted. A trend test, however, showed no association between score and re-consultation ($p = 0.16$). A multiple logistic regression model adjusted for covariates revealed no association between re-consultation within 30 days and absence of a cough, temperature ≥38.5°C, cervical lymphadenitis, tonsillar coating or antibiotic prescription (data not shown).

After 3 months, 15% of all patients had re-consulted for a sore throat. Again, the highest proportion, 26%, was among patients with a Centor score of 4 ($p = 0.054$).

After 2 years, 43% of the patients and 18% of the controls had consulted again for a sore throat ($p < 0.001$). At this point, however, there were no significant differences among patients with regard to aetiology at inclusion.

Antibiotics were prescribed to 44% of the patients at inclusion and more often to patients with a Centor score of 3–4 (70%) than to patients with a Centor score of 0–2 (28%) ($p < 0.001$). Patients with GAS as the sole finding had the highest proportion of prescriptions (96%), followed by patients with "only bacteria" (73%), "only *F. necrophorum*" (30%), no found pathogen (22%) and "only viruses" (14%). However, antibiotic prescription was not associated with the rate of new visits for a sore throat within 30 days or 2 years, neither in the patient group as a whole nor within the different aetiological groups (data not shown).

Registry data

In the RADT cohort, 8.6% of the patients made a new visit for pharyngotonsillitis within 30 days, 41% of these within 7 days (median 12, IQR 4–16).

In patients with a positive RADT, antibiotic prescription was associated with a lower proportion of pharyngotonsillitis within 30 days, compared with no prescription (8.7% vs. 12%; $p = 0.02$). In contrast, in patients with a negative RADT, antibiotics were associated with a higher proportion of pharyngotonsillitis (9.7% vs 7.9; $p = 0.01$). The choice of antibiotic was unrelated to the rate of new visits (data not shown).

In the culture cohort, 20% of the patients made a new visit for pharyngotonsillitis within 30 days, and 80% of these within 7 days (median 3, IQR 2–6). The highest proportion was registered among patients with a negative culture (23%), followed by patients with SDSE (22%), patients with *F. necrophorum* (21%), and patients with GAS (16%) ($p = 0.2$).

In patients with SDSE, antibiotic prescription was associated with a lower proportion of pharyngotonsillitis within 30 days, compared with no prescription (15% vs. 29%; $p = 0.03$). In contrast, in patients with a negative culture, antibiotics were associated with about twice as large a proportion of pharyngotonsillitis (29% vs. 18%; $p = 0.01$). In patients with GAS and patients with *F. necrophorum,* there was no association between antibiotic prescription and re-consultation for pharyngotonsillitis. The choice of antibiotic was unrelated to the rate of new visits (data not shown).

Table 8
Overview of follow-up periods and clinical outcomes for Studies I–IV.

	Studies I+II		Study III		Study IV	
Outcome, n (%)	Patients	Controls	Patients	Controls	Cohort 1	Cohort 2
Pharyngotonsillitis						
30 days	21/207 (10)	4/108 (3.7)			1160/13460 (8.6)	269/1343 (20)
90 days	32/207 (15)		12/77 (16)	0/34		
2 years	90/207 (43)	19/108 (18)				
Complication						
30 days	1/205 (0.49)	0/108			214/13460 (1.6)	51/1343 (3.8)
60 days					315/13207 (2.4)	59/1303 (4.5)
90 days			0/77	0/34		
Tonsillectomy						
90 days	0/178	0/108	0/77	0/34	36/12987 (0.28)	15/1282 (1.8)
2 years	5/178 (2.8)	0/108				

Studies I+II = prospective recruitment of patients 15–45 years old attending PHC for acute sore throat; Study III = prospective recruitment of patients 0–14 years old attending PHC for acute sore throat; Study IV = registry study of patients of all ages with a diagnosed pharyngotonsillitis.

Cohort 1 = rapid antigen detection test for group A streptococci cohort; cohort 2 = throat culture cohort.

Complications

The prospective cohorts

In the prospective cohort of children 0–14 years old, none of the 77 patients and 34 controls had a complication or were hospitalised within 3 months from inclusion.

In the prospective cohort of young adults, 1 (0.49%) patient had a complication within 30 days from inclusion (acute sinusitis). This patient had *F. necrophorum* at inclusion and no antibiotic treatment.

Registry data

In the RADT cohort, 1.6% of the patients had a diagnosed complication within 30 days and 42% of these within 7 days (median 12, IQR 3–21). The most common complication was peritonsillitis, which comprised 29% of the complications, and was diagnosed in 0.47% of the patients, 73% of these within 7 days (median 3, IQR 2–14).

There were no significant associations between antibiotic prescription and complication rates, regardless of RADT result. However, there was an association

between antibiotic choice and peritonsillitis rates both in patients with a positive RADT and in patients with a negative RADT, with the lowest rates associated with penicillin V (data not shown).

In the culture cohort, 3.8% of the patients had a diagnosed complication within 30 days, 86% of these within 7 days (median 3, IQR 2–5). Peritonsillitis comprised most complications (82%) and was diagnosed in 3.1% of the patients, 88% of these within 7 days (median 2, IQR 2–5).

A complication within 30 days of the index visit was most frequent among patients with a single finding of *F. necrophorum* (9/75; 12%), followed by patients with a negative culture (22/381; 5.8%), patients with a single finding of SDSE (2/171; 1.2%), and patients with a single finding of GAS (1/190; 0.53%) ($p < 0.001$). As peritonsillitis comprised most complications, the numbers were very similar for peritonsillitis within 30 days: *F. necrophorum* (8/75; 11%), negative culture (19/381; 5.0%), SDSE (2/171; 1.2%), and GAS (1/190; 0.53%) ($p < 0.001$).

Antibiotic prescription was not associated with the complication rate in any aetiological group except for patients with a negative culture, where antibiotics were associated with a higher incidence of complications (data not shown). The choice of antibiotic was unrelated to the complication rate in this cohort (data not shown).

Tonsillectomy

The prospective cohorts

In the prospective cohort of children 0–14 years old, none of the 77 patients or 34 controls had tonsillar surgery within 3 months from inclusion.

In the prospective cohort of young adults, none of the 178 patients without previous tonsillectomy had tonsillar surgery within 3 months from inclusion. However, after 2 years, 5 (2.8%) had either undergone or been planned for surgery. The microbial test results at inclusion for these patients were "only viruses" (n=1), "only *F. necrophorum"* (n = 1), "only GAS" (n = 1) and "no pathogen" (n = 2). None of the 108 controls had tonsillar surgery within 2 years from inclusion.

Registry data

In the RADT cohort, 0.28% of the patients had a tonsillectomy within 90 days from the index visit. This proportion was almost the same regardless of RADT result and antibiotic treatment (0.26–0.31%).

In the culture cohort, none of the patients with GAS or SDSE had a tonsillectomy within 90 days from the index visit, whereas 5.6% of patients with *F. necrophorum* and 2.5% of the patients with a negative extended culture did ($p = 0.002$). With regard to antibiotics, there were no significant differences between treated and untreated patients within the aetiological groups.

Discussion

Main findings

GAS was the most common pathogen in pharyngotonsillitis in both adults and children, whereas SDSE and *F. necrophorum* were rare in children. There was a high prevalence of bacteria and viruses in throat swabs from healthy children, making it difficult to judge the relevance of an aetiological finding in symptomatic patients.

The clinical presentation of viral and bacterial pharyngotonsillitis overlaps extensively in both children and adults, and no single or combined symptom is sufficient to perfectly predict the presence of any aetiology – however, cough and coryza rule out GAS with a high probability. The Centor score was more predictive of any bacterial finding than for GAS specifically. A positive RADT for GAS greatly increased the positive predictive value of GAS in patients with a Centor score of 3–4.

GAS was associated with more return visits for pharyngotonsillitis within 30 days than other pathogens in the prospective cohort of adults. However, after 2 years, there were no differences between aetiologies. In the registry-based study, patients with *F. necrophorum* or a negative culture had a higher incidence of peritonsillitis within 30 days than patients with GAS or SDSE.

There were no associations between antibiotic prescription and new visits for pharyngotonsillitis or complications within 30 days in the small prospective studies. In the registry-based study, antibiotics were associated with fewer return visits for pharyngotonsillitis within 30 days in patients with a positive RADT for GAS, compared with no antibiotics. However, regardless of aetiology, antibiotics were not associated with fewer purulent complications in any cohort of the registry study.

Meaning of the results

In this section, the main findings of the thesis will be discussed thematically and put into the context of previous research.

Presence of bacteria and viruses

Prospective cohort of children

GAS was by far the most prevalent pathogen in children with pharyngotonsillitis, which was expected from previous research [35], and was detected in half of the patients. Second to GAS were *H. influenzae* and *S. aureus*, which were both part of the standard reporting by the laboratory used in Study III but are not typically considered pathogenic in pharyngotonsillitis and probably reflect a carriage state in most patients [132]. As expected from previous studies using PCR, rhinovirus was the most common virus [27, 73], present in 9% of the patients, whereas the overall prevalence of viruses (36%) was much lower than we had expected [27]. This might be explained by sampling errors or differences in age distribution and epidemiologic situations. Notably, adenovirus, often reported as the most common virus in acute sore throat [26, 28, 133], was uncommon in our population. RSV, metapneumovirus, and parainfluenza virus were only found in patients and not in controls, supporting the idea of these viruses as pathogens [73]. SDSE and *F. necrophorum* were only found in one patient each, supporting previous reports of a low occurrence in children [44, 56]. *M. catarrhalis*, which is often part of the nasopharyngeal microbiome of children [94], was not detected.

In addition to the patients, a clear majority of the controls also had viruses and bacteria in their samples, most often GAS, but also *H. influenzae, S. aureus*, SDSE, and several viruses. The asymptomatic carriage of GAS (32%) was so high [35] that we first suspected a local outbreak, but the pattern repeated itself in all three study centres. The high rate of viruses and bacteria in healthy children makes it hard to judge the importance of a microbial finding in patients, as the patients are thought to be just as likely as healthy children to carry potentially pathogenic microorganisms at the time of infection [108]. The finding might thus represent an "innocent bystander" rather than the causative pathogen (see below, under Clinical implications) [44, 45, 54].

The high prevalence of GAS in both patients and controls in children contrasts the prospective study of adults by Hedin et al. [29], where GAS was found in 30% of the patients but only 2.3% of controls, using similar methods of sampling and analysis. The prevalence of viruses in patients was comparable between children and adults, whereas the overall carriage rate of viruses and bacteria was much higher in healthy children than in healthy adults.

Registry data

The registry-based study was not well suited to estimate the prevalence of different pathogens due to the highly selected population, but one can still attempt a cautious interpretation of the results.

The RADTs were positive for GAS in 67% of adults and 80% of children, which was much higher than we had expected from previous studies [5, 29, 34]. These results are not straight-forward to interpret: according to the national guideline in

Sweden, only patients with a Centor score of 3–4 should have an RADT [91], i.e., if the physicians in Region Kronoberg adhere to this guideline, the RADT cohort would be a selection of the more severe cases of pharyngotonsillitis. Though we lack the clinical data to verify this hypothesis, it is directly challenged by the results of Study II, where 94% of the adult patients had an RADT – regardless of Centor score. Moreover, even if the adherence to the guideline would be high, a Centor score of 3–4 predicts that only about every second patient will have a GAS growth in culture, so the proportion of 67% still seems high. Therefore, a more reasonable hypothesis is that there exists a classification bias among physicians, where patients with a positive RADT are more likely to receive a diagnosis of pharyngotonsillitis, but patients with a negative RADT are more likely to receive a diagnosis of unspecific upper respiratory infection (an ICD code that we did not include in our study). This hypothesised interplay between test result and ICD coding is in line with previous research, which has shown that the choice of diagnosis code or disease label is associated with the clinical management; for example, patients labelled with tonsillitis were more likely to receive antibiotics than patients labelled with pharyngitis, upper respiratory tract infection, or sore throat [134, 135].

The throat culture results are even more difficult to interpret, partly because the Swedish guideline does not recommend cultures for primary diagnosis, but instead for patients with worsening symptoms, persistent infection, therapy failure, or recurrent infections [91], and partly because the reasons for the physicians to order these cultures were unknown to us. Some cultures might have been ordered in line with the guidelines, whereas others were probably due to individual differences among physicians or to patients' expectations, as illustrated by the fact that most cultures were ordered on the same day as the first visit and not at a follow-up visit. Regardless of the cause, the throat cultures in the registry-based study were indeed not ordered randomly, which is also evident from the results, where GAS was no more common than the other bacteria despite 67% of the RADTs in the study being positive for GAS. *F. necrophorum* was detected in 15% of prolonged anaerobic cultures, and unless something in the clinical presentation led the physician to suspect this bacteria, this suggests that samples for regular cultures might also harbour *F. necrophorum*. In support of this, two recent meta-analyses of patients diagnosed with acute sore throat in PHC reported an 18–19% prevalence of *F. necrophorum* [45, 136]. In our material, *F. necrophorum* and SDSE were most common in patients 15–29 years old and least common in children, which is in line with previous reports [29, 42, 44, 45, 137].

Clinical findings

Prospective cohort of children

In the prospective study of children, the clinical presentation was very similar across aetiologies. Except for coryza, which was more common in patients with viruses

than in patients with GAS and in line with previous reports [4, 74, 75], no sign or symptom differed with statistical significance between single viruses or bacteria or between viruses and bacteria as groups nor did the combination of symptoms in the Centor score. The reason for this might have been the small size of the study, but the findings are in line with previous reports [26, 27, 96, 138]. Fever at examination was surprisingly uncommon, possibly due to antipyretics taken before the visit. This affected the Centor score of the patients and not a single child reached 4 points.

The symptom diaries revealed that 95% of the respondents were symptom-free at day 10, albeit with a few experiencing early relapses. Patients with GAS treated with antibiotics had the fastest resolution of symptoms, which is in line with trials with adult patients [6]; however, this result was not statistically significant.

Prospective cohort of young adults

Among young adults, the "viral features" cough and coryza [4, 74, 75] and a history of frequent sore throats were more common in patients with only viruses than in patients with only bacteria. On the other hand, tonsillar coating was more common in patients with only bacteria than in patients with only viruses. Fever, lymphadenitis and the duration of symptoms before consultation were similar between the two groups.

Diagnosis and prediction

The prospective studies of children and young adults estimated the prevalence of different bacteria and viruses in patients with pharyngotonsillitis in PHC and registered the frequency of different signs and symptoms coupled to these microorganisms. With this information combined, it is possible to make aetiological predictions – i.e., how probable it is to detect a certain microorganism given a clinical sign or set of signs. The prevalence is important because almost no clinical sign is unique to a certain microorganism, not even the scarlatine rash (which is often thought of as a sign of GAS). Moreover, signs that are very typical of a pathogen become less predictive if that pathogen is rare. For example, a cough is typical of viruses but can also be elicited by bacteria; therefore, if there was a low prevalence of viruses in the studied population and most infections were caused by bacteria, a cough might be just as predictive for bacteria as for viruses.

Prospective cohort of children

Due to the similar presentation of most viral and bacterial microorganisms in this study, the predictive values of clinical signs and symptoms were overall low, far from the 85% certainty that would approach the performance of an RADT [138]. The typical viral feature coryza was significantly more common in patients with viruses than in patients with bacteria, but the lower-than-expected prevalence of viruses resulted in a low PPV for viruses for this symptom. Only 24% of the patients

with GAS had a cough, so this symptom was more helpful in ruling out GAS than for ruling in viruses.

Prospective cohort of young adults

Cough and coryza were more common in patients with viruses than in patients with bacteria. However, as bacteria were more prevalent than viruses in this population and more than a quarter of the patients had no detected aetiology, the PPVs to detect viruses remained low. In contrast, as relatively few patients with GAS had a cough or coryza, these symptoms proved helpful in ruling out GAS. Tonsillar coating and absence of cough were significantly associated with GAS and any bacteria, in line with a previous meta-analysis [76]. However, no sign or symptom could rule in GAS or any bacteria with 85% certainty.

Combining symptoms into a Centor score increased the PPVs for GAS and "any bacteria", raising the probability with each point. However, only patients with the highest score of 4 had a probability of GAS exceeding that of not having GAS. As previously reported [42, 43], the Centor score was useful also for bacteria other than GAS, resulting in an overall prediction that was better for "any bacteria" than for GAS alone. In contrast, low Centor scores were not very predictive of viruses.

Rapid antigen detection tests for group A streptococci

Due to the low precision of clinical signs and symptoms, aetiological tests such as the RADT for GAS are often used in PHC. This was also evident in the prospective cohorts, where RADTs were used in 69% of the children and 94% of the young adults without being part of the study protocol. The sensitivity was excellent in both cohorts, which is in line with previous reports [99], whereas the specificity was lower than expected in children due to many false positives. The PPV for GAS was overall fair but increased with each Centor score. Interestingly, we observed that the test's sensitivity in young adults was lower in patients with low Centor scores, a phenomenon called spectrum bias, which might be explained by a lower presence of bacteria in these patients [139–141]. However, the higher risk of missing a true GAS infection in patients with a score of 0–2 is countered by the fact that these patients should not be tested in the first place, according to many guidelines [5, 74, 91]. The NPV was high in both children and adults, so a negative RADT is useful for ruling out GAS infection, theoretically lowering antibiotic prescription by half [74, 142, 143]. At the same time, too liberal use of RADTs in patients with low scores could lead to the treatment of patients with no proven benefit [9] and contribute to medicalisation [78]. The widespread routine use of RADTs in both studies is a major deviation from Swedish national guidelines [91].

A focus on RADTs for GAS will inevitably disregard other bacteria such as SDSE and *F. necrophorum* that would otherwise be picked up by clinical scoring systems like the Centor score and the FeverPAIN score [42, 43, 53, 78]. In the end, it becomes a question of which bacteria are important to treat and which are not. Until

that question is settled, overly relying on clinical scoring systems alone will lead to antibiotic overuse [99].

Presence of viruses and bacteria in healthy people

So far, we have discussed how different bacteria and viruses can be predicted using clinical findings and RADTs. However, this discussion assumed that the detected microorganism is responsible for the infection and ignored the fact that there is a widespread presence of potentially pathogenic bacteria and viruses not only in healthy children but also in symptomatic patients [35, 108]. Somehow, we need to deal with this carriage and try to estimate how likely the detected pathogen caused the infection. Therefore, it is essential to know the background prevalence in healthy controls.

In children, GAS was detected in 49% of the patients and 32% of the controls, resulting in a positive EPV of only 54%. This EPV implies that GAS was probably only the true causative pathogen in half of the patients where it was detected. To test this assumption, one could use the reference standard for detecting an actual GAS infection, namely a series of serological markers for GAS that would indicate that the patient's immune system had elicited a response to the pathogen. Such studies exist, and a recent meta-analysis established that only 56% of children with GAS-positive pharyngotonsillitis had a serologically confirmed infection [34], a fraction that is very close to our EPV.

In young adults, the low prevalence of viruses and bacteria in healthy controls implies that most cases of microbial detection in symptomatic patients represent a true infection, not carriage. The calculated positive EPVs for GAS and "any bacteria" in symptomatic patients confirm this as they were 95% and 84%, respectively. Therefore, aetiological diagnosis is meaningful in young adults.

Follow-up

New visits for pharyngotonsillitis

In the prospective cohort of children, 16% of the patients made a new visit for a sore throat within 3 months, and 33% of these were early re-consultations due to worsening or non-resolving symptoms. Most of the children with new episodes had GAS at inclusion, and some had antibiotic treatment. Unfortunately, this study was not dimensioned for evaluating differences among the few patients who re-consulted.

In the prospective cohort of young adults, 10% of the patients made a new visit for a sore throat within 1 month, of which only a minority were due to worsening or non-resolving symptoms. As with the children, patients with GAS re-consulted more frequently than others. Despite being the second most common bacteria, none of the patients with *F. necrophorum* as the sole finding re-consulted within the first month. In line with previous findings [144], neither individual nor combined clinical

signs were very helpful in predicting which patients would re-consult. After 2 years, 43% of the patients had made a new visit for a sore throat. At this point, there were no differences as to initial microbial findings, implying that aetiology might be important in the short term but not in the long term, where instead other determinants for consultation such as personality traits and patients' beliefs and behaviours could be more significant [19]. Antibiotics were prescribed to 44% of the patients, most often to patients with GAS and more commonly to patients with high Centor scores than those with low scores. There was no association between prescription and the rate of re-consultation, neither within 1 month nor within 2 years. However, despite adjusting for confounders with logistic regression, the results are hard to interpret due to the observational nature of this study, its small size, and the complex interplay between aetiology, clinical presentation, and antibiotic prescription.

In the large registry-based study, patients with a positive RADT and antibiotic prescription had a lower re-consultation rate for pharyngotonsillitis than patients with a positive RADT and no prescription. Although observational data, this suggests a protective role for antibiotics in patients with GAS, which challenges previous reports of increased re-attendance after immediate prescriptio as an effect of changed expectations and behaviour (i.e., medicalisation) [145–147]. In contrast, antibiotic prescription was associated with a higher re-consultation rate in patients with a negative RADT, pointing to either a medicalising effect, ineffective treatment, or a confounding by indication, where the more severely ill patients were more likely to be offered treatment [148]. Unfortunately, in the absence of medical file reviews and interviews with physicians, these are mere hypotheses.

Apart from RADTs, the registry data also covered a smaller cohort of patients with throat cultures. Overall, the re-consultation rate for pharyngotonsillitis was similar across the aetiological groups. However, we found an association between antibiotic prescription and a lower re-consultation rate in patients with SDSE, suggesting that a subset of patients with a negative RADT could benefit from antibiotics. As with the patients with a negative RADT, we found that antibiotic prescription was associated with a higher re-consultation rate in patients with a negative culture, possibly explained by the factors mentioned above.

Complications

The complication rate was very low in the prospective cohorts of children and young adults: no child was diagnosed with a complication within 3 months, and only 1 (0.49%) young adult was diagnosed with a complication during the first month. This rate was expected from previous studies [6], but the small sizes of these studies make it hard to evaluate factors that could influence the incidence of complications.

In the registry-based study, the complication rate was highest among patients with *F. necrophorum*, followed by patients with a negative extended culture. Although the study was primarily designed to investigate the effect of antibiotic treatment

within each aetiological group rather than to compare aetiologies, these results support the view of an association between *F. necrophorum* and peritonsillitis.

Antibiotic prescription was not associated with complication rates, regardless of the RADT result, which contrasts with a meta-analysis showing a protective role of antibiotics [6]. This raises the question once again as to whether patients who received a prescription were more ill to start with, which might explain why they were more likely to develop a complication. Despite the large number of participants, the study might still have been undersized to detect such differences, as most patients with a positive RADT received a prescription, and complications are rare outcomes.

The complication rate was higher in the throat culture cohort than in the RADT cohort, pointing to a selection bias of this population, but antibiotic prescription was not associated with the complication rate in this group either, except for a higher rate in patients with a negative culture. This was a somewhat surprising finding with regard to *F. necrophorum*, which is thought to be involved in the pathogenesis of peritonsillitis, possibly through the advancement of a tonsillar infection to the peritonsillar tissue [149].

Tonsillectomy

None of the children or young adults in the prospective cohorts had a tonsillectomy during the follow-up period of 3 months. Among young adults, however, 5 (2.8%) had tonsillar surgery within 2 years. These patients had different aetiologies at inclusion, but only *F. necrophorum* in one case. Due to the long follow-up period, it is hard to determine the relevance of these microorganisms as to the subsequent removal of the tonsils.

In the registry-based study, 0.28% of the patients with an RADT had their tonsils removed within 3 months, but there was no association between antibiotic prescription and tonsillectomy rate. In the culture cohort, none of the patients with findings of GAS or SDSE had a tonsillectomy within 3 months, whereas 5.6% of the patients with *F. necrophorum* and 2.5% of the patients with a negative extended culture did, suggesting an association with peritonsillitis. However, there was no association between antibiotic prescription and tonsillectomy rate in this cohort either.

Methodological considerations

Several possible methods come to mind to address the aims of this thesis. These methods will be discussed together with the strengths and limitations of each method.

Observational cohorts

To study the prevalence of viruses and bacteria in PHC as well as the related clinical presentation, patients with a sore throat could either be actively recruited through advertising, which would include individuals who would otherwise manage their infection at home with self-care or passively recruited as they contacted a PHCC on their own accord. The latter would mirror everyday clinical practice, and ideally, all eligible patients at the clinic would be included in a consecutive or randomised manner to minimise selection bias. Also, the PHCCs would ideally be selected in a randomised rather than strategic way to avoid bias. Although some prevalence studies are carried out with a cross-sectional design, the study of pharyngotonsillitis requires time, as patients become ill on different occasions. The inclusion protocol would be similar for all patients, but they would be included at different times, creating a so-called inception cohort. As both the aetiologies and the incidence of pharyngotonsillitis vary over the seasons, a study period should cover at least one year. Moreover, as many microorganisms can be found in asymptomatic individuals, including a matched control group of healthy people would be advised to adjust for background prevalence and calculate EPVs [108]. The disadvantages of these studies are that they might interfere with the clinical work and are time-consuming, and that it is difficult to involve all relevant staff. Therefore, many patients may not be asked to participate, which introduces bias. As with all observational studies that are not randomised, there will always be some degree of confounding that alters the result despite the best efforts to adjust for possible differences between study groups.

A strength of this thesis is that three of four studies were based on such controlled inception cohorts, with patients passively recruited as they contacted their PHCC. Therefore, these studies were performed with the very patients that we see in our day-to-day work as PHC physicians in contrast to the abundance of studies of pharyngotonsillitis that were set in hospital clinics or secondary ambulatory care. A limitation, however, is that it was hard to engage all doctors and nurses at the PHCCs, resulting in both a convenience sampling (i.e., patients were only recruited when they had contact with a staff that remembered that there was an ongoing study) and, for the cohort of children, a study population that was much smaller than we aimed for despite running the study for many years. In addition, the controls were not individually matched in age and gender in the prospective studies but rather on a group level.

Aetiological testing

Aetiological testing for viruses and bacteria can be performed in different ways. Many bacteria grow well in culture, albeit under specific conditions, so such a method may suffice for most species – e.g., for the detection of GAS, throat culture has long been considered the reference standard [108]. The identification of bacteria

can be greatly enhanced with advanced technology such as MALDI-TOF, where the combination of an organic solvent (matrix) is excited by a laser resulting in bacterial peptides being ionised and identified by mass spectrometry [124]. Each bacterium has its unique pattern and can therefore be identified to the species level. Sources of error in culture include faults associated with sampling technique, specimen transport conditions, bacterial isolation and processing techniques [71]. Bacteria can also be detected with PCR as long as there are proper primers and probes, and in recent years, many multiplexed assays have been developed that enable fast analysis of a wide range of pathogens. As with culture, there are sources of error associated with sampling, transportation and analysis. In most PHCCs, antigens of GAS can be detected within minutes using immunoassays, and although not perfect, the sensitivity and specificity are acceptable.

It has almost become a truth that viral infection is the most common aetiology in pharyngotonsillitis. The studies that support this have typically relied on serum antibodies to viruses [26, 133], viral antigen detection [133] or viral culture [28]. However, with the emerging PCR technique, diagnosing viruses has become easier and more precise.

To ensure the most representative sample, the sampling location in studies of pharyngotonsillitis is usually the throat; however, the nasopharynx might be a better alternative for virus detection [150], although sampling from this location is less pleasant for the patient.

Regardless of the test, an aetiological study can only find the pathogens that it was designed to detect, and a negative finding could therefore hypothetically be explained by the presence of a causative pathogen not in the scope of the test.

A strength of the prospective cohorts is that we used PCR to detect viruses and some bacteria. In many earlier studies of pharyngotonsillitis, aetiological detection has been limited to GAS, non-GAS streptococci, non-streptococci or no pathogen, of which the last group is very unspecific but often thought to imply viruses. Moreover, only a single swab was needed in the study of children, minimising both the risk of sampling errors and the potential discomfort, which was a strength also from an ethical perspective. A limitation in the analyses of pathogens was the small sizes of the cohorts, which made it impossible to draw any strong conclusions regarding single pathogens and probably introduced type II errors. Another limitation was the lack of serological markers or other host responses, so we had no reference standard for confirming a true infection. This would have been valuable when interpreting the clinical findings associated with the different viruses and bacteria, especially in light of the high carriage rate.

Clinical signs and symptoms

A structured protocol is needed to study clinical symptoms of patients with pharyngotonsillitis to ensure that all patients are evaluated in the same way regardless of the physician they meet. Nonetheless, physicians might differ in their

perception of so-called objective signs (e.g., temperature, swollen glands, and tonsillar coating), so ideally, the inter-rater reliability should be quantified. Moreover, because thermometers might differ, all patients should be measured with the same type of thermometer.

To study the clinical course, patients can use symptom diaries to register symptoms every day as long as they experience illness. Diaries ensure maximum reliability as the risk of recall bias is eliminated. On the other hand, the patient must remember to fill in the diary and send it back to the researcher. Online diaries may be an alternative, alerting the researcher if a patient forgets to register.

In the prospective cohorts, all patients were evaluated with regard to medical history, signs, and symptoms in a structured way. However, the inter-rater reliability was never tested, which might have introduced bias. In the cohort of children, printed symptom diaries were used, but unfortunately, a large proportion of the diaries were never returned, despite reminders and pre-paid stamped envelopes.

Prospective studies

In a longitudinal, prospective study, patients are followed over time to measure some predefined outcomes. The follow-up may be active and include diaries, surveys, phone calls, e-mails, and return visits, or be performed with a review of medical records from routine care. As the interpretation of medical records might differ between researchers, the inter-rater reliability should be quantified. Other obstacles to follow-up include patients moving away from the region or medical records being unavailable to the researcher.

In the prospective cohorts, both active and passive methods were used. A symptom diary was used in the cohort of children, and in both cohorts, EMR reviews were performed. Inter-rater reliability of file review was not tested for, but in the adult cohort, one researcher did all the review work, which was both a strength and a weakness. The EMR system in Region Kronoberg is comprehensive and covers both PHC and hospitals, which was a huge advantage in the review work.

Registry-based studies

Registry-based studies offer a huge amount of data and therefore are suitable for studying rare outcomes that would be impossible to measure in prospective cohorts. Apart from the ethical question about personal integrity, from a researcher's perspective, some of the disadvantages are the lack of clinical data, the lack of information about the clinical circumstances, the lack of reasons for ordered tests and prescribed medications, and the resulting uncertainty about the plausibility of the diagnoses.

A strength of our registry study is that it was based on complete data from our region and that almost 15 000 patients could be included, compared with 220

patients in the prospective cohort of adults and 77 patients in the cohort of children. This opened up for a much better understanding of the incidence of complications. However, the rate of ordered throat cultures was low, about 6%, leading to a probable undersize of this subset of patients, and the reason for performing each throat culture was unknown to us – although most likely, it was not random.

Randomised controlled trials

Perhaps the most significant concern of observational studies is that one can never truly isolate the trait or property of interest in the participants, keeping everything else similar between groups – there will always be some bias or confounding left that the researcher did not think of or could not adjust for. This residual confounding affects the result but to an unknown degree. The simple solution is to allocate the participants randomly, let chance even out dissimilarities, and blind both the patient and the researcher to the treatment. The downside, however, is the enormous amount of time and money required, which also limits the number of possible participants. Therefore, rare outcomes are not suited for this design. Moreover, to compare the property of interest, most other variables need to be kept to a minimum, so some patients will be excluded because they are, for example, too ill, too old, or have too many other diseases or medications, creating a somewhat artificial population that will diminish the generalisability of the results.

With this said, a controlled trial would still be the best alternative to investigate the effect of antibiotic treatment on the clinical course of pharyngotonsillitis, including longitudinal follow-up for complications and new episodes of pharyngotonsillitis.

A method related to the explanatory randomised controlled trial is the pragmatic trial, which can, for example, compare a new treatment with the currently best alternative (i.e., usual care) [151]. Such "real world" trials generally allow most patients with the sought-after condition to be included, better mirroring everyday practice. The downside of this approach is that it is harder to interpret the results.

The effect of antibiotic treatment

The effect of antibiotics is hard to estimate without conducting an experiment (i.e., a randomised controlled trial) as the degree of confounding in observational studies is high.

In the prospective cohorts, the participants were too few to draw any conclusions. In the registry-based study, almost all patients with GAS were prescribed antibiotics, leaving few patients for comparison. Moreover, it is unclear if those not prescribed antibiotics had something in common (i.e., milder symptoms) or if prescription occurred randomly, but the risk of confounding by indication is high. In the subset of patients with a throat culture, the low number of participants

probably introduced type II errors. Here too, it was impossible to infer why patients were or were not prescribed antibiotics.

Case-control studies

Instead of selecting a group of healthy people hoping that some of them will develop the outcome of interest over time, a retrospective case-control study starts with people who have already contracted the disease. It then compares these people with those who stayed healthy in the hope of finding some trait or exposure that predisposed to the outcome. Therefore, a case-control study could be suitable for studying rare complications, such as peritonsillitis or Lemierre's syndrome. The downside is that it can never answer how many people were at risk and therefore cannot calculate the relative risk of the outcome given the exposure.

This thesis does not include a case-control study, although complications to pharyngotonsillitis have been an area of interest. The registry-based study could give some answers about complications, but the entry to this study was an aetiological test, which excluded all patients who were not tested but subsequently developed a complication.

The primary health care context

As is evident from the introduction, most cases of acute sore throat occur and resolve spontaneously far from the health care clinics, and people generally do fine. Should they still want to see a doctor, the absolute majority visit their general practitioner, while only a fraction of the patients are referred to secondary care. This fraction, of course, typically represents the most severe or unusual presentations of acute sore throat and its complications, and it would therefore be both unfair and meaningless to extrapolate findings from secondary care research into PHC. If we want to learn how dangerous or harmless the average episode of pharyngotonsillitis is in a patient who attends for his or her symptoms, we need to perform that study in PHC. This is, of course, not unique for sore throat but goes for most other infections and diseases.

As noted in the introduction, the bulk of antibiotic prescriptions occur in PHC, and the fight against resistance must start there. This is not to say that prescription patterns in secondary and tertiary care are unimportant, but they cannot be our priority. The antibiotic prescription in PHC is excessive, and we need to take measures to lower the rates. Information and communication are central to this work, but the facts must be put there by researchers performing studies in PHC.

As for methodological considerations, only imagination limits what we can do. The tools are there to use – both quantitatively and qualitatively – from

observational studies, registry-based studies, and controlled trials to questionnaires and interview studies. Research in PHC is both feasible and necessary.

Clinical implications

GAS was the most common pathogen in the prospective cohort studies, where it was also associated with the highest re-consultation rate for a sore throat; therefore, it should still be considered the most important pathogen in pharyngotonsillitis, both in children and adults. SDSE and *F. necrophorum* were rare in children and do not merit any special attention in this age group. In young adults, SDSE and *F. necrophorum* might cause complications and recurrent disease; however, the findings are not strong enough to suggest physicians to use a throat culture at first visit. Instead, it might be feasible to search for these bacteria in patients with worsening symptoms.

The high carriage rate of GAS in children poses a diagnostic problem, and the clinician needs to keep in mind that perhaps only every second detection of GAS represents a true infection. Viral features such as a cough and coryza are rare in patients with GAS infection and should raise the suspicion of another aetiology. In young adults, on the other hand, most findings of GAS truly represent an infection.

With the limitation that these studies did not measure any host response to confirm a true infection with the detected pathogen, the clinical symptoms were insufficient to predict any aetiology, alone and in combination, both in children and young adults and points to the need for aetiological tests for confirmation. The RADT for GAS has high sensitivity and specificity when used on a selected group of patients with a higher likelihood of GAS infection. Most patients with a Centor score of 3–4 and a negative RADT had a bacterial finding, showing that the Centor score also predicts other bacteria such as SDSE and *F. necrophorum*. This could be helpful in patients with worsening symptoms, where high Centor scores should raise the suspicion of a bacterial infection.

In the registry-based study, antibiotics seemed to protect against early re-consultation for pharyngotonsillitis in patients with a positive RADT for GAS. This suggests that the concern of a medicalising effect of treatment might be exaggerated. Although with a cautious interpretation, antibiotics also seemed to have a similar protective effect in patients with SDSE but not in patients with *F. necrophorum*. The overall complication rate was low but markedly higher in patients with *F. necrophorum* than in patients with GAS or SDSE. Antibiotics, however, did not seem to protect against complications within any of the aetiological groups. This suggests that clinicians should not prescribe antibiotics out of fear of complications but instead adopt strategies such as safety-netting or free re-consultation for patients who experience worsening symptoms.

Future perspectives

As discussed frequently in this thesis, there is a continuing need for controlled randomised trials that evaluate the effect of antibiotic treatment of patients with SDSE or *F. necrophorum*. Nonetheless, because a trial sized for evaluating the short-term clinical course of these patients would be too small to measure rare outcomes such as peritonsillitis and tonsillectomy, the sizing and power calculation would need close attention to study these outcomes as well.

The widespread carriage of microorganisms in healthy children and, to some extent, in adults identifies a vulnerability in the routine detection of bacteria and viruses. There is an obvious need for biomarkers of infection to separate infected patients from patients with a mere carriage of the detected pathogen. Serological markers develop too slowly to be clinically helpful in acute disease, and there is an apparent lack of alternatives. CRP has not proven useful in this matter, but other markers, such as myxovirus resistance protein A (MxA) that has increased activity in patients with viral infections, might be used for rapid diagnosis. As most patients with pharyngotonsillitis seek medical care in PHC, the tests would need to be point-of-care tests and provide a result within minutes.

Although already explored, more research seems to be needed to explain why physicians keep overprescribing antibiotics or what measures need to be taken to change this behaviour. There is also a need for alternative products or medications for symptom relief, as today's antibiotic prescriptions function in large as advanced painkillers.

Rather than treating a disease that has already occurred, new vaccines might prevent the disease altogether. Although not the typical area of research in PHC, such studies might be performed in collaboration with vaccine researchers.

Lastly, we need to continue to monitor the incidence of severe complications over time due to both potential shifts in virulence factors of the pathogens and a decreased use of antibiotics. Just as rheumatic fever disappeared half a century ago, the disease panorama and complications might alter in the future.

Conclusions

The findings in this thesis suggest that GAS remains the most important pathogen in pharyngotonsillitis, both in children and adults. SDSE was rare in children and uncommon in young adults and did not distinguish itself as a major cause of acute pharyngotonsillitis, recurrent infections, or complications. *F. necrophorum* was rare in children but commonly detected in young adults. In the registry-based study, *F. necrophorum* was associated with a higher incidence of peritonsillitis than GAS and SDSE. In children, there was a high prevalence of respiratory viruses and bacteria in both patients and healthy controls, making it challenging to judge the diagnostic relevance of an aetiological finding. In adults, because the carriage rate in healthy controls was lower, an aetiological finding represented a true infection in most cases.

Because clinical signs and symptoms of viruses and bacteria overlapped extensively in both children and adults, neither single nor combined symptoms could predict GAS or other aetiologies with a high probability. The Centor score, designed to predict GAS in culture, was even more predictive of bacterial infection in general. Patients with a Centor score of 3–4 and a positive RADT had the highest probability of GAS.

GAS was associated with a higher re-consultation rate for pharyngotonsillitis within 30 days compared with other aetiologies in the prospective cohort of adults, but after 2 years, there were no differences between different aetiologies.

In the registry-based study, an antibiotic prescription was associated with fewer return visits for pharyngotonsillitis in patients with a positive RADT for GAS, implying a protective role of antibiotics, but with more visits in patients with a negative RADT for GAS or a negative throat culture. Moreover, antibiotics seemed to protect against return visits for pharyngotonsillitis in patients with SDSE. Antibiotics were not associated with a lower incidence of purulent complications regardless of aetiological finding.

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Study I

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RESEARCH ARTICLE Open Access

A 2-year follow-up study of patients with pharyngotonsillitis

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Abstract

Background: Longtime follow-up studies on patients with pharyngotonsillitis are rare. We aimed to describe the patterns of new visits for a sore throat, complications and tonsillectomy during 2 years in a cohort of patients with pharyngotonsillitis and non-infected controls.

Methods: A retrospective chart review was performed on a cohort of patients with acute sore throat ($n = 207$), and non-infected controls ($n = 108$). New visits, complications and tonsillectomy within 2 years was recorded and analyzed in relation to microbiological findings at inclusion.

Results: Patients with Group A streptococci (GAS) (12/66) reconsulted more often within 30 days than patients with no GAS (9/141) ($p = 0.009$) and patients with *F. necrophorum* (2/29). After 2 years, we observed no significant differences in reconsultations with regard to aetiology at inclusion. A single complication was recorded and 5 patients were planned for tonsillectomy.

Conclusions: Group A streptococci were the sole aetiological agent associated with recurrent sore throat while *F. necrophorum* did not distinguish itself as a major cause of either recurrent infection or complications in this cohort. More studies, preferably with the focus on adolescents, are needed before *F. necrophorum* can be considered an important cause of pharyngotonsillitis.

Keywords: Pharyngitis, Etiology, Primary healthcare, Fusobacterium necrophorum

Background

Acute pharyngotonsillitis constitutes one fifth of all visits for respiratory tract infections in Swedish primary healthcare [1]. The most common causative agent is *Streptococcus pyogenes* (Group A streptococcus, GAS) [2] but several other bacteria and viruses have also been associated with the condition [2, 3], among these Streptococcus group C and G, *Mycoplasma pneumoniae* and *Arcanobacterium haemolyticum*. Furthermore, *Fusobacterium necrophorum* has been suggested as a possible pathogen in tonsillitis [4–8] and reported to be the second most common bacterial finding [6]. However, no one has so far studied the course of these patients, and studies on the course of patients with pharyngotonsillitis where modern diagnostic approaches and treatment recommendations have been used are also lacking.

Pharyngotonsillitis is associated with short-term complications such as sinusitis, otitis and peritonsillar abscess in a small percentage of patients [9]. Historically, post-streptococcal acute rheumatic fever and glomerulonephritis were dreaded conditions, but these are now uncommon in industrialized countries [2]. In some cases, recurrent infections lead to tonsillectomy [10, 11], but the long-term complications of an episode of pharyngotonsillitis have very rarely been studied, especially in relation to the aetiology of the condition.

Recently, we performed a case-control study on the aetiology of pharyngotonsillitis in young Swedish adults with a special focus on the importance of *F. necrophorum* as a possible pathogen [6]. The present study is a follow-up on that study with the purpose of observing patients over a 2-year period after a pharyngotonsillitis episode together with a cohort of non-infected patients. Specifically, our objective was to quantify the proportion of patients who would have a new doctor's appointment for a sore throat within 2 years; have a complication of pharyngotonsillitis

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within 30 days; undergo or be planned for tonsillectomy within 2 years. These outcomes were studied in relation to the identified microorganism at inclusion.

Methods

As previously described [6], a prospective case-control study was performed in 5 primary healthcare centres in southern Sweden during 2 subsequent winter periods (October–March, 2010–12). Patients aged 15–45 years presenting with acute sore throat and assessed to be in need of seeing a physician according to Swedish guidelines [12], were asked to participate [6]. Samples were collected from throat, nasopharynx and blood and screened for 20 different viruses and bacteria, using either culture, PCR or serology [6]. The following microorganisms were analyzed: β-hemolytic streptococci (Lancefield group A, C, and G), *Fusobacterium necrophorum, Mycoplasma pneumoniae, Chlamydophila pneumoniae*, Epstein-Barr virus, Adenovirus, Bocavirus, Coronavirus NL63, Coronavirus OC43, Coronavirus HKU1, Coronavirus 229E, Enterovirus, Influenza A virus, Influenza B virus, Metapneumovirus, Parainfluenzavirus, Rhinovirus and Respiratory syncytial virus. Controls were recruited among patients aged 15–45 years who presented at the healthcare centre for any other reason than respiratory tract infections.

Follow-up

For both the patient and the control cohort medical files were reviewed retrospectively for the 2 subsequent years following inclusion. Data was retrieved from the comprehensive countywide electronic medical record system that covers both general practice and hospital care (Cambio Cosmic, Cambio Healthcare Systems, Linköping, Sweden) in Kronoberg county, Sweden. A standardized protocol was constructed to facilitate the review. Information about new visits for a sore throat, complications within 30 days after inclusion and tonsillectomy was retrieved from routine records. Based on previous studies [9, 13], we defined a complication as one of the following conditions occurring within 30 days after inclusion: sinusitis, peritonsillitis, media otitis, cellulitis, meningitis, sepsis, glomerulonephritis or rheumatic fever. A new visit was defined as a new doctor's visit in either primary or secondary healthcare with an acute sore throat as the main symptom, including both non-resolving cases and recurrence with a symptom-free interval. Surgery was defined as either tonsillectomy or tonsillotomy, or being planned for this after consultation with an otorhinolaryngologist.

To minimize documentation bias, entries were read through in full and assessed for possible re-labelling of ICD-10 codes for outcomes. The review was performed by the principle author (JP), who was blinded to study data from the inclusion. Ambiguous medical entries were discussed with KH. Both researchers are general practitioners. Study subjects leaving the county during the follow-up period were not reviewed but confirmed alive through the Swedish population register.

Statistical analysis

Protocol data was merged with inclusion data and transferred to SPSS 23.0 software (IBM, Armonk, NY, USA) for descriptive statistics and for two-sided χ^2-testing of proportions of categorical variables. Where expected numbers were low, a two-sided Fisher's exact test was used.

In accordance with Hedin et al. [6], the microorganisms were grouped as: no pathogen",," only viruses",," only bacteria",," only GAS (*Streptococcus pyogenes*)",," only *F. necrophorum*" and" only Influenza B". Consequently, all groups were not mutually exclusive.

For calculations of new visits, we used "30 days" and "2 years" after inclusion as points in time.

Power estimation and sample sizing was primarily calculated by Hedin et al. [6] for the aetiological study, and not for the follow-up.

Results

Characteristics of patients and controls

All 220 patients and 128 controls originally included were confirmed alive at follow-up after 2 years. Thirteen patients and 18 controls had moved away from the county, one control was already included as a patient and yet another control was mistakenly registered twice, leaving 207 patients (94%) and 108 controls (86%) eligible for follow-up (Fig. 1). Median age was 34 among patients (range 15–48) and 33 among controls (range 16–46). Other characteristics of the 2 groups are presented in Table 1. Among the patients and controls lost to follow-up, the median age was 22 and 24, respectively (Table 2).

New visits for a sore throat

Of all patients 90/207 (43%) visited a doctor at least once for a sore throat during the 2-year follow-up period, compared to 19/108 (18%) in the control group ($p < 0.001$). At 30 days after inclusion, the corresponding proportions were 21/207 (10%) among patients and 4/108 (4%) among controls ($p = 0.045$). Of the 21 patients 4 had non-resolving symptoms and 17 presented with a new episode.

In the group with GAS as the sole microbiological finding at inclusion, 9/46 (20%) patients made a new visit within 30 days, which was significantly higher than among patients with no GAS (9/141 (6.4%); $p = 0.018$, Fisher's). This difference remained even if the GAS group included the 20 additional patients where GAS was found together with other pathogens (12/66 (18%); $p = 0.009$).

None of the 10 patients with *F. necrophorum* as the only finding reconsulted for a sore throat within 30 days, in contrast to 19/178 (11%) of patients with no *F. necrophorum* ($p = 0.08$, Fisher's). When considering all patients where *F.*

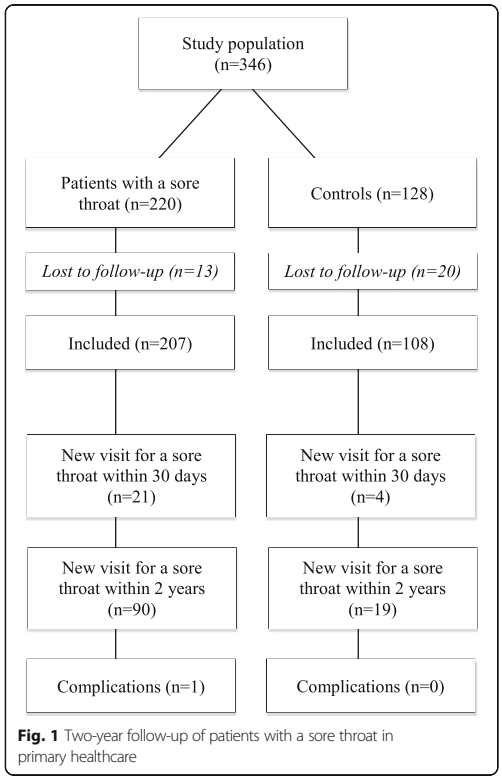

Fig. 1 Two-year follow-up of patients with a sore throat in primary healthcare

necrophorum was found, either alone or together with other pathogens, 2/29 (7%) reconsulted within 30 days ($p = 0.74$, Fisher's, compared to no *F. necrophorum*).

The differences observed at 30 days were less evident after 2 years, although the group with GAS as the only finding still had the highest proportion (52%) of at least one reconsultation. At this point, however, the differences were not statistically significant (Table 3). The temporal distribution of new visits is presented in Fig. 2, with separate cumulative percentages for "all patients", "controls", "all patients with GAS" and "all patients with *F. necrophorum*".

Table 1 Characteristics of the study population

	Percent		
	Patients (n = 207)	Controls (*n = 108*)	χ^2 *p*
Female	65	76	0.051
Smoker	14	8	0.090
History of often having a sore throat	34	6	<0.001
Previous tonsillectomy	14	13	0.70
Antibiotic treatment in the past month	7	3	0.11

Table 2 Characteristics of the missing cases in the follow-up of patients with sore throat in primary healthcare in relation to aetiology at inclusion

	Number		
	Patients	Female	Median age (yy)
All	13	6	22
No pathogen	5	4	21
Only viruses	3	0	29
Only bacteria	5	2	19
GAS	–	–	–
F. necrophorum (only)	4	2	19
Influenza B	–	–	–

Complications and surgery

We excluded 2 patients from follow-up due to an ongoing complication (sinusitis and peritonsillitis) already at inclusion. In these two, no pathogen had been found. Among the remaining patients, 1 of 205 presented with sinusitis within 30 days, as compared to no complications in the control group. This patient had *F. necrophorum* as a single pathogen at inclusion. Among patients without previous tonsillar surgery, 5/178 (2.8%) either underwent or were planned for surgery during the follow-up period, as compared to none in the control group. In these 5 cases, the microorganisms at inclusion were the following: only viruses ($n = 1$), only *F. necrophorum* (n = 1), only GAS (n = 1) and no pathogen found ($n = 2$).

Antibiotics

Of the 207 patients, 91 (44%) received an antibiotic prescription at inclusion. Antibiotics were prescribed more often to patients with a Centor score of 3–4 (56/80 (70%)) than to patients with Centor score 0–2 (35/127 (28%)) ($p < 0.001$). When comparing aetiological groups, patients with only GAS had the highest proportion of prescriptions: 44/46 (96%), followed by the group with

Table 3 Proportion (%) of patients and controls attending for a sore throat within 30 days and 2 years, respectively, in relation to microbiological findings at inclusion

Microbiological finding at inclusion	Percent	
	30 days	2 years
Controls ($n = 108$)	4	18
Patients ($n = 207$)	10	43
No pathogen ($n = 60$)	7	38
Only viruses ($n = 49$)	8	47
Only bacteria ($n = 80$)	14	50
GAS only ($n = 46$)	20	52
F. necrophorum only ($n = 10$)	–	40
Influenza B only ($n = 13$)	–	46

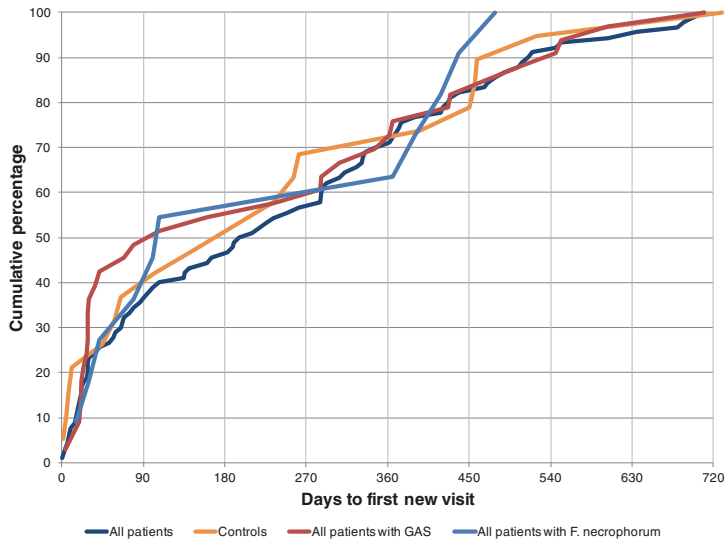

Fig. 2 Time to first new visit for a sore throat. The graph illustrates the pattern of new visits for sore throat over time where the cumulative percentage of 100 corresponds to the total number of new visits in each patient group within 2 years from inclusion. The overall percentage of new visits were: 90/207 (43%) for "all patients", 19/108 (18%) for "controls", 33/66 (50%) for "any GAS" and 11/29 (38%) for "any *F. necrophorum*". A "new visit" was only counted once for each patient

only bacteria: 58/80 (73%), only *F. necrophorum*: 3/10 (30%), no pathogen found: 13/60 (22%) and only viruses: 7/49 (14%). The patient who developed sinusitis as a complication did not receive antibiotic treatment at inclusion.

No significant difference was seen between treated or untreated patients regarding new visits for a sore throat, either within 30 days or after 2 years. This observation held true both within the different aetiological groups and in the patient group as a whole (data not shown).

Further analysis of the group with only GAS based on Centor criteria, revealed that antibiotics were prescribed equally often irrespective of Centor score (Table 4).

Table 4 Proportion of patients with GAS only ($n = 46$) and new visits, in relation to Centor Score and antibiotics prescribed at first visit

	Percent (*n*)	
	30 d	2 years
Centor 0–2 ($n = 15$)	20 (3)	60 (9)
Antibiotics ($n = 13$)	15 (2)*	54 (7)**
Antibiotics ($n = 2$)	50 (1)	100 (2)
Centor 3–4 ($n = 31$)	19 (6)	48 (15)
Antibiotics ($n = 31$)	19 (6)	48 (15)
Antibiotics ($n = 0$)	–	–

*$p = 0.37$ (Fisher's exact test), **$p = 0.49$ (Fisher's exact test), when + "antibiotics" is compared to - "antibiotics"

Discussion

In this study, we followed a well-described cohort of patients with pharyngotonsillitis and non-infected controls in primary healthcare for 2 years after inclusion, with special focus on the aetiology [6]. We observed a high tendency in patients to return with a sore throat within 2 years irrespective of microbiological finding at inclusion, while patients with GAS more often returned within 30 days as compared to patients with other possible aetiology of their disease. Only one complication was recorded (sinusitis) and 2.8% of the patients underwent tonsillectomy within 2 years after inclusion.

The main strength of this study is that it links the aetiological study on pharyngotonsillitis [6], where modern techniques were used, with both short- (30 days) and long-term (2-year) follow-up data.

The medical file review was carried out in a comprehensive electronic medical record system that covered both general practice and hospital care in the county. This increased the possibility to catch all relevant events. Possibly, a few patients may have sought medical advice outside the county.

The main weakness of this study, however, is its small size, being powered rather for the aetiological mapping than for prospective follow-up of uncommon events. This has increased the risk of missing true differences between groups, as well as it prevented from adjusting

for confounders such as smoking, age, socioeconomic status and morbidity. Hedin et al. did, however, only find smoking and tonsillar coating to be associated with *F. necrophorum* at inclusion [6]. The rate of complications and surgery was also in line with previous reports [9].

Research on children has suggested that immediate prescription increases the risk of both relapse and recurrent infections [14], and Little et al. found that prescribing antibiotics lead to medicalisation and increased re-attendance in patients with sore throat [15]. In our study, the group of patients with only GAS had the highest proportion of reconsultations within 30 days. In Swedish primary care, rapid antigen detection tests for GAS are readily available, and one hypothesis could be that the mere identification of GAS changes the way physicians communicate with their patients. This may in turn affect the patients' view on relapsing symptoms and hence lower their threshold for re-attendance. The fact that most patients with GAS were prescribed antibiotics, and equally often regardless of Centor score, could reflect both an excessive use of rapid antigen tests and GP's making treatment decisions based on microbiological findings rather than clinical severity. The high prescription rate among patients with GAS and *F. necrophorum* (despite the physicians being unaware of the latter) may have reduced the number of complications observed in this study. However, a study on respiratory tract infections in general practice found that even a large reduction in antibiotic prescribing was only associated with a small increase in the number of complications [16].

While *F. necrophorum* was the second most prevalent pathogen in the aetiological study [6], it does not seem to compete with GAS aetiology regarding new visits in the short-term perspective. Rather, the patients with *F. necrophorum* were positioned with the groups with "only viruses" and "no pathogen" detected. However, the power of this result was somewhat diminished by 4 young patients with *F. necrophorum* leaving the county before follow-up.

As the proportion of patients with new visits evened out between groups over time, the aetiology did not seem to matter in the long perspective. This finding, together with the finding that the patients had more new visits than the controls, might suggest that a subset of the general population more often than the average experience a sore throat (as subjectively reported in the background characteristics) and/or have a lower threshold for attending medical care. The proportion of controls that re-attended was higher than we had anticipated. It must also be pointed out that a sore throat can have non-infectious causes, and that this study might have miss-classified some of the new visits as infectious.

According to current guidelines, the main reason for treating an acute sore throat with antibiotics is to alleviate symptoms in patients with more severe presentations, rather than preventing complications or surgery [2]. This study does not contradict these recommendations.

The significance of *F. necrophorum* in an acute sore throat has been debated: we saw previously that the bacterium was highly prevalent (15%) in the studied cohort, only outnumbered by GAS [6], and Centor found it to be even more common (20% prevalence) in a student population aged 15 to 30 [7]. Similarly, other researchers have identified *F. necrophorum* more often in patients than in controls [5, 8, 17] and Klug states that the role of *F. necrophorum* in acute tonsillitis seems significant but has to be clarified [18]. These studies were all focused on the acute illness and did not include a follow-up study. Jensen, however, analysed throat swabs retrospectively among patients aged 10 to 40 and found *F. necrophorum* in 11% of patients with acute non-streptococcal group A tonsillitis, but in 23% of patients with recurrent tonsillitis, which supports the view that the bacterium could be especially involved in such conditions [5]. Similarly, our group has found *F. necrophorum* to be common before tonsillectomy but also prevalent (16%) six months post-tonsillectomy, despite the fact that all these patients were then asymptomatic. This emphasizes the hypothesis that *F. necrophorum* may only cause a throat infection under certain circumstances [19]. In this study, we have not found any support for *F. necrophorum* as a more pathogenic finding than other pathogens in patients with pharyngotonsillitis with regard to new visits, complications or surgery within 2 years of infection.

Conclusions

This study verifies that Group A Streptococci still is to be considered the most important pathogen in pharyngotonsillitis, associated with a higher number of new visits within 30 days, and that *F. necrophorum* did not distinguish itself as a major cause of recurrent infection or complications. These results do not merit any expansion of the aetiological paradigm of pharyngotonsillitis as suggested by others [20]. More studies, preferably treatment studies with the focus on the aetiology (and especially *F. necrophorum*) in adolescents with a sore throat, are needed before *F. necrophorum* can be confirmed or discarded as an important pathogen in pharyngotonsillitis.

Abbreviations

GAS: Group A Streptococcus

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Availability of data and materials
The datasets generated and analysed during the current study are not publicly available due to Swedish legislation (the Personal Data Act) but are available from the corresponding author on reasonable request.

Authors' contributions
JP: data acquisition, data analysis and interpretation, manuscript drafting and final manuscript approval. MS, KH: study conception and design, data interpretation, critical manuscript revision and final manuscript approval. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Ethical approval was obtained from the regional ethics review board in Linköping, Sweden: 2010/267–31. Two amendments were approved for this study: 2013/286–32 and 2015/146–32. All participants gave written informed consent before inclusion and could withdraw at any time. In line with the decision from the ethics review board and with Swedish law (The Act concerning the Ethical Review of Research Involving Humans (2003:460)), research subjects aged 15 to 18 years could consent on their own, without the involvement of a parent or legal guardian. Personal information was treated confidentially.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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Study II

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RESEARCH ARTICLE Open Access

The aetiology of pharyngotonsillitis in primary health care: a prospective observational study

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Abstract

Background: Few studies on pharyngotonsillitis have examined the clinical presentation of different aetiologies where pathogens have been detected using molecular methods. We aimed to assess how well clinical signs and symptoms can predict (1) the presence or absence of a broad range of viruses and bacteria, and (2) reconsultations for a sore throat or a complication.

Methods: In this descriptive observational prospective study in primary health care 220 patients aged 15–45 with suspected pharyngotonsillitis were sampled from nose, throat and blood and screened for 20 bacteria and viruses using polymerase chain reaction (PCR), culture and serology. Odds ratios (OR) and predictive values with 95% confidence intervals (CI) were used to show association between microbiological findings and clinical signs and symptoms. Patients were followed up after 3 months by reviewing electronic medical records.

Results: Both cough and coryza were more common in patients with only viruses (67%) than in patients with only bacteria (21%) ($p < 0.001$), whereas tonsillar coating was more common in patients with only bacteria (53%) than in patients with only viruses (29%) ($p = 0.006$). Tonsillar coating (adjusted OR 6.0; 95% CI 2.5–14) and a lack of cough (adjusted OR 3.5; 95% CI 1.5–8.0) were significantly associated with *Streptococcus pyogenes* (group A streptococci; GAS) and with any bacterial finding. A Centor score of 3–4 had a positive predictive value of 49% (95% CI 42–57) for GAS and 66% (95% CI 57–74) for any bacterial findings. The use of rapid antigen detection test for GAS increased the positive predictive value for this group to 93%.

Conclusions: Signs and symptoms, both single and combined, were insufficient to rule in GAS or other pathogens. However, both cough and coryza were useful to rule out GAS. The results support the clinical approach of restricting rapid antigen detection testing to patients with 3–4 Centor criteria. The low carriage rate of bacteria among asymptomatic controls implied that most detections in patients represented a true infection.

Keywords: Pharyngotonsillitis, Predictive values, Primary health care, Group A streptococci, Symptoms

Background

Acute sore throat, or pharyngotonsillitis, is one of the most common reasons for consultation in primary health care [1]. Throat infections are most often of viral aetiology [2] but can also be caused by bacteria, of which *Streptococcus pyogenes* (group A streptococcus; GAS) is the most important and the only one to have a definitive indication for treatment in many guidelines, e.g. the Infectious Diseases Society of America [2] and The Sore Throat Guideline Group within the European Society for Clinical Microbiology and Infectious Diseases [3]. The clinical presentation of pharyngotonsillitis, however,

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overlaps broadly in GAS and non-GAS aetiology and individual signs and symptoms are not sufficient to discriminate between the two [4]. Attempts have therefore been made to group signs and symptoms into clinical scoring systems to increase the diagnostic accuracy [4–7]. The four item Centor score, invented in 1981 [8], is a well-calibrated and validated score [4, 5, 8] for detecting GAS in throat cultures, as is the newer FeverPAIN score [6]. Though easy to use, these scores only increase the positive predictive values to modest levels, especially in low-prevalence settings [7, 9], which is why several guidelines in North America and Europe recommend the addition of a rapid antigen detection test (RADT) for GAS [2, 3, 10]. In Sweden the Medical Products Agency recommends the use of such a test in patients with a Centor score of 3–4 (out of a maximum of 4 points), if they are thought to benefit from antibiotics, and to only prescribe antibiotics to patients who test positive [10].

Looking beyond GAS, there is some support for group C and G streptococci to present in a similar manner to group A [3, 11, 12]. The same goes for the anaerobic bacteria *Fusobacterium necrophorum*, most often detected in young adults with pharyngotonsillitis [13–15], though it has also been associated with a cough [14]. These alleged similarities between different bacteria [3, 11–15] has led some researchers to suggest that clinical scoring systems in fact predict the presence of bacteria, rather than GAS only [11–13]. For instance, the creators of the FeverPAIN score claim that their score detects both GAS and group C and G streptococci and recommend that treatment be guided by score rather than aetiology [12]; Centor et al. have argued that the Centor score predicts not only group A, but also group C and G streptococci [13] and *F. necrophorum* [13]; and Lindbaek et al. have also suggested that the Centor score predicts group C and G streptococci in addition to GAS [11]. Nevertheless, the studied pathogens in these papers have been restricted to a narrow range of bacteria [11–13], and there is still a lack of studies that investigate the clinical signs and symptoms of a broad range of bacteria and viruses using polymerase chain reaction (PCR) technique [3].

We previously published an aetiological prospective case–control study on young adults with pharyngotonsillitis in primary health care [14], with a subsequent 2-year follow-up study on the same patients and controls [16]. The present study was a reanalysis of these data sets, but with a shifted focus to a clinician's perspective, examining how different signs and symptoms predict the presence or absence of various viruses and bacteria.

Our aims of this study were to describe how different signs and symptoms are associated with a wide range of aetiologies in pharyngotonsillitis, and to assess the association between the clinical presentation and return visits for a sore throat or for a complication within 30 days, or for a sore throat or tonsillectomy within three months. As we later discovered that 94% of the patients had been subjected to a RADT for GAS, we also aimed to describe both the performance of this test in our population and the underlying aetiologies in individuals who test negative.

Methods

Design and setting

This prospective observational study on young adults with pharyngotonsillitis in Swedish primary health care was a renewed analysis of data collected by Hedin et al. in a prospective aetiological case–control study of pharyngotonsillitis [14], and by Pallon et al. in a subsequent follow-up study [16]. While the previous studies compared aetiological findings between patients and asymptomatic controls, the current study focused on the clinical signs and symptoms of different aetiologies in patients. However, the controls still played a small part in this study, as they were used to calculate aetiological predictive values (see "Statistical analyses").

The study took part in Kronoberg County in the south of Sweden, which during the study period had a population of approximately 190,000, or about 2% of the Swedish population. To serve this population were two hospitals and 34 primary health care centres (PHCC), five of which participated in the study [14]. The participating PHCCs were located in urban areas and were chosen by convenience.

Participants

Patients aged 15–45 years who presented to the phone triage nurse with an acute sore throat as a major complaint and who were sufficiently ill to motivate a doctor's visit according to national guidelines [10], were asked to participate. The national guidelines advise that patients with compelling signs of viral infection should neither be tested for GAS nor treated with antibiotics; that only patients with 3–4 Centor criteria should be tested for GAS; and that patients with severe symptoms or immunosuppression should always be examined by a doctor [10]. If the doctor interpreted the symptoms as infectious pharyngotonsillitis, the patient was recruited after signing a form for informed consent. Asymptomatic controls were recruited from patients 15–45 years old who belonged to the same primary health care centre and consulted for non-infectious causes. We aimed for a consecutive sampling of all eligible patients, but ended up with a convenience sample as it was hard to engage all nurses and doctors in recruitment. The intended ratio of patients to controls was one (see "Statistical analyses"),

but turned out closer to two; neither did we manage to fully match the controls in age and sex with the patients.

Data collection

We asked the doctors to approach each participant as they would normally do, with the addition of completing a form with data about background characteristics, signs and symptoms, diagnosis, tests and treatment.

Microbiological procedures

As previously described [14], all patients and controls were sampled from the nasopharynx, throat and blood and screened with routine culture for β-haemolytic streptococci (Lancefield group A, C, and G); with anaerobic culture for *Fusobacterium necrophorum*; with serology for Epstein–Barr virus; with single PCR for Influenza A and B viruses and *Mycoplasma pneumoniae;* and with multiplex real-time PCR for two intracellular bacteria and 13 viruses: *M. pneumoniae*, *Chlamydophila pneumoniae*, Adenovirus, Bocavirus, Coronavirus NL63, Coronavirus OC43, Coronavirus HKU1, Coronavirus 229E, Enterovirus, Influenza A virus, Influenza B virus, Metapneumovirus, Parainfluenzavirus, Rhinovirus and Respiratory syncytial virus. The primers and probes used in the multiplex PCR have been described elsewhere [17].

RADTs for GAS are routinely used at most Swedish primary health care centres. The only RADT kit available in Region Kronoberg during the study period was QuickVue Dipstick Strep A (Quidel Corporation, San Diego, CA, USA), a lateral-flow immunoassay using antibody-labelled particles. The test detects either viable or nonviable organisms directly from throat swabs.

Follow-up

We reviewed all electronic medical records from the primary health care and hospitals for the 3 months following inclusion, to see if the patients had made any reconsultations for a sore throat, for a complication—defined here as sinusitis, peritonsillitis, media otitis, mastoiditis, lymphadenitis, necrotizing fasciitis, meningitis, sepsis, glomerulonephritis or rheumatic fever—or for tonsillectomy (ICD-codes for the studied outcomes are provided in Additional file 1: Table S1).

Statistical analyses

Data was analysed using SPSS 23.0 software (IBM, Armonk, NY, USA) and MedCalc (MedCalc Software Ltd, Ostend, Belgium). Due to non-normal distribution and small sample sizes continuous variables were reported as median (interquartile range [IQR]). Confidence intervals for sensitivity and specificity were calculated using the binomial (Clopper–Pearson) "exact" method. Confidence intervals for positive and negative predictive values were calculated as standard logit confidence intervals according to Mercaldo et al. [18]. Receiver operating characteristic (ROC) curves with area under the curve (AUC) were calculated to evaluate the diagnostic performance of a RADT for GAS at different levels of Centor score. For comparison of independent categorical data, we used two-sided Pearson χ^2-test, Fisher's exact test and Mantel–Haenzel trend test. p-values < 0.05 were considered as significant. Multiple logistic regression was used to predict aetiology from signs and symptoms: in the crude model, univariate odds ratios (OR) with 95% confidence intervals were calculated for each sign and symptom, using the "Enter" method; in the multiple model, adjusted odds ratios (aOR) were calculated with 95% confidence intervals. To ensure that there would be at least ten participants per variable in the multiple model, the variables were limited to the four Centor criteria (no cough, lymphadenitis, fever, and tonsillar coating), age and rapid attendance (duration ≤ 3 days). "No coryza" was excluded from the model due to collinearity with "no cough". Univariate ORs for Centor score 1 through 4 were calculated with logistic regression using Centor 0 as reference category.

This study is based on clinical data previously collected in conjunction with an aetiological case–control study [14], where the intended sample size of 150 patients and 150 controls was primarily chosen so that each participant would represent a percentage larger than one. Moreover, this sample size was also calculated to be able to detect a 10% difference in the prevalence of *F. necrophorum* between patients and controls with a power of 0.8 and an α value of 0.05 [14], which was hypothesized from a small pilot study by one of the authors (MS), where *F. necrophorum* was detected in 11% of patients and 5% of controls (unpublished data). Due to the small numbers of single pathogens, we created mutually exclusive groups before analysis: "only viruses", "only bacteria", "viruses and bacteria", and "no pathogen". In addition, we grouped all patients with a bacterial finding into "any bacteria", and all patients with GAS positive culture into "GAS". A Centor score [8] for each patient was calculated by adding one point each for absence of cough, temperature ≥ 38.5 °C, cervical lymphadenitis and tonsillar coating (for a maximum score of 4).

As there exists no reference standard to determine if a throat infection is caused by GAS or if the detection rather represents a GAS colonisation with a concomitant viral infection, regular predictive values only indicate the presence of GAS, not the presence of disease. *Aetiological predictive value*, on the other hand, is a statistical method that adjusts for asymptomatic carriage when interpreting an aetiological test [19], and it provides positive and negative predictive values with 95% confidence intervals. The

requisites for a calculation are: (1) the prevalence of the pathogen among both patients and asymptomatic individuals, (2) the sensitivity of the test, and (3) the "theta" value—the ratio of GAS prevalence in asymptomatic individuals and in patients with a sore throat caused by another pathogen. Based on previous work [19], we assumed a 90% sensitivity of throat culture to detect GAS and a theta value of 0.9.

Results

Characteristics of patients and controls

We included 220 patients with a median age of 33 (range 15–48). Their characteristics are presented in Table 1. To be able to calculate aetiological predictive values we also included 126 controls, with a median age of 31 (range 16–46). The controls differed from the patients in having a higher proportion of women (76%) and a lower proportion with frequent episodes of a sore throat (7%). A full table of characteristics of patients and controls has been published elsewhere [14], but is also provided in Additional file 2: Table S2.

Detected aetiology

The microbial findings in patients and controls were previously reported by Hedin et al. [14]. In summary, 155/220 patients (71%) had at least one of the 20 targeted microorganisms. Bacteria were found in 103 patients (47%) and viruses in 70 patients (32%). GAS was the most common finding (66 patients; 30%). Among controls, 3/126 (2.4%) had GAS and 17/126 (13%) had a bacterial finding.

Clinical signs and symptoms

Table 1 presents the frequencies of clinical signs and symptoms in different aetiological groups. Cough and coryza were more common in patients with only viruses compared to patients with only bacteria, as was a history of frequent sore throats. Tonsillar coating was more common in those with only bacteria, as was a Centor score

Table 1 Clinical signs and symptoms of different aetiologies in patients with a sore throat, number (%) if not otherwise stated

		Mutually exclusive groups					
Clinical signs and symptoms	**Total n = 220**	**Only viruses n = 52**	**Only bacteria n = 85**	**Viruses + bacteria n = 18**	**No pathogen n = 65**	**GAS n = 66**	**Any bacteria n = 103**
Age (years), median (IQR)	33 (23–39)	28 (21–38)	34 (24–40)	35 (26–38)	32 (23–40)	36 (33–40)	34 (26–39)
Female	141/220 (64)	34 (65)[a]	58 (68)[a]	8 (44)	41 (63)[a]	43 (65)	66 (64)
Smoker	30/215 (14)	7 (13)[a]	11 (13)[a]	2 (11)	10 (15)[a]	5 (8)	13 (13)
Days with symptoms, median (IQR)	4 (3–7)	4 (3–7)	3 (3–5)	3 (3–4)	6 (3–10)	3 (3–4)	3 (3–5)
Longstanding sore throat before inclusion	69/215 (32)	15 (29)[a]	22 (26)[a]	4 (22)	28 (43)[a]	16 (24)	26 (25)
Frequent sore throats	72/216 (33)	24 (46)[a,†]	24 (28)[a]	6 (33)	18 (28)[a]	18 (27)	30 (29)
Tonsillectomised	29/219 (13)	8 (15)[a]	11 (13)[a]	2 (11)	8 (12)[a]	11 (17)	13 (13)
Antibiotics last month	17/216 (8)	6 (12)[a]	7 (8)[a]	0 (0)	4 (6)[a]	3 (5)	7 (7)
Coryza	89/220 (40)	35 (67)[†††]	20 (24)	7 (39)	27 (42)	12 (18)	27 (26)
Cough	88/220 (40)	35 (67)[†††]	18 (21)	9 (50)	26 (40)	12 (18)	27 (26)
Temperature ≥ 38.5 °C	128/215 (60)	32 (62)[a]	56 (66)[a]	13 (72)	27 (42)[a]	48 (73)	69 (67)
Lymphadenitis	130/215 (60)	32 (62)[a]	56 (66)[a]	10 (56)	32 (49)[a]	44 (67)	66 (64)
Tonsillar coating	84/207 (41)	15 (29)[a,††]	45 (53)[a]	7 (39)	17 (26)[a]	36 (55)	52 (50)
Palatal petechiae	25/207 (12)	9 (17)	10 (12)	3 (17)	3 (5)	8 (12)	13 (13)
Duration ≤ 3 days	96/213 (45)	21 (40)	43 (51)	10 (56)	22 (34)	36 (55)	53 (51)
Centor 0	16/220 (7)[a]	5 (10)[a]	3 (4)[a]	1 (6)	7 (11)[a]	2 (3)	4 (4)
Centor 1	50/220 (23)[a]	14 (27)[a]	8 (9)[a]	4 (22)	24 (37)[a]	6 (9)	12 (12)
Centor 2	69/220 (31)[a]	20 (38)[a]	26 (31)[a]	5 (28)	18 (28)[a]	16 (24)	31 (30)
Centor 3	54/220 (25)[a]	10 (19)[a]	28 (33)[a]	7 (39)	9 (14)[a]	24 (36)	35 (34)
Centor 4	31/220 (14)[a]	3 (6)[a]	20 (24)[a]	1 (6)	7 (11)[a]	18 (27)	21 (20)
Centor 3–4	85/220 (39)	13 (25)[†††]	48 (56)	8 (44)	16 (25)	42 (64)	56 (54)

GAS group A streptococci

[†] p = 0.03 compared to "only bacteria"

[††] p = 0.006 compared to "only bacteria"

[†††] p < 0.001 compared to "only bacteria"

[a] These numbers were previously published by Hedin et al. [14] but are republished here for the sake of completeness

of 3–4. Prevalence of fever, lymphadenitis, petechiae and seeing a doctor within 3 days were similar between the two groups. Patients with no detected pathogen waited the longest before seeing a doctor, with a median of 6 days of symptoms prior to the visit. They also more commonly reported a sore throat lasting a long time compared to the other groups ($p=0.02$). GAS comprised the majority of bacterial findings, with the frequencies of this group resembling those of "only bacteria" and "any bacteria".

Among the 85 patients with a Centor score of 3–4, bacteria were found in 56 (66%), and any microorganism was found in 69 (81%). Thus, bacteria were detected in 56/69 (81%) patients with a microbial finding. Clinical signs and symptoms of the 85 patients with a Centor score of 3–4, grouped by "only viruses", "only bacteria", "viruses + bacteria", and "no pathogen" are presented in Additional file 3: Table S3. Among the 16 (19%) patients with "no pathogen", the frequencies of signs and symptoms resembled those of "only bacteria" most closely.

Predictive values of clinical findings

Odds ratios and predictive values for GAS and any bacterial findings are presented in Table 2. In the multiple logistic regression model, tonsillar coating and absence of a cough were significantly associated both with GAS and any bacterial findings, whereas fever and lymphadenitis were not.

The positive predictive values were low to moderate for single symptoms, and generally were better at predicting any bacteria than GAS specifically. The negative predictive values were the highest for absence of a cough and absence of coryza, indicating that a finding of cough or coryza would rule out most cases of GAS.

A regression analysis of the Centor score with 0 as reference category revealed a positive association between odds ratios for GAS and any bacteria and increasing score, which was mirrored in the predictive values. Again, the analysis showed a better prediction of any bacteria than of GAS. Adding the result of RADT for GAS to patients with Centor 3–4 increased the positive predictive value from 49 to 93% (Table 2).

Aetiological predictive values

As the carriage rate of GAS in controls was only 3/126 (2.4%), compared to 30% in symptomatic patients, the Aetiological predictive value of a positive culture for GAS reached 95% (95% CI 81–100), implying an infection in most detected cases. The carriage rate of any bacteria was however higher (13%), resulting in an Aetiological predictive value of any bacterial finding in culture or PCR that was somewhat lower: 84% (95% CI 62–95). Aetiological predictive values for single symptoms in addition to a sore throat were higher than for a sore throat alone, as presented in Table 2.

Performance of the rapid antigen detection test

In total, 207/220 patients (94%) had an RADT for GAS, despite the test not being a mandatory part of the original study protocol. The 13 patients not tested were evenly distributed with regard to Centor score. Table 3 shows sensitivity, specificity, predictive values, and area under the curve of the test based on our data set, and Fig. 1 displays the ROC-curves. Both sensitivity and positive predictive values increased with higher Centor scores, whereas the negative predictive values were overall high. Of the 37 test negative patients with Centor score 3–4, the underlying aetiology was "any bacteria" in 14 cases (38%), "only viruses" in 12 cases (32%) and "no pathogen" in 11 cases (30%). The detected bacteria were *F. necrophorum* ($n=9$), group G streptococci ($n=3$), group C streptococci ($n=2$), and GAS ($n=1$).

Follow-up

A total of 207 patients (94%) could be followed up. Of these, 21 (10%) reconsulted for a sore throat within 30 days, 17 of whom had a new episode and 4 had non-resolving symptoms. One patient (0.5%) had a complication (sinusitis). Patients with Centor score 4 reconsulted most frequently (5/27; 19%), in contrast to Centor score 0, where none did that. A trend test, however, showed no evidence of a positive association between score and reconsultation (Mantel–Haenzel $p=0.16$). A multiple logistic regression model adjusted for covariates, revealed no association between reconsultation and absence of a cough, temperature ≥ 38.5 °C, cervical lymphadenitis, tonsillar coating, or antibiotic prescription (data not shown).

After 3 months a total of 32 patients (16%) had reconsulted for a sore throat. Again, the highest proportion was among patients with Centor score 4 (7/27; 26%) (Mantel–Haenzel $p=0.054$).

Discussion

Principal findings

In this prospective observational study on young adults visiting primary health care with pharyngotonsillitis, we reanalysed data from Hedin et al. [14] from a more clinical perspective, to study how various clinical signs and symptoms could predict the detected aetiologies. In addition, we followed the patients for three months to analyse any associations between the clinical presentation at inclusion and subsequent reconsultation for a sore throat or a complication.

No single sign or symptom was sufficiently useful to rule in bacteria or viruses, and combining them into a

Table 2 Odds ratios and predictive values for the presence of group A streptococci or any bacterial finding among patients with a sore throat (n = 220)

Clinical findings	Group A streptococci (n = 66)					Any bacteria (n = 103)				
	OR (95% CI)	aOR, model 1 (95% CI)	PPV (95% CI)	NPV (95% CI)	EPV (95% CI)	OR (95% CI)	aOR, model 1 (95% CI)	PPV (95% CI)	NPV (95% CI)	EPV (95% CI)
No coryza	2.8 (1.5–5.4)††	–	41 (37–46)	87 (79–92)	97 (88–100)	3.2 (1.8–5.6)†††	–	58 (52–63)	70 (61–77)	90 (75–97)
No cough	4.4 (2.2–8.8)†††	6.0 (2.5–14)†††	41 (36–46)	86 (79–92)	97 (88–100)	3.1 (1.7–5.4)†††	2.7 (1.4–5.1)††	58 (52–63)	69 (61–77)	90 (74–97)
Temperature ≥ 38.5 °C	2.7 (1.4–5.1)††	1.4 (0.60–3.1)	38 (33–43)	79 (71–86)	96 (86–100)	2.1 (1.2–3.7)††	1.5 (0.76–2.8)	54 (48–59)	61 (53–69)	88 (70–97)
Lymphadenitis	1.7 (0.90–3.1)	1.6 (0.71–3.4)	34 (29–39)	74 (66–81)	96 (83–100)	1.6 (0.93–2.8)	1.2 (0.65–2.3)	51 (45–56)	56 (48–64)	86 (65–96)
Tonsillar coating	3.1 (1.7–5.8)†††	3.5 (1.5–8.0)††	43 (35–51)	76 (70–81)	97 (88–100)	3.0 (1.7–5.4)†††	2.5 (1.3–4.7)††	62 (53–70)	59 (53–64)	92 (77–98)
Palatal petechiae	1.2 (0.48–2.9)	–	32 (18–51)	68 (66–70)	95 (54–100)	1.4 (0.59–3.1)	–	52 (34–69)	51 (48–53)	87 (41–98)
Duration ≤ 3 days	1.9 (1.1–3.5)†	1.4 (0.65–3.0)	38 (31–45)	74 (68–80)	96 (85–100)	1.9 (1.10–3.3)†	1.3 (0.69–2.5)	55 (48–62)	57 (51–63)	89 (69–97)
Age, years	1.09 (1.05–1.13)†††	1.12 (1.07–1.18)†††	–	–	–	1.02 (0.99–1.05)	1.02 (0.99–1.06)	–	–	–
Centor 3–4	4.5 (2.4–8.3)†††	–	49 (42–57)	82 (77–87)	98 (91–100)	3.6 (2.0–6.4)†††	–	66 (57–74)	65 (60–70)	94 (81–99)
Centor 3–4 + positive RADT	5.8 (1.4–24)†	–	93 (81–97)	97 (84–100)	–	–	–	–	–	–
Centor 0[a]	1	–	13 (3–38)	69 (67–70)		1	–	25 (10–50)	51 (50–53)	
Centor 1	0.96 (0.17–5.3)	–	12 (6–23)	65 (62–68)		0.95 (0.26–3.5)	–	24 (15–36)	47 (43–50)	
Centor 2	2.1 (0.43–10)	–	23 (16–33)	67 (63–71)		2.4 (0.72–8.3)	–	45 (36–55)	52 (48–57)	
Centor 3	5.6 (1.2–27)†	–	44 (34–56)	75 (71–78)		5.5 (1.6–20)††	–	65 (53–75)	59 (55–63)	
Centor 4	9.7 (1.9–50)††	–	58 (42–73)	75 (72–77)		6.3 (1.6–25)††	–	68 (51–81)	57 (54–59)	

Crude (univariate) odds ratio (OR) and adjusted ORs (aOR) from the logistic regression are presented with 95% confidence intervals. The multiple model adjusts for the four Centor criteria (no cough, lymphadenitis, fever, and tonsillar coating), age and duration ≤ 3 days. "No coryza" was excluded from the model due to collinearity with "no cough"

PPV positive predictive value (true positives/all positives), *NPV* negative predictive value (true negatives/all negatives), *EPV* positive aetiological predictive value, *95% CI* 95% confidence interval, *RADT* rapid antigen detection test

† $p < 0.05$; †† $p < 0.01$; ††† $p < 0.001$

[a] Odds ratios for Centor score 1 through 4 are calculated with Centor 0 as reference category

Table 3 Sensitivity, specificity and predictive values for a rapid antigen detection test (RADT) for group A streptococci (GAS) at different Centor scores

Centor score	All patients (n = 220)		Patients tested with RADT (n = 207)						
	n (%)	Prevalence of GAS in culture n (%)	Tested n (%)	Positive n (%)	Sensitivity[a] (95% CI)	Specificity[a] (95% CI)	PPV[a] (95% CI)	NPV[a] (95% CI)	AUC[a] (95% CI)
0	16 (7)	2 (13)	15 (94)	3 (20)	100 (16–100)	92 (64–100)	67 (23–93)	100	0.96 (0.86–1.0)
1	50 (23)	6 (12)	46 (92)	5 (11)	50 (12–88)	95 (83–99)	60 (24–88)	93 (85–97)	0.73 (0.46–0.99)
2	69 (31)	16 (23)	67 (97)	18 (27)	81 (54–96)	90 (79–97)	72 (52–86)	94 (85–98)	0.86 (0.74–0.98)
3	54 (25)	24 (44)	52 (96)	24 (46)	96 (78–100)	93 (77–99)	92 (74–98)	96 (80–99)	0.94 (0.87–1.0)
4	31 (14)	18 (58)	27 (87)	18 (67)	100 (80–100)	90 (56–100)	94 (73–99)	100	0.95 (0.84–1.0)
Total	220 (100)	66 (30)	207 (94)	68 (33)	89 (79–95)	92 (87–96)	84 (74–90)	95 (90–97)	0.91 (0.86–0.96)

RADT rapid antigen detection test, *GAS* group A streptococci, *PPV* positive predictive value (true positives/all positives), *NPV* negative predictive value (true negatives/all negatives), *AUC* area under the curve, *95% CI* 95% confidence interval

[a] All numbers for RADT are calculated with throat culture as reference standard for the detection of group A streptococci

Centor score of 3–4 only modestly raised the positive predictive values, to 49% for GAS and 66% for any bacterial finding. Cough and coryza were rare in patients with GAS and had a negative predictive value of 86%, making these symptoms useful to rule out this pathogen. Aetiological predictive values were high for both GAS and any bacterial finding, meaning that a positive finding represents a true infection rather than carriage in most cases

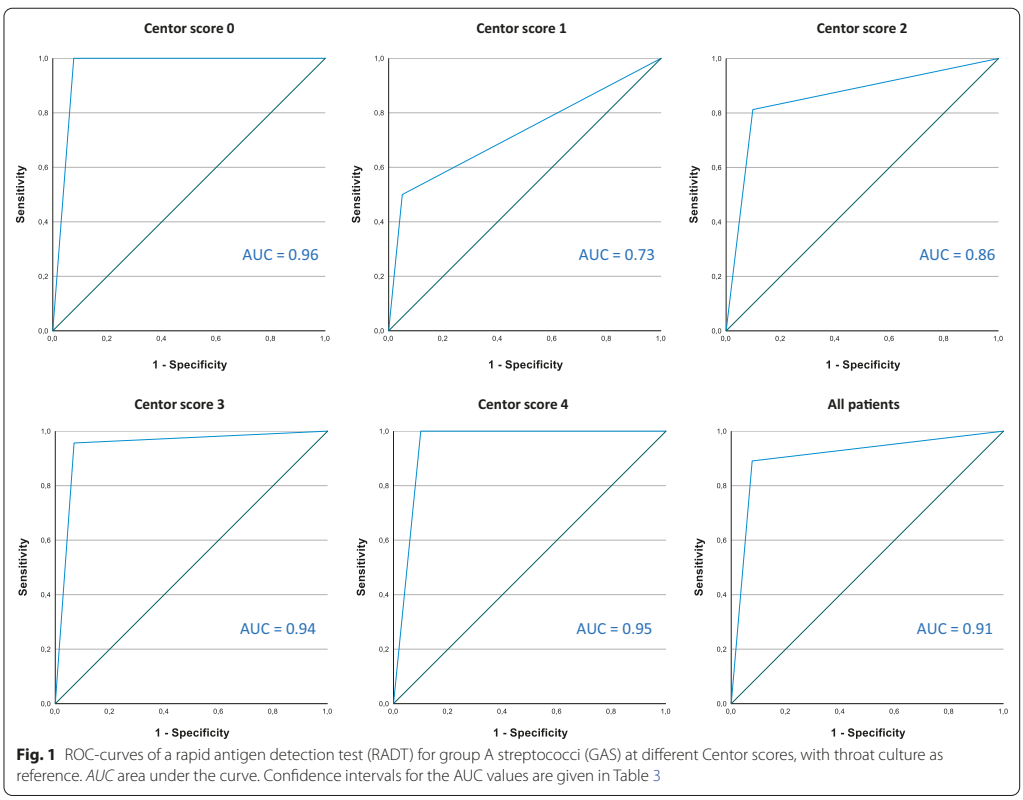

Fig. 1 ROC-curves of a rapid antigen detection test (RADT) for group A streptococci (GAS) at different Centor scores, with throat culture as reference. *AUC* area under the curve. Confidence intervals for the AUC values are given in Table 3

[19]. The RADT was excellent at ruling out GAS regardless of Centor score, whereas the positive predictive value was only acceptable for patients with Centor 3–4. We found no evidence of an association between increasing Centor score and reconsultation within 3 months.

Strengths and weaknesses

Though some of these results were previously published [14, 16], those articles aimed to describe the clinical characteristics of different pathogens. In this study we attempted to shift the focus to a clinician's perspective, examining how any given sign and symptom in a patient can predict the presence or absence of different viruses and bacteria. The use of both culture and PCR has enabled us to classify the aetiology as viral or bacterial with greater certainty than with culture alone. In addition, we made no assumptions of aetiology in patients with no detected pathogen, but instead grouped them separately. A limitation of the renewed analysis, however, was that the small size of the study forced us to analyse most microorganisms grouped instead of individually.

Though we could not include all consecutive patients due to busy offices, and though the summer season was excluded, the study interfered minimally in the everyday clinical management of the patients, and thus mirrors typical patients and conditions in Swedish primary health care. This is also the case for the evaluation of signs and symptoms, which is partly a subjective task.

Registering details from the clinical management made it possible to describe the performance of the RADT for GAS. As this test was not asked for, we gave no specific instructions on sampling technique to the participating centres, but RADTs are routinely used in Swedish primary care, and both doctors and laboratory staff are trained in the sampling procedure; it thus mirrors everyday clinical practise.

Other strengths of the study were the sampling of asymptomatic controls, which enabled us to measure the presence of bacteria and viruses and calculate aetiological predictive values; registering individual signs and symptoms rather than the total Centor score; and the prospective approach, which enabled us to follow patients over time.

Pharyngotonsillitis is more common in children than in adults [20, 21], but the prevalence of bacterial pathogens differs with age [22, 23] and we therefore found it reasonable to focus on children and adults separately. At the time of this study we had already started to plan such a project on paediatric sore throat.

Interpretation

Though many symptoms of pharyngotonsillitis require very large sample sizes to discriminate between GAS and non-GAS aetiology [4], cough and coryza are generally considered viral features [2], and our study also found these symptoms more frequently in patients with microbiological analyses positive for only viruses than in patients with only bacteria. On the other hand, tonsillar coating was more frequent in patients where only bacteria were found. The regression analysis found tonsillar coating and absence of cough to be significantly associated with both findings of GAS and any bacteria, which is in line with a large meta-analysis that showed "any exudates" to have the strongest discriminatory power for GAS [4]. As presented by others [4, 9, 24], no single sign or symptom, however, reached sufficiently high positive predictive values to diagnose GAS or any bacterial finding with certainty. However, both cough and coryza, which are often found together, had a negative predictive value for GAS > 85%, making these symptoms useful for ruling out this pathogen, though not any bacterial findings.

Combining single symptoms into Centor score increased the predictive values for both GAS and any bacterial findings. The positive predictive value increased with every point, and at Centor score 3–4 the positive predictive value was greater than for any single symptom. However, only patients with a score of 4 had a probability for GAS that was greater than the probability of not having GAS. In line with previous reports [11, 13], Centor score was better at predicting any bacterial findings than GAS alone, which is explained by high scores also in many patients with group C or G streptococci or *F. necrophorum*.

The negative predictive value of Centor score 3–4 for GAS was modest, and for any bacterial findings even lower. Furthermore, low Centor scores were not very predictive of viruses.

Adding more items to a score could increase the predictive values, but at the cost of usefulness. The comprehensive nine-item score of Joachim et al. [25], for instance, was created to diagnose GAS in low-resource settings, but is hard to remember.

To our surprise, almost a fifth of the patients with Centor score 3–4 had no detected pathogen, though an infectious aetiology rather than non-infectious seems more likely at these levels. This absence of pathogens might be explained by errors made during sampling, handling, transportation, or analysis [26]. Although one can only speculate about the underlying aetiology, 81% of the patients with a Centor score of 3–4 and a detected aetiology had a bacterial finding, and the frequencies of clinical signs and symptoms in patients with "no pathogen" most closely resembled those of patients with "only bacteria" (Additional file 3: Table S3).

The problem with insufficient precision of clinical scores in diagnosing GAS can be overcome with rapid antigen detection tests, which have great sensitivity and specificity [27]. Several guidelines recommend such a test [2, 3, 10], but it should be restricted to patients with Centor score 3–4 as this is the only group shown to benefit from antibiotics [28]. Another reason to restrict testing to these patients, which was apparent in our study, is that both sensitivity and specificity of the RADT increase with Centor score [29], leading to false positives and false negatives in patients with Centor score 0–2. The large number of patients with low scores in this study, together with a lower sensitivity of the RADT, reduced the positive predictive values, which were only 60–70% at these levels.

The negative predictive values of RADT were high at all levels of Centor score, in line with previous reports [27], and this shows that a negative test result rule out most cases of GAS. Correctly used, an RADT could therefore lower the antibiotic prescription rate to half [2, 30, 31]. On the other hand, a too liberal use of the test at lower scores, as was the case in our study group, will encourage antibiotic treatment in patients with no apparent benefit, and this could contribute to medicalisation and changed expectations among patients [12]. The fact that an overwhelming majority of the patients were tested is a major deviation from National guidelines [10], and deserves a study on its own with regard to doctor's attitudes.

If group C and G streptococci and *F. necrophorum* are considered important pathogens, the RADT will miss them, whereas both the Centor score and the FeverPAIN score will detect many of them [11–13, 15]. It then becomes a question of which bacteria to treat [12, 13, 15]. Little et al. [12] showed that basing antibiotic treatment on an RADT for GAS did not improve the outcomes regarding pain and time to recovery, compared to using the FeverPAIN score, which, in essence, is a comparison of treating only GAS with treating any bacteria. However, before we have stronger evidence for the benefits of treating other bacteria than GAS, the clinical scores may lead to antibiotic overuse [27].

A commonly overlooked problem in aetiological diagnosis is the possibility of asymptomatic carriage, especially in children [32]. This applies not only to GAS, but also to other streptococci and *F. necrophorum*, and occludes the meaning of a positive test [19]. To correctly assess a finding, one must therefore adjust for the carriage rate. The aetiological predictive value [19] does exactly that, with the assumption that the carriage rate is the same in symptomatic patients and symptomatic controls. In our study, we found a low carriage rate of both GAS and other bacteria, implying that most detected bacteria were responsible for the symptoms, and that aetiological diagnosis is thus meaningful.

The follow-up revealed no strong evidence for an association between individual or combined signs and symptoms and reconsultation, adjusted for antibiotic treatment. This was in line with a previous study, that only found previous medical problems, sex, temperature and muscle aches to be independently but weakly associated with reconsultation [33]. Signs and symptoms thus seem to be inadequate as predictors of future visits for a sore throat, and the clinician should rather focus on other factors that seem to have a greater impact on the tendency to consult for respiratory infections, such as young age, female gender, anxiety, and perceived threats [34]. This could be accomplished by promoting self-management to targeted groups of patients, and providing broader information, such as leaflets and public campaigns [34].

Conclusions

Signs and symptoms, both single and combined, were insufficient to diagnose GAS or other pathogens; a greater use may instead lie in ruling out GAS, as cough and coryza both exhibited great NPVs. The Centor score was more predictive of any bacterial finding than of GAS, which indicates an overlapping clinical presentation of many bacteria. The RADT was excellent at ruling out GAS regardless of Centor score, whereas the PPV for GAS was only acceptable for patients with a Centor score of 3–4. The low carriage rate of bacteria among asymptomatic controls implied that most detections in patients represented a true infection.

Abbreviations

aOR: Adjusted odds ratio; GAS: Group A streptococci; EPV: Positive aetiological predictive value; IQR: Interquartile range; NPV: Negative predictive value; OR: Odds ratio; PCR: Polymerase chain reaction; PPV: Positive predictive value; RADT: Rapid antigen detection test; ROC: Receiver operating characteristic.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-021-06665-9.

Additional file 1: Table S1. List of ICD-codes for outcomes in the follow-up study of 220 patients with a sore throat in primary health care.

Additional file 2: Table S2. Characteristics of 220 patients 15–45 years old with acute sore throat in primary health care, and 126 asymptomatic controls 15–45 years old.

Additional file 3: Table S3. Clinical signs and symptoms of different aetiologies in 85 patients with a sore throat and a Centor score of 3–4, number (%).

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Authors' contributions
JP: Conceptualisation, data curation, formal analysis, funding acquisition, investigation, writing—original draft preparation, writing—review and editing. MR: conceptualisation, formal analysis, writing—review and editing. MS: formal analysis, writing—review and editing. KH: conceptualisation, data curation, formal analysis, funding acquisition, project administration, resources, supervision, writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials
The data sets generated and analysed during the current study are not publicly available due to Swedish legislation (the Personal Data Act) but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from the Regional Ethics Review Board, Linköping University, Linköping, Sweden (Reference Number 2010/267-31, with two amendments: 2013/286-32 and 2015/146-32). All participants gave informed consent in writing before inclusion and could withdraw at any time. In line with the decision from the ethics review board and with Swedish law (The Act concerning the Ethical Review of Research Involving Humans (2003:460)), research subjects aged 15 to 18 years could consent on their own, without the involvement of a parent or legal guardian. Personal information was treated confidentially.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Study III

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ORIGINAL PAPER

Presence of microorganisms in children with pharyngotonsillitis and healthy controls: a prospective study in primary healthcare

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Abstract

Purpose Most studies on paediatric pharyngotonsillitis focus on group A streptococci. This study, however, analyses a broad spectrum of bacteria and viruses related to paediatric pharyngotonsillitis and evaluates their associated clinical symptoms and courses.

Methods This observational prospective study in primary healthcare includes 77 children aged < 15 with a sore throat and 34 asymptomatic children, all of whom were sampled from the tonsils with an E-swab® for analysis with culture and PCR for 14 bacteria and 15 viruses. Patients were evaluated clinically, and their symptoms recorded in diaries for 10 days. Participants were followed up for 3 months by reviewing medical records.

Results A pathogen was detected in 86% of patients and in 71% of controls ($P = 0.06$). Bacteria were found in 69% of patients and 59% of controls ($P = 0.3$), and viruses in 36% and 26%, respectively ($P = 0.3$). Group A streptococci was the most common finding, with a prevalence of 49% and 32%, respectively ($P = 0.1$). Clinical signs were not useful for distinguishing pathogens. None of the controls and 16% of the patients reconsulted for a sore throat within 3 months.

Conclusion Bacteria were more common than viruses in both study groups. The high rate of pathogens in asymptomatic children interferes with diagnoses based on aetiology.

Keywords Pharyngotonsillitis · Aetiology · Children · Primary healthcare · PCR · Prospective

Abbreviations

GAS Group A streptococci
PCR Polymerase chain reaction

Introduction

Pharyngotonsillitis accounts for 6% of all primary healthcare visits by children [1] and leads to antibiotic prescriptions in 53–60% of the cases [1–4]. The most important pathogen is the bacterium *Streptococcus pyogenes* (group

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A streptococcus; GAS), which can cause both severe non-suppurative complications such as acute rheumatic fever and glomerulonephritis and immediate suppurative complications such as peritonsillar abscess, otitis media, and sinusitis. Non-suppurative complications are almost absent in high-income countries, and suppurative complications are too rare to justify antibiotic treatment. Current guidelines note that acute sore throat is a self-limiting infection that usually subsides within a week without antibiotic treatment, so the benefits of antibiotics must be weighed against adverse effects [5, 6].

Although GAS is the most common bacterial aetiology, it is only found in every third child with an acute sore throat and even less so in children younger than 5 years old [7]; that is, a majority of throat infections are caused by other pathogens, including respiratory viruses and other streptococcal species [8]. However, previous studies have often focused on a narrow spectrum of pathogens and relied on older methods such as culture and antigen detection [9, 10]. Moreover, GAS is also found in 12% of asymptomatic children [7], which poses problems diagnosing test-positive patients.

A few studies of unselected children with an acute sore throat in primary healthcare have investigated a broad range of respiratory pathogens using both culture and molecular methods [6]. In addition, there is a knowledge gap regarding the presentation and clinical course associated with these pathogens as well as their carriage rate in healthy children.

This study has three aims: (1) to estimate the prevalence of 29 respiratory pathogens in children with an acute sore throat and in healthy controls; (2) to relate signs, symptoms, and clinical course to aetiology; and (3) to measure the incidence of complications and return visits for a sore throat within 3 months after clinical examination.

Materials and methods

Design and setting

In this prospective inception cohort study, we recruited children with an acute sore throat in primary healthcare and studied their symptoms and clinical course in relation to detected pathogens. For comparison, we also included non-infected controls. Both groups were followed for 3 months regarding recurrence and complications. Four primary healthcare centres in three counties in southern Sweden participated. Inclusion was open between 12 September 2014 and 17 October 2017.

Participants

Patients with suspected pharyngotonsillitis were initially identified by a triage nurse during a telephone assessment. During office hours for ordinary ambulatory care, these patients and their parents were recruited to participate by the authors and other physicians. These patients were eligible if they were 0–14 years old and had a sore throat lasting less than 7 days as a major complaint (or signs of pharyngotonsillitis on clinical examination in the youngest). Exclusion criteria were imminent complications associated with a sore throat (peritonsillitis, sinusitis, acute otitis media, or lymphadenitis colli), symptoms of obstructive airway disease, and difficulties understanding Swedish. Apart from study-related procedures, all patients received care-as-usual, including any required tests or prescriptions.

The control group was recruited from asymptomatic children aged 0–14 who belonged to the same primary healthcare centre and sought care for non-infectious conditions.

We set out for a consecutive sampling of all eligible patients, but as the researchers were not always in the office and the triage nurses at times forgot about the study, we ended up using convenience sampling.

Data collection

After informed consent, the physician recorded background information on all participants. For patients, the physician also recorded signs and symptoms, working diagnosis, and decisions about antibiotics and ordered tests.

Symptom diary

We asked the parents to keep a structured diary for 10 days and record symptoms (e.g., sore throat, stuffed up or runny nose, pain when swallowing, cough, hoarseness, diarrhoea, vomiting, and resting more than half the day), analgesics use, antibiotics use, and morning temperature. We also asked them to assess daily if their child was still unwell and if their children missed preschool or school due to their illness. After completion, they returned the diary by mail in a prepaid envelope. Two weeks after inclusion, we called each patient as a reminder.

Microbiological sampling

Either the physician or trained staff at the primary healthcare centre collected a throat specimen from each participant by rolling a single nylon-flocked swab (E-Swab®, Copan Diagnostics Inc., Murrieta, CA) repeatedly against both tonsils. The swab was transferred to liquid Amies medium in the accompanying container and stored in a refrigerator for overnight transport. All samples were analysed the following day at the Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, Sweden. To ensure analysis was performed the day following collection, we limited inclusion to Monday

through Thursday between 8 a.m. and 4 p.m. The laboratory staff were blinded to clinical data and any point-of-care test results.

Bacterial culture

A calibrated loop (10 µl) of diluted tonsillitis secretion was inoculated onto horse blood agar, *Streptococcus* agar, *Haemophilus* agar, and *Arcanobacterium haemolyticum* agar (all prepared in-house at Clinical Microbiology, Sahlgrenska University Hospital). The agar plates were incubated for 1 day at 34–36 °C in air with 5% CO_2, and after inspection incubated for another day at 34–36 °C in air, or for the *Arcanobacterium* agar, in air with 5% CO_2. Group A, B, C, and G streptococci, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and Gram-negative rods were enumerated and identified using standard bacteriological methods. *A. haemolyticum* was identified with a CAMP inhibition test.

PCR detection of Fusobacterium necrophorum

Bacterial DNA was extracted and purified from 500 µl of diluted tonsillitis secretion using Amplicor Respiratory Specimen Preparation kits (Roche Diagnostics, Mannheim, Germany). *F. necrophorum* ssp. *funduliforme* was detected with a real-time PCR using previously published primers for the *rpo* gene (partial) [11], and SYBR green for detection of the amplified PCR product. The PCR conditions were as follows: initial denaturation at 95 °C for 2 min, followed by 40 cycles, each cycle consisting of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 20 s, all performed in a Rotor-Gene Q (Qiagen, Sollentuna, Sweden). After a pre-incubation step at 75 °C for 90 s, a melting curve analysis was performed from 75 to 95 °C, rising by one degree each step, to confirm the correct *F. necrophorum rpo* gene amplification.

PCR detection of viral and other bacterial pathogens

Nucleic acids from 200 µl of the tonsillitis secretion were extracted with a MagNA Pure LC instrument (Roche Diagnostics, Mannheim, Germany) using Total Nucleic Acid Isolation kits (Roche Diagnostic). Next, a multiplex real-time PCR was performed to detect 15 respiratory tract viruses (adenovirus, bocavirus, coronavirus 229E, OC43, NL63 and HKU-1, enterovirus, influenza A and B virus, metapneumovirus, parainfluenza virus 1–3, rhinovirus and respiratory syncytial virus, RSV) and five bacteria (*S. pneumoniae*, *H. influenzae*, *Bordetella pertussis*, *Chlamydophila pneumoniae,* and *Mycoplasma pneumoniae*) [12].

Follow-up

Three months after inclusion, we reviewed the medical records of all patients and controls regarding return visits for a sore throat during the period and for a complication (peritonsillitis, sinusitis, acute otitis media, lymphadenitis colli, glomerulonephritis, or rheumatic fever) within 30 days of inclusion. We had access to relevant data from primary healthcare and hospitals at all study sites.

Statistical analyses

Based on earlier reports [7, 9], we estimated that 100 patients and 100 controls would be sufficient to describe the epidemiologic situation and to reveal possible differences in aetiological prevalence between groups, primarily regarding GAS.

Data were analysed with SPSS 23.0 (IBM, Armonk, NY, USA). Continuous variables with non-normal distribution or with small sample sizes were reported as median (interquartile range, IQR). For comparison of three or more groups of variables not normally distributed, we used Kruskal–Wallis *H* test, reported with the *H* statistic, degrees of freedom and *P* value. For comparison of categorical data, we used either Pearson χ^2 or Fisher's exact test for independent groups, and McNemar's test for paired data.

Before analysis, the participants were grouped by age: < 1 year (before preschool), 1–5 years (preschool), and 6–14 years (school). The microorganisms were also grouped, partly because of small numbers and partly to reflect clinical usefulness: "GAS" (corresponding to a positive culture or a rapid antigen detection test), "any bacteria" (positive in culture and/or PCR), "only viruses" (no benefit from antibiotics), and "no detected pathogen". We chose to use Centor score (one point each for fever, absence of cough, tonsillar coating, and tender cervical lymph glands) [13] rather than McIsaac score (age-adjusted Centor score) [14] to describe the summarized clinical features, because Centor score mirrors Swedish guidelines [15] and the two scoring systems are similar in the age group 3–14 years.

Aetiological predictive value, introduced by Gunnarsson and Lanke [16], is a statistical method that accounts for asymptomatic carriage when interpreting an aetiological test. As microbial carriage is also seen in symptomatic people, a positive finding could mean either infection or carriage. To correctly assess the test outcome, the level of uncertainty must first be quantified. Positive and negative predictive values with 95% confidence intervals can be calculated with known data for the prevalence of the pathogen (in our case GAS) for both patients and healthy subjects as well as the sensitivity of the test. It is also necessary to estimate "theta"—i.e., the ratio of GAS carriage in healthy individuals and in patients with a sore throat caused by a

virus. Based on Gunnarsson and Lanke, we assumed a 90% sensitivity of throat culture to detect GAS and a theta of 0.9.

Results

Characteristics

The study included 79 patients and 34 controls. Two patients were later excluded from analysis due to withdrawn consent or symptoms lasting more than 7 days. Patients and controls were included in parallel, and most patients (63 of 77, 82%) and controls (28 of 34, 82%) were recruited during cold months (October–April). The age distribution was similar in both groups, with a median value of 7.8 years in patients (IQR 4.6–11) and 7.7 years in controls (IQR 4.2–10). Among the patients, 71 of 77 (92%) were aged 3 or older. The median number of days with symptoms before consultation was 3 (IQR 2–5). Other background characteristics of the study population are presented in Table 1.

Detected pathogens

Prevalence

In 66 of 77 patients (86%) and 24 of 34 controls (71%), we detected at least one of the 29 targeted pathogens ($P=0.06$). Bacteria were found in 69% of the patients and 59% of the controls ($P=0.3$), and viruses in 36% and 26%, respectively ($P=0.3$). That is, bacteria were more common than viruses among both patients ($P=0.001$) and controls ($P=0.02$). Thirteen of the pathogens were never detected in the patients, and 17 were never detected in the controls.

GAS was the most prevalent pathogen in patients, making up a majority of bacterial findings, followed by *H. influenzae*, *S. aureus*, influenza B virus, and rhinovirus. In controls, GAS was also the most prevalent pathogen, followed by rhinovirus (Tables 2 and 3). We detected two or three concomitant pathogens in 23 patients (30%), 15 of which were a combination of bacteria and viruses. The most common combination was GAS and influenza B virus ($n=4$). Nine (26%) of the controls had two or three concomitant pathogens. GAS was mostly detected as a sole pathogen (in 71% of patients and 55% of controls with GAS, respectively).

Aetiology and age

No pathogen was detected in the two patients who were under 1 year old. In the two older age groups, the distribution of pathogens in each group mirrored the overall pattern, and we found no differences between patients and controls that were statically significant (i.e., $P<0.05$). The relationship between age group and microbial findings is presented in Table 3.

Aetiological predictive value for group A streptococci

With a prevalence of 49% for patients and 32% for controls, the positive aetiological predictive value for GAS was 54% (95% CI 0–92%). Restricting the calculation to patients with a Centor score of 3–4, the corresponding value was 67% (95% CI 0–97%).

Clinical symptoms and management

Symptoms and aetiology

The median number of days with a sore throat before consultation was similar between the mutually exclusive groups "any bacteria", "only viruses", and "no pathogen" ($H=2.5$, 2 *d.f.*, $P=0.3$) (Table 4). Swollen tonsils were found in 47% of patients with GAS and 31% of patients

Table 1 Characteristics of the study population

	Number (%)		
	Patients ($n=77$)	Controls ($n=34$)	χ^2 (Fisher) *P* value
Age 0	2 (3)	0	1 (Fisher)
Age 1–5	27 (35)	11 (32)	0.8
Age 6–14	48 (62)	23 (68)	0.8
Female	52 (68)	16 (47)	0.04
Smoker in household	11 (14)	5 (15)	1
A history of recurring sore throat	25 (32)	3 (9)	0.008
Previous tonsillectomy	5 (6)	2 (6)	1 (Fisher)
Antibiotic treatment in the last month	10 (13)	0	0.03 (Fisher)
Prone to infections (parents' view)	16 (21)	1 (3)	0.02
Sore throat in family member in the last month	49 (64)	13 (38)	0.01

Table 2 Bacteria and viruses detected by culture or PCR in children with a sore throat and in controls

	Number of patients (%)		
	Patients ($n=77$)	Controls ($n=34$)	Fisher or χ^2 P
Bacteria			
Group A streptococci	38 (49)	11 (32)	0.1[e]
Group C streptococci	1 (1)	3 (9)	0.08
Group G streptococci	–	1 (3)	0.3
Haemophilus influenzae	9 (12)[a]	2 (6)[a]	0.5
Fusobacterium necrophorum	1 (1)	1 (3)	0.5
Mycoplasma pneumoniae	–	1 (3)	0.3
Staphylococcus aureus	7 (9)	3 (9)	1
Gram-negative rods	5 (6)[b]	3 (9)[c]	0.7
Any bacteria	53 (69)	20 (59)	0.3[e]
Viruses			
Adenovirus	4 (5)	–	0.3
Bocavirus	–	2 (6)	0.1
Coronavirus NL63	1 (1)	1 (3)	0.5
Coronavirus OC43	1 (1)	–	1
Enterovirus	4 (5)[d]	1 (3)	1
Influenza A virus	2 (3)	–	1
Influenza B virus	6 (8)	–	0.2
Metapneumovirus	3 (4)	–	0.6
Parainfluenzavirus 1	1 (1)	–	1
Rhinovirus	7 (9)	7 (21)	0.1[e]
Respiratory syncytial virus	2 (3)	–	1
Any virus	28 (36)	9 (26)	0.3[e]

The following bacteria and viruses were not detected: *Arcanobacterium haemolyticum*, *Bordetella pertussis*, *Chlamydophila pneumoniae*, group B streptococci, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, Coronavirus 229E and HKU-1, Parainfluenzavirus 2 and 3

[a] *Haemophilus influenzae* was detected in patients both as the sole finding ($n=2$), and concomitant with group A streptococci ($n=2$), *S. aureus* ($n=3$), and viruses ($n=3$). Among controls, it was detected together with a virus ($n=1$) and *M. pneumoniae* ($n=1$)

[b] *Enterobacter cloacae* ($n=1$), *Klebsiella pneumoniae* ($n=4$)

[c] *Pseudomonas* spp. ($n=3$)

[d] In one patient, the analysis could not differentiate between enterovirus and rhinovirus

[e] χ^2 test was used

with only viruses ($P=0.3$), and had a positive predictive value of 67% for GAS (95% CI 51–80%). Tender cervical lymph glands were common both in patients with GAS and in patients with "no pathogen" and had a positive predictive value of 53% for GAS (95% CI 41–64%). Coryza was more common in patients with only viruses than in patients with GAS ($P=0.04$), but it had a low positive predictive value for viruses (24%; 95% CI 16–35%). A cough was present in 46% of patients with only viruses and 24% of those with GAS ($P=0.2$). A lack of a cough had a positive predictive value for GAS of 55% (95% CI 47–62%).

Centor scores

In total, 47 of 77 patients (61%) had a Centor score of 0–2, and 30 patients (39%) had a score of 3 (Table 4). As there were few patients with fever at consultation ($n=9$), no patient had a score of 4. A Centor score of 3 was seen in 45% of patients with GAS and in 31% of patients with only viruses ($P=0.5$). The positive predictive value of a Centor score of 3–4 for GAS was 57% (95% CI 43–70%) and the negative predictive value was 55% (95% CI 46–64%).

Table 3 Aetiology vs. age in children < 15 years with a sore throat

	Aetiology, *n* (%)								
	All ages[a]			Age 1–5			Age 6–14		
	Patients (*n* = 77)	Controls (*n* = 34)	*P* value	Patients (*n* = 27)	Controls (*n* = 11)	*P* value	Patients (*n* = 48)	Controls (*n* = 23)	*P* value
Any pathogen	66 (86)	24 (71)	0.06	24 (89)	9 (82)	0.6[b]	40 (83)	15 (65)	0.09
Any bacteria	53 (69)	20 (59)	0.3	18 (67)	6 (55)	0.7[b]	33 (69)	14 (61)	0.5
GAS	38 (49)	11 (32)	0.1	13 (48)	2 (18)	0.1[b]	25 (52)	9 (39)	0.3
Only viruses	13 (17)	4 (12)	0.5	6 (22)	3 (27)	1[b]	7 (15)	1 (4)	0.3[b]

P values are for Pearson χ^2 test

GAS group A streptococci

[a]"All ages" also includes the two patients aged < 1 year

[b]Fisher's exact test

Table 4 Clinical signs vs. pathogen findings in children < 15 years with a sore throat, *n* (%)

	All patients (*n* = 77)	Any bacteria (*n* = 53)	GAS (*n* = 38)	Only viruses (*n* = 13)	No pathogen (*n* = 11)
Days with a sore throat prior to visit, median (IQR)	3 (2–5)	3 (2.3–4.8)	3 (2–4.5)	3 (2–5.5)	2 (1–4)
Cough	24 (31)	16 (30)	9 (24)	6 (46)	2 (18)
Coryza	33 (43)	20 (38)	11 (29)	8 (62)	5 (45)
Tender cervical lymph glands	35 (45)	24 (45)	19 (50)	4 (31)	7 (64)
Tonsillar coating	19 (25)	13 (25)	9 (24)	3 (23)	3 (27)
Tonsillar erythema	54 (70)	38 (72)	29 (76)	8 (62)	8 (73)
Swollen tonsils	27 (35)	21 (40)	18 (47)	4 (31)	2 (18)
Petechiae	5 (6)	4 (8)	3 (8)	1 (8)	–
Raspberry tongue	1 (1)	1 (2)	1 (3)	–	–
Scarlatine rash	1 (1)	1 (2)	1 (3)	–	–
Impetigo	–	–	–	–	–
Temperature ≥ 38.5 °C	7 (9)	3 (6)	3 (8)	3 (23)	1 (9)
Centor score					
0	6 (8)	3 (6)	2 (5)	3 (23)	–
1	19 (25)	14 (26)	6 (16)	2 (15)	3 (27)
2	22 (29)	16 (30)	13 (34)	2 (15)	2 (18)
3	30 (39)	20 (38)	17 (45)	4 (31)	6 (55)
4	–	–	–	–	–

GAS group A streptococci

Clinical course

Symptom diaries

We received complete diaries from 55 of 77 patients (71%). The response rate differed slightly between the groups: 74% for "any bacteria", 77% for "only viruses", and 55% for "no pathogen" ($P = 0.4$, Fisher). Most of these patients (52 of 55) reported a resolution of their sore throat within 10 days, although five experienced recurrent symptoms.

The median duration of a sore throat after consultation differed between groups, with the fastest resolution in GAS patients treated with antibiotics (median 3 days; IQR 1.5–3.5) and the slowest resolution in GAS patients not treated with antibiotics (median 4.5 days; IQR 2.3–8.8). The difference, however, was not statistically significant ($H = 6.2$, 3 *d.f.*, $P = 0.1$). The gradual resolution of a sore throat is illustrated in Fig. 1.

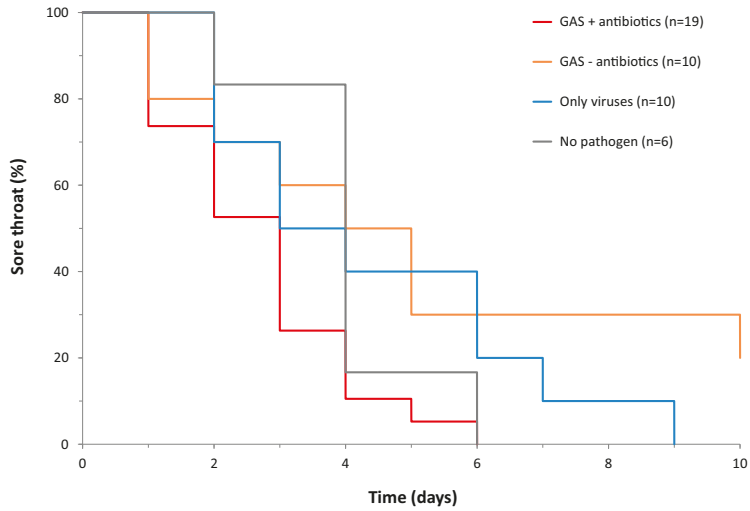

Fig. 1 Duration of a sore throat after a visit to a physician, as reported in symptom diaries of 55 children aged 0–14. *GAS* group A streptococci, with and without antibiotic treatment

Self-reported prevalence of a sore throat, fever, and absence from preschool or school at days 3 and 7 in the different groups are presented in Supplementary Table 1.

Three-month follow-up

All 77 patients and 34 controls were followed up after 3 months. Twelve patients (16%) had made return visits for a sore throat after a median of 25 days (IQR 18–53). None of the patients and controls had a complication and none were hospitalized.

Four of the twelve patients reported worsened or non-resolving symptoms, and they all had non-treated GAS at inclusion. The other eight patients reported a new episode, and five of these had GAS at inclusion, three of whom received antibiotics. None of the controls consulted for a sore throat during the follow-up.

Discussion

In this prospective observational study on pharyngotonsillitis in children presenting to primary healthcare, we found a high prevalence of bacteria and viruses in both patients (86%) and controls (71%). Bacteria were more common than viruses in both groups, and GAS was the most common pathogen. The observed differences in signs and symptoms between bacteria and viruses were not specific enough to be clinically useful. The fastest resolution of symptoms was seen in GAS patients treated with antibiotics. After 3 months, 16% of the patients had made return visits for a sore throat, but without a clear association to detected pathogens.

Strengths and weaknesses

To our knowledge, this is the first study on self-referred and unselected children with pharyngotonsillitis in primary healthcare that takes advantage of PCR to screen for a broad range of pathogens in both patients and controls and associates those findings with clinical symptoms and the course of the infection. Despite the lack of specific demographic data, we believe that this multicentre study is representative of children presenting to primary care with an acute sore throat. The data were collected in both urban and rural areas over three seasons, and the findings are reported by age strata to further increase their usefulness. Whether our findings can be replicated elsewhere depends on the epidemiological situation in those locations.

The low number of participants, especially controls, was less than we aimed for, and this could have introduced type II errors. Based on previous data, we expected to recruit a sufficient number of participants in one season, but failed to do so, mainly because the clinics were unable to provide enough resources. We also learned that children visiting for non-infectious causes are scarce, and they may not want to participate in a study while suffering from a sprained ankle or upset stomach. Aware of this limitation, we urge the reader to consider this an exploratory study.

Some methodological limitations need to be discussed. First, as we did not ask about fever previous to the visit, we might have missed important information, especially since

the proportion of children with fever at the clinic was lower than expected (possibly explained by uncalibrated thermometers, use of antipyretics, or many visits in the morning). Second, although a throat swab may be more convenient for children than a nasopharyngeal swab and better reflect the pathogens of a pharyngeal infection, this technique could be an inferior way to detect viruses and result in false negatives [17]. Regardless of technique, because aetiological tests only test for the specified microorganisms, we probably missed other pathogens. By adding biomarker tests, we might have been able to classify the infection as viral or bacterial [18]. Third, as many diaries were never returned, we should have used other ways to obtain the information and help the parents, for example, by offering web-based forms.

Interpretation

Group A streptococcus (GAS) was the most prevalent pathogen in both patients and controls, a finding in line with the previous reports [7]. However, the high carriage rate made us wonder if there had been an outbreak of GAS during the study period; analysis of the temporal variations revealed no such fluctuations (data not shown). Normally, GAS in children under 5 years old is less prevalent than in older children, but our study could only confirm this in the controls, not in the patients. Group C or G streptococci were only found in one patient but in four of 34 controls, a finding congruent with a large observational study that suggests both an increasing incidence with age and a likely carriage state in children [19].

Haemophilus influenzae, *S. aureus*, and *K. pneumoniae* were found in a quarter of patients, as well as in controls. Although these bacteria can be associated with disease in children, they are more likely to represent a colonization [20]. *M. catarrhalis*, another common bacterium in the nasopharyngeal microbiome of children, was never detected [5].

The anaerobic bacterium *Fusobacterium necrophorum* has been suggested as a possible pathogen in adolescents with pharyngitis [21–23]. We detected *F. necrophorum* in only one patient, aged 14 and with a concomitant finding of influenza B virus, and in one control, aged 3. These findings are in line with a previous report of a 2% prevalence in children under 15 years old [23].

The prevalence of viruses in our study was much lower than the prevalence of viruses from a previous study using PCR [18]. This unexpected finding could be the result of the sampling errors described above, age distribution differences between our study and the previous studies, and epidemiologic differences between our settings and the previous study's settings. Among children, viruses become less prevalent with age [18], and two-thirds of our patients were 6–14 years old.

Rhinovirus was the most prevalent virus in both patients and controls, which is congruent with studies using PCR [18, 24], while adenovirus, the most prevalent virus in older studies [9, 10, 25], was less common. In our study, parainfluenzavirus, metapneumovirus, and RSV were only found in patients, which supports the findings of a study on young children with acute respiratory infection [24].

The high rate of bacteria and viruses in asymptomatic children makes it difficult to interpret a positive finding in patients, as there is good reason to assume that they have similar carriage rates [16]. This is especially true for GAS [7], rhinoviruses [24, 26], and adenoviruses [18]. The fact that most findings in our study were single pathogens does not contradict the idea of a simultaneous carriage and infection, as we had no test for aetiological causality and no estimate of false-negative findings. Both rapid antigen tests and the Centor criteria are used to detect GAS, not to distinguish between infection and colonization.

While detection of microorganisms is insufficient for determining causality, measuring the host response may get us closer. Repeated testing for streptococcal antibodies could retrospectively determine a likely infection with GAS [27], but this will not help the clinician at the time of visit. C-reactive protein (CRP) and procalcitonin are biomarkers that have been suggested to distinguish bacterial from viral infections, but their usefulness lies in repeated measures in hospitalized patients, and have not been proven useful in diagnosing pharyngitis in adults [6]. Myxovirus resistance protein A (MxA) is a marker for viral infections, and a recent study found a clear association between elevated MxA levels and detection of viruses in children with febrile pharyngitis [18]. However, the differential diagnostic value for bacterial infection was poor, as an elevated MxA does not exclude a concomitant finding of GAS. Combining MxA with CRP could be a better approach, but this needs more evaluation [18]. Transcriptional profiling is another promising technique to differentiate viral detection from an active viral infection [26].

Rather than relying on biomarkers, the statistical method etiologic predictive value (EPV) considers asymptomatic carriage when interpreting an aetiological finding in patients [16]. Although this approach does not answer the question of causality, it does provide an important indication of the uncertainty. In our study, we found that the EPV of a GAS-positive culture was only 54%, no more than flipping a coin, and with an incredibly wide confidence interval due to the high carriage rate. Incidentally, a recent meta-analysis found that only 56% of children with GAS-positive had a serologically confirmed infection [28].

The large diagnostic uncertainty must also be weighed against the small clinical benefits of antibiotic treatment, the low risk of complications in untreated patients, and the adverse effects of antibiotics. Except for patients with severe

symptoms, no prescription or a back-up prescription could therefore be a better approach, which is in line with current guidelines [5, 6].

Our study adds to previous knowledge [9, 18, 29, 30] by noting that the clinical presentation for viruses and bacteria was very similar. Viral features like cough and coryza were less common in patients with GAS, but as GAS was highly prevalent, the positive predictive values for viruses for these symptoms were still low. No single symptom was specific enough for GAS or viruses to change the post-test probability to > 85%, a level of reasonable certainty that approaches the performance of a rapid antigen detection test [30]. Although pointing to difficulties in aetiological diagnosis in children with pharyngotonsillitis, we do not consider the results of this small descriptive study robust enough to change clinical guidelines.

Conclusion

With a high carriage rate of both viruses and bacteria among controls, it is likely that symptomatic patients also harbour these microorganisms alongside their active infection. Together with the low predictive values of signs and symptoms, this makes causal aetiological diagnosis in children with pharyngotonsillitis very challenging, even where rapid antigen detection tests are available. The development of a fast, specific, and cheap point-of-care marker for active infection would be of great value.

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Author contributions JP conceptualization, data curation, formal analysis, investigation, visualization, writing—original draft preparation, and writing—review and editing. MS conceptualization, formal analysis, and writing—review and editing. MR formal analysis, and writing—review and editing. PD conceptualization, investigation, and writing—review and editing. TN conceptualization, investigation, and writing—review and editing. SS investigation, writing—original draft preparation, and writing—review and editing. JS conceptualization, investigation, and writing—review and editing. KH conceptualization, formal analysis, funding acquisition, project administration, resources, supervision, and writing—review and editing.

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Availability of data and materials The datasets generated and analysed during the current study are not publicly available due to Swedish legislation (the Personal Data Act), but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval The study was approved by the regional ethics review board in Lund, Sweden: 2014/314, with one amendment: 2016/157.

Consent to participate Older children received verbal information about the study and, depending on reading ability, written information in a language adapted for children was provided. More detailed information was given to parents or legal guardians. The participants and their parents or legal guardians were given the opportunity to ask questions. Consent was obtained verbally from the child and in writing from parents or legal guardians. If only one parent or legal guardian was present, we asked that they inform the other person as soon as possible and contact us if he or she did not want to participate in the study. Participants could withdraw their consent at any time.

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Study IV

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RESEARCH Open Access

Association between bacterial finding, antibiotic treatment and clinical course in patients with pharyngotonsillitis: a registry-based study in primary healthcare in Sweden

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Abstract

Background: The role of non-group A streptococci and *Fusobacterium necrophorum* in pharyngotonsillitis has been disputed and few prospective studies have evaluated the effect of antibiotic treatment. This study uses registry data to investigate the relation between antibiotic prescription for pharyngotonsillitis in primary healthcare and return visits for pharyngotonsillitis, complications, and tonsillectomy.

Methods: Retrospective data were extracted from the regional electronic medical record system in Kronoberg County, Sweden, for all patients diagnosed with pharyngotonsillitis between 2012 and 2016. From these data, two cohorts were formed: one based on rapid antigen detection tests (RADT) for group A streptococci (GAS) and one based on routine throat cultures for β-haemolytic streptococci and *F. necrophorum*. The 90 days following the inclusion visit were assessed for new visits for pharyngotonsillitis, complications, and tonsillectomy, and related to bacterial aetiology and antibiotic prescriptions given at inclusion.

Results: In the RADT cohort (n = 13,781), antibiotic prescription for patients with a positive RADT for GAS was associated with fewer return visits for pharyngotonsillitis within 30 days compared with no prescription (8.7% vs. 12%; $p = 0.02$), but not with the complication rate within 30 days (1.5% vs. 1.8%; $p = 0.7$) or with the tonsillectomy rate within 90 days (0.27% vs. 0.26%; $p = 1$). In contrast, antibiotic prescription for patients with a negative RADT was associated with more return visits for pharyngotonsillitis within 30 days (9.7% vs. 7.0%; $p = 0.01$). In the culture cohort (n = 1 370), antibiotic prescription for patients with *Streptococcus dysgalactiae* ssp. *equisimilis* was associated with fewer return visits for pharyngotonsillitis within 30 days compared with no prescription (15% vs. 29%; $p = 0.03$).

Conclusions: Antibiotic prescription was associated with fewer return visits for pharyngotonsillitis in patients with a positive RADT for GAS but with more return visits in patients with a negative RADT for GAS. There were no differences in purulent complications related to antibiotic prescription.

Keywords: Pharyngotonsillitis, *Fusobacterium necrophorum*, Group A streptococci, Aetiology, Primary healthcare, *Streptococcus dysgalactiae* subspecies *equisimilis*

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Background

Infectious pharyngotonsillitis can be caused by a wide array of viruses and bacteria, of which *Streptococcus pyogenes* (group A streptococci, GAS) is the most important pathogen and the only one that warrants antibiotic treatment according to most guidelines [1–4]. The indication of antibiotic therapy, however, is confined to reducing symptoms as non-purulent complications of GAS such as rheumatic fever and glomerulonephritis are rare in high-income countries [3] and purulent complications such as peritonsillitis, sinusitis, and media otitis occur in less than 1% of patients [5].

The Sore Throat Guideline Group within the European Society for Clinical Microbiology and Infectious Diseases advocates using the Centor scoring system (one point each for fever, cervical lymphadenitis, tonsillar coating, and absence of cough) [6] to select patients with a higher likelihood of GAS infection (i.e., 3–4 criteria) and considering using a Rapid Antigen Detection Test (RADT) for these patients [3]. Throat cultures are not necessary for routine diagnosis of GAS nor after a negative RADT [3]. Penicillin V, twice or three times daily for 10 days, is the recommended treatment of GAS [3], but should be avoided in patients with Centor score 0–2 as these patients do not seem to benefit from antibiotics [3]. The Swedish Medical Products Agency has adopted this guideline for the most part but stresses that an RADT should only be performed in patients with Centor scores 3–4 as these are the patients who could benefit from antibiotic treatment [1].

In addition to GAS, *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE), formerly described as large colony group C or G streptococci in the Lancefield classification system [7], has been detected in 9 to 15% of young adults with pharyngotonsillitis [8–10], and the anaerobe *Fusobacterium necrophorum* has been detected in 18–19% of patients with pharyngotonsillitis in primary healthcare (PHC) [11, 12]. Both bacteria, however, are also recovered from healthy controls, and their roles as pathogens in pharyngotonsillitis are still disputed [10–14]. *F. necrophorum*, the main pathogen causing the severe but unusual Lemierre's syndrome [15], has been associated with peritonsillar abscesses [16] and several case reports have described complications following pharyngotonsillitis associated with group C and group G streptococci [3]. Most cases of peritonsillitis, however, are not preceded by a recorded pharyngotonsillitis [17] and few prospective studies have approximated the incidence of complications after an episode of pharyngotonsillitis. Furthermore, no randomised controlled study has shown that antibiotic treatment of pharyngotonsillitis caused by SDSE or *F. necrophorum* lowers the complication rate [10, 12].

This study uses registry data to prospectively follow patients with a PHC-recorded pharyngotonsillitis for 90 days and to quantify the incidence of new visits for pharyngotonsillitis, complications, and tonsillectomy in relation to initial aetiology and antibiotic prescription.

Methods

Study population and setting

This study was conducted in Kronoberg County in southern Sweden. The Swedish healthcare system is mainly tax-funded and is equally accessible to all inhabitants, with the services decentralised to 21 regional councils. PHC is provided by approximately 1 200 PHC centres (PHCC) dispersed throughout the country. People are encouraged to contact their PHCC before seeking emergency care at hospitals. Therefore, sore throat and other respiratory infections are usually managed by the PHCC.

During the study period (2012–16), the median population in Kronoberg County was 189 292, about 2% of the Swedish population. This population was served by two hospitals and 34 PHCCs, 31 of which participated in the study. The PHCCs were generally open between 08:00 and 17:00, and two out-of-hours centres also served patients between 17:00 and 21:00. In most cases, patients were first assessed over the telephone by a triage nurse, who decided if a physician's visit was necessary. All visits with a physician required that the physician register a diagnosis code according to the 10th revision of the *International Statistical Classification of Diseases and Related Health Problems* (ICD-10) or its modified Swedish PHC edition (KSH97-P) [18].

This paper is reported following the STROBE statement [19] and the RECORD statement [20].

Data extraction

Retrospective data for the years 2012–16 were extracted from the regional electronic medical record (EMR) system (Cambio Cosmic, Cambio Healthcare Systems, Linköping, Sweden) and the laboratory information system (ADBAKT, Autonik, Nyköping, Sweden). The data extraction was performed in four steps.

In the first step, all patients were identified who received a diagnosis code for pharyngotonsillitis (J02 or J03) from a PHCC or hospital clinic physician during the study period (Step 1, Fig. 2). Data regarding age, sex, RADTs, throat cultures, and antibiotic prescriptions from PHCCs and hospital clinics were then extracted and linked using the Swedish personal identification number and visit date. As the indication for antibiotic treatment could not be extracted from the EMR system, the following antibiotics relevant for treating pharyngotonsillitis in accordance with Swedish guidelines [1] were identified: phenoxymethylpenicillin (penicillin V), cefadroxil, and

clindamycin. In addition, amoxicillin, erythromycin, and azithromycin were included as they are approved by the Swedish Medical Products Agency for treating pharyngotonsillitis. However, data were unavailable that would confirm whether patients collected their medication at a pharmacy or complied with prescribed treatment regime.

In the second step, patients who had at least one eligible visit to a PHCC with aetiological testing (see below) were selected (Step 2, Fig. 2). Five exclusion criteria for a visit were used: (1) visit date during the first 30 days of the study period; (2) a diagnosed pharyngotonsillitis or complication (defined as peritonsillitis, media otitis, sinusitis, lymphadenitis or sepsis, see Additional file 1: Table S1) the previous 30 days; (3) antibiotic prescription (as defined above) the previous 30 days; (4) a complication diagnosed on the same day as the visit; and (5) prescription of an antibiotic not indicated for a sore throat (Fig. 2). Aetiological testing was defined as an RADT for GAS performed on the same date as the visit or a throat culture performed within seven days. RADTs performed on the first day and cultures performed within a week were included because this routine mirrors clinical practice. Early descriptive analysis also revealed that most cultures were performed on the same day as the index visit, and an absolute majority within 7 days.

In the third step, a cohort (cohort 1) was formed with all patients from step 2 where an RADT had been performed (Step 3, Fig. 2). The first eligible visit for each patient was denoted as the index visit.

In the fourth step, using the same patients as in step 2, a new, explanatory cohort was created (cohort 2), with all patients who had been cultured (step 4, Fig. 2). As before, the first eligible visit with a culture was denoted as the index visit. As most patients had an RADT performed before they were cultured, many patients in cohort 2 were also in cohort 1, with common index visit dates.

In both cohorts, patients were grouped by antibiotic prescription on the day of their index visit, as early descriptive analysis revealed that most patients with a prescription were prescribed antibiotics during their index visit, whereas subsequent prescriptions were often made during a new visit, which we wanted to count as an outcome.

Although the main criteria for inclusion in this study was a visit to a PHCC, the outcomes were defined as a visit to either a PHCC or a hospital clinic (Fig. 1). This was especially important for tonsillectomy, as it is never coded for in PHC, as well as for peritonsillitis, as these patients sometimes visit an emergency department at a hospital without first visiting a PHCC. If a patient had separate index visit dates for the two cohorts, the exclusion criteria made sure that no index visit would be registered as an outcome in the other cohort.

Microbiological procedures

The RADT kit for GAS used in Kronoberg County during the study period was QuickVue Dipstick Strep A (Quidel Corporation, San Diego, CA, USA), a lateral-flow immunoassay using antibody-labelled particles [21]. The test detects viable and nonviable organisms directly from throat swabs.

Routine throat cultures for the recovery of large colony β-haemolytic streptococci used standard procedures, as previously described [9]. Starting in 2013, the laboratory

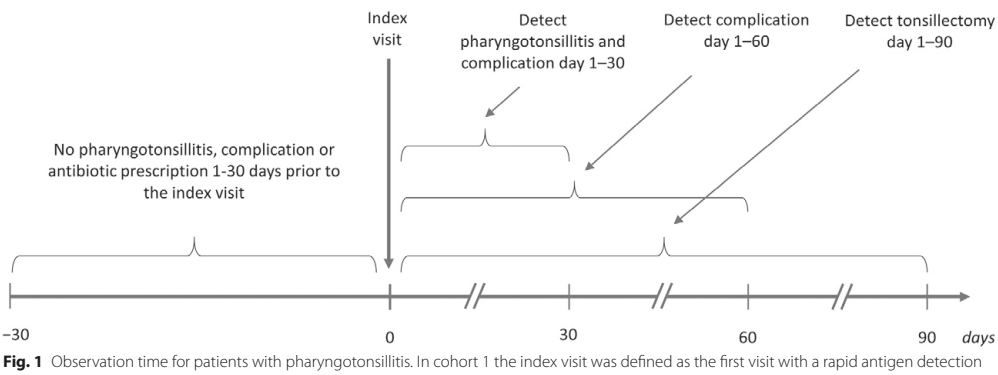

Fig. 1 Observation time for patients with pharyngotonsillitis. In cohort 1 the index visit was defined as the first visit with a rapid antigen detection test (RADT) for group A streptococci (GAS) performed on the same day. In cohort 2, the index visit was defined as the first visit where a throat culture was performed within seven days. For a visit to be eligible there should neither be a visit for pharyngotonsillitis or a complication nor an antibiotic prescription during the last 30 days. In the follow-up, each patient was assessed for new visits for pharyngotonsillitis, a complication, and tonsillectomy up to 90 days from the index visit

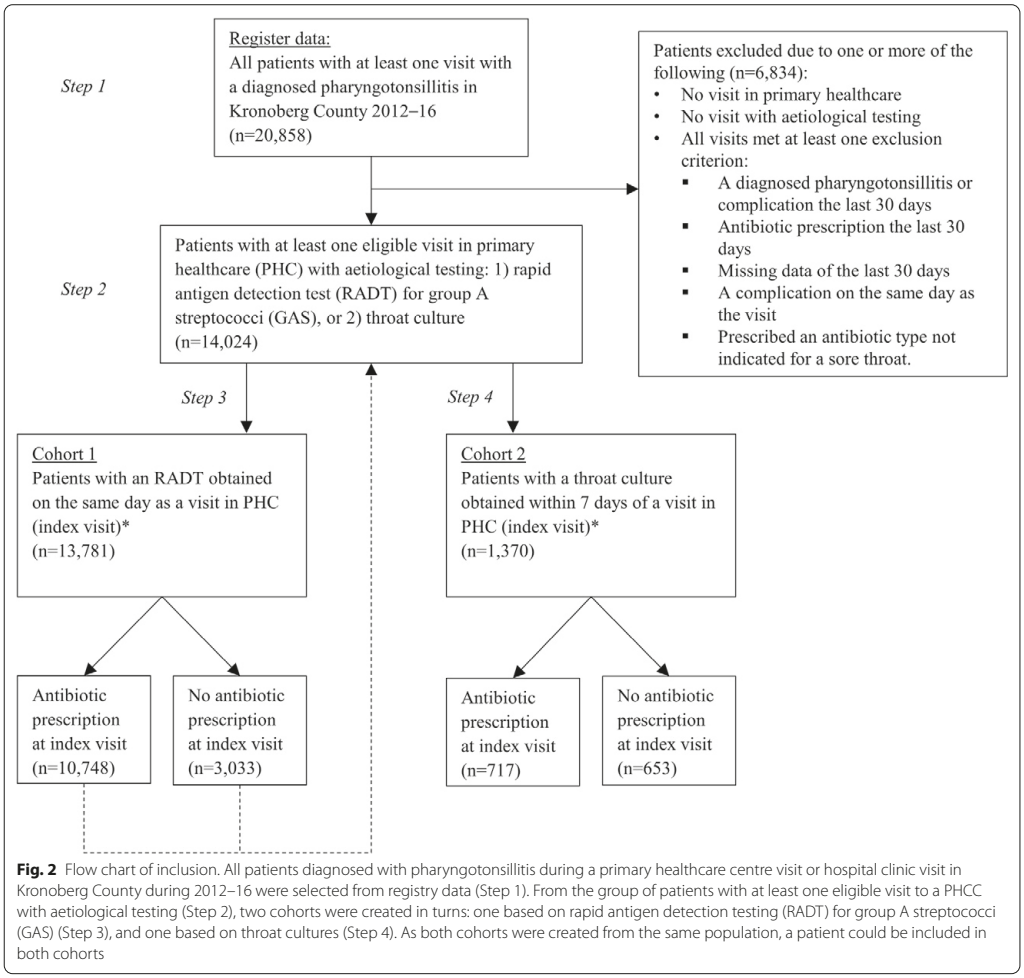

Fig. 2 Flow chart of inclusion. All patients diagnosed with pharyngotonsillitis during a primary healthcare centre visit or hospital clinic visit in Kronoberg County during 2012–16 were selected from registry data (Step 1). From the group of patients with at least one eligible visit to a PHCC with aetiological testing (Step 2), two cohorts were created in turns: one based on rapid antigen detection testing (RADT) for group A streptococci (GAS) (Step 3), and one based on throat cultures (Step 4). As both cohorts were created from the same population, a patient could be included in both cohorts

also offered an extended throat culture that added an anaerobic plate for the recovery of *F. necrophorum* [9]. In late 2013, with the introduction of matrix-assisted laser desorption/ionization with time-of-flight mass spectrometer (MALDI-TOF), the reporting of streptococci transitioned from Lancefield classification to species identification. As a result, GAS was reported as *S. pyogenes* and most group C and G streptococci were reported as SDSE. In this study, group C or G streptococci were reported as SDSE. During the study period, before the transition, group C and G streptococci constituted 40% of all β-haemolytic streptococci in throat cultures; after the transition, the corresponding proportion for SDSE was 42% (data not shown).

Statistical methods

Data were cleaned and analysed using Excel 2019 (Microsoft, Redmond, WA, USA) and SPSS 25.0 software (IBM, Armonk, NY, USA). Continuous variables with non-normal distribution or small sample sizes were reported as median (interquartile range, IQR). Categorical data were compared with two-sided Pearson χ^2-test or Fisher's exact test for independent groups, and McNemar test or

Cochran's Q test for dependent groups. A p-value < 0.05 was considered significant.

Results

* For patients with multiple eligible visits, the first visit was denoted index visit. Due to double aetiological testing or multiple eligible visits, 1 127 patients were included in both cohorts.

Study population

Between 2012 and 2016, 20,858 patients were diagnosed with pharyngotonsillitis during at least one PHCC or hospital clinic visit. Of these, 14,024 had at least one eligible visit to a PHCC with aetiological testing, and from these patients two cohorts were formed (Fig. 2). Most index visits in cohort 1 and 2 took place during office hours (84% and 88%, respectively).

Aetiology

In cohort 1, the RADT was positive for GAS in 9 170 patients (67%). In cohort 2, a regular culture was performed in 745 (54%) patients and an extended culture was performed in 625 (46%) patients. Of the 1 370 cultures registered within seven days of the index visit, 1 128 (82%) were performed on the same day as the index visit (Additional file 1: Table S2). Bacterial growth was found in 231 (31%) of the regular cultures and 234 (37%) of the extended cultures. Overall, GAS was detected in 201 (15%) patients and SDSE in 190 (14%) patients. *F. necrophorum* was detected in 95 (15%) patients who had extended cultures.

Characteristics in relation to aetiology

GAS was most prevalent in children aged 0–14, with 80% of RADTs positive, whereas SDSE and *F. necrophorum* were most prevalent in patients aged 15–29. Table 1 lists the background characteristics of the patients in relation to aetiology.

Frequency of outcomes

In the RADT cohort, 8.6% of the patients made a new visit for pharyngotonsillitis within 30 days (median = 12 days, IQR 4–16) and 1.6% made a new visit for a complication within 30 days (median = 12 days, IQR 3–21). Peritonsillitis accounted for 29% of these complications (median = 3 days, IQR 2–14) (Additional file 1: Table S3).

In the culture cohort, 20% of the patients made a new visit for pharyngotonsillitis within 30 days (median = 3 days, IQR 2–6) and 3.8% made a new visit for a complication within 30 days (median = 3 days, IQR

Table 1 Characteristics of patients who performed a RADT for GAS or a throat culture

	RADT for GAS (cohort 1)			Throat culture (cohort 2)				
	Positive n = 9170	**Negative n = 4611**	**All n = 13,781**	***S. pyogenes*[1] n = 201**	***S. dysgalactiae* ssp. *equisimilis*[1] n = 190**	***F. necrophorum*[1,2] n = 95**	**Negative n = 905**	**All[2] n = 1 370**
Female, n (%)	5 068 (55)	2 585 (56)	7 653 (56)	111 (55)	119 (63)	57 (60)	509 (56)	785 (57)
Age, years, median (IQR)	19 (7–36)	23 (16–38)	21 (9–37)	27 (12–38)	20 (17–29)	21 (17–26)	23 (17–38)	23 (17–36)
Age 0–14, n (%)	4 056 (44)	1 024 (22)	5 080 (37)	64 (32)	24 (13)	2 (2.1)	146 (16)	236 (17)
Age 15–29, n (%)	1 882 (21)	1 928 (42)	3 810 (28)	45 (22)	119 (63)	75 (79)	422 (47)	646 (47)
Age 30 + , n (%)	3 232 (35)	1 659 (36)	4 891 (35)	92 (46)	47 (25)	18 (19)	337 (37)	488 (36)
RADT performed (cohort 2), n (%)				139 (69)	154 (81)	73 (77)	660 (73)	1 011 (74)
RADT positive/all RADT, n (%)				104 (75)	6 (3.9)	6 (8.2)	83 (9.2)	196 (19)
Antibiotic treatment[3], n (%)	8 751 (95)	1 997 (43)	10 748 (78)	151 (75)	97 (51)	52 (55)	429 (47)	717 (52)
Penicillin V, n (% of treated)	7 894 (90)	1 630 (82)	9524 (89)	106 (70)	73 (75)	34 (65)	324 (76)	527 (74)
Clindamycin, n (% of treated)	339 (3.9)	181 (9.1)	520 (4.8)	21 (14)	14 (14)	17 (33)	73 (17)	123 (17)
Cefadroxil, n (% of treated)	345 (3.9)	116 (5.8)	461 (4.3)	20 (13)	8 (8.2)	0	24 (5.6)	52 (7.3)
Other, n (% of treated)	173 (2.0)	70 (3.5)	243 (2.3)	4 (2.6)	2 (2.1)	1 (1.9)	8 (1.9)	15 (2.1)

Characteristics of patients who had a RADT for GAS performed on the same day as a visit to a primary healthcare centre or a culture performed to determine aetiology within seven days of a visit to a primary healthcare centre, in relation to aetiology

RADT Rapid Antigen Detection Test; *GAS* Group A streptococci (*S. pyogenes*)

[1] Refers to any finding (21 patients had a concomitant finding of two bacteria)

[2] To detect *F. necrophorum*, an extended culture was needed (see Methods section). In total, 625/1 370 (46%) of the patients had an extended culture

[3] Refers to antibiotics approved by the Swedish Medical Products Agency for treating pharyngotonsillitis (see Methods section) prescribed on the same day as the index visit. Antibiotic types are expressed as percentages of treated patients

Table 2 Rapid Antigen Detection Test (RADT) for GAS result and antibiotic prescription in relation to outcomes

RADT for GAS[1]	Antibiotics[2]	Pharyngotonsillitis	Complication		Peritonsillitis[3]		Tonsillectomy
		30 d	30 d	60 d	30 d	60 d	90 d
Positive	All	791/8928 (8.9%)	136/8928 (1.5%)	211/8728 (2.4%)	33/8928 (0.37%)	37/8728 (0.42%)	23/8561 (0.27%)
	Antibiotics +	743/8528 (8.7%)	129/8528 (1.5%)	199/8338 (2.4%)	30/8528 (0.35%)	34/8338 (0.41%)	22/8182 (0.27%)
	Antibiotics −	48/400 (12%)	7/400 (1.8%)	12/390 (3.1%)	3/400 (0.75%)	3/390 (0.77%)	1/379 (0.26%)
	p	0.02	0.7	0.4	0.2†	0.2†	1†
Negative	All	369/4532 (8.1%)	78/4532 (1.7%)	104/4479 (2.3%)	30/4532 (0.66%)	34/4479 (0.76%)	13/4426 (0.29%)
	Antibiotics +	190/1965 (9.7%)	32/1965 (1.6%)	43/1939 (2.2%)	16/1965 (0.81%)	19/1939 (0.98%)	6/1918 (0.31%)
	Antibiotics −	179/2567 (7.0%)	46/2567 (1.8%)	61/2540 (2.4%)	14/2567 (0.55%)	15/2540 (0.59%)	7/2508 (0.28%)
	p	0.01	0.7	0.7	0.3	0.1	0.8

Rapid antigen detection test (RADT) for group A streptococci (GAS) and antibiotic prescription in relation to outcomes in patients where an RADT was performed on the same day as a visit to primary healthcare (n = 13 781)

† Fisher's exact test

[1] Refers to RADTs performed on the same day as the index visit

[2] Refers to antibiotics approved by the Swedish Medical Products Agency for treating pharyngotonsillitis (see Methods section) prescribed on the same day as the index visit

[3] Patients with peritonsillitis are also included in "Complication"

2–5). Peritonsillitis accounted for 78% of these complications (median = 2 days, IQR 2–5). Of the 51 patients with a complication, 61% were cultured on the same day as the

Table 3 Throat culture result and antibiotic prescription in relation to outcomes

Throat culture result[1]	Antibiotics[2]	Pharyngotonsillitis	Complication		Peritonsillitis[3]		Tonsillectomy
		30 d	30 d	60 d	30 d	60 d	90 d
S. pyogenes	All	30/190 (16%)	1/190 (0.53%)	3/188 (1.6%)	1/190 (0.53%)	1/188 (0.53%)	0/185
	Antibiotics +	21/143 (15%)	1/143 (0.70%)	2/142 (1.4%)	1/143 (0.70%)	1/142 (0.7%)	0/140
	Antibiotics −	9/47 (19%)	0/47	1/46 (2.2%)	0/47	0/46 (0%)	0/45
	p	0.5	1†	0.6†	1†	1†	-
S. dysgalactiae ssp. *equisimilis*[4]	All	37/171 (22%)	2/171 (1.2%)	2/170 (1.2%)	2/171 (1.2%)	2/170 (1.2%)	0/168
	Antibiotics +	13/87 (15%)	2/87 (2.3%)	2/86 (2.3%)	2/87 (2.3%)	2/86 (2.3%)	0/85
	Antibiotics −	24/84 (29%)	0/84 (0%)	0/84 (0%)	0/84 (0%)	0/84 (0%)	0/83
	p	0.03	0.5†	0.5†	0.5†	0.5†	-
F. necrophorum[5]	All	16/75 (21%)	9/75 (12%)	10/75 (13%)	8/75 (11%)	9/75 (12%)	4/72 (5.6%)
	Antibiotics +	11/41 (27%)	3/41 (7.3%)	4/41 (9.8%)	2/41 (4.9%)	3/41 (7.3%)	1/38 (2.6%)
	Antibiotics −	5/34 (15%)	6/34 (18%)	6/34 (18%)	6/34 (18%)	6/34 (18%)	3/34 (8.8%)
	p	0.2	0.3†	0.5†	0.1†	0.3†	0.3†
Negative (extended cultures only)	All	89/381 (23%)	22/381 (5.8%)	23/370 (6.2%)	19/381 (5.0%)	19/370 (5.1%)	9/363 (2.5%)
	Antibiotics +	56/194 (29%)	16/194 (8.2%)	17/188 (9.0%)	15/194 (7.7%)	15/188 (8.0%)	4/184 (2.2%)
	Antibiotics −	33/187 (18%)	6/187 (3.2%)	6/182 (3.3%)	4/187 (2.1%)	4/182 (2.2%)	5/179 (2.8%)
	p	0.01	0.04	0.02	0.01	< 0.001	0.8

Antibiotic prescription and results from throat cultures performed within seven days from the index visit for pharyngotonsillitis in relation to outcomes (n=1 370)

† Fisher's exact test

[1] Refers to findings of single pathogens within seven days of the index visit

[2] Refers to antibiotics approved by the Swedish Medical Products Agency for treating pharyngotonsillitis (see Methods section) prescribed on the same day as the index visit

[3] All cases with peritonsillitis are also included in "Complication"

[4] Before 2013, *S. dysgalactiae* ssp. *equisimilis* was reported as either group C or G streptococci, which is detailed in the Methods section

[5] To detect *F. necrophorum*, an extended culture was needed (see Methods section). In total, 625/1 370 (46%) of the patients had an extended culture

Table 4 Aetiological test results and antibiotic choice in relation to outcomes

Aetiological test	Antibiotics[1]	Pharyngotonsillitis	Complication		Peritonsillitis[2]		Tonsillectomy
		30 d	30 d	60 d	30 d	60 d	90 d
RADT for GAS							
Positive	PcV	652/7685 (8.5%)	108/7685 (1.4%)	169/7504 (2.3%)	23/7685 (0.30%)	27/7504 (0.36%)	16/7358 (0.22%)
	Clindamycin	37/333 (11%)	10/333 (3.0%)	14/331 (4.2%)	4/333 (1.2%)	4/331 (1.2%)	3/328 (0.91%)
	Cefadroxil	31/340 (9.1%)	5/340 (1.5%)	7/335 (2.1%)	2/340 (0.59%)	2/335 (0.60%)	1/331 (0.30%)
	p	0.2	0.07†	0.06	0.02†	0.04†	0.04†
Negative	PcV	145/1604 (9.0%)	25/1604 (1.6%)	33/1582 (2.1%)	10/1604 (0.62%)	13/1582 (0.82%)	3/1564 (0.19%)
	Clindamycin	25/178 (14%)	5/178 (2.8%)	6/176 (3.4%)	5/178 (2.8%)	5/176 (2.8%)	2/174 (1.1%)
	Cefadroxil	13/115 (11%)	1/115 (0.87%)	2/115 (1.7%)	0/115	0/115	1/115 (0.87%)
	p	0.08	0.4†	0.4†	0.02†	0.046†	0.052†
Throat culture[3]							
S. pyogenes	PcV	15/101 (15%)	1/101 (0.99%)	1/100 (1.0%)	1/101 (0.99%)	1/100 (1.0%)	0/98
	Clindamycin	3/19 (16%)	0/19	0/19	0/19	0/19	0/19
	Cefadroxil	3/19 (16%)	0/19	1/19 (5.3%)	0/19	0/19	0/19
	p	1†	1†	0.5†	1†	1†	-
S. dysgalactiae ssp. *equisimilis*[4]	PcV	10/64 (16%)	2/64 (3.1%)	2/63 (3.2%)	2/64 (3.1%)	2/63 (3.2%)	0/62
	Clindamycin	3/13 (23%)	0/13	0/13	0/13	0/13	0/13
	Cefadroxil	0/8	0/8	0/8	0/8	0/8	0/8
	p	0.5†	1†	1†	1†	1†	-
F. necrophorum[5]	PcV	7/25 (28%)	1/25 (4.0%)	2/25 (8.0%)	0/25	1/25 (4.0%)	1/22 (4.5%)
	Clindamycin	4/15 (27%)	2/15 (13%)	2/15 (13%)	2/15 (13%)	2/15 (13%)	0/15
	Cefadroxil	0/0	0/0	0/0	0/0	0/0	0/0
	p	1†	0.6†	0.6†	0.1†	0.6†	1†
Negative	PcV	83/318 (26%)	20/318 (6.3%)	23/311 (7.4%)	17/318 (5.3%)	18/311 (5.8%)	4/307 (1.3%)
	Clindamycin	17/70 (24%)	6/70 (8.6%)	7/70 (10%)	6/70 (8.6%)	6/70 (8.6%)	2/69 (2.9%)
	Cefadroxil	9/24 (38%)	0/24	0/24	0/24	0/24	0/23
	p	0.4	0.4†	0.3†	0.3†	0.4†	0.5†

† Fisher's exact test; *RADT* Rapid Antigen Detection Test; *GAS* Group A Streptococci

[1] Antibiotics prescribed on the same day as the index visit. In Sweden, PcV is the recommended antibiotic for pharyngotonsillitis and Clindamycin and Cefadroxil are alternatives. All antibiotics are recommended for ten days of treatment

[2] All cases with peritonsillitis are also included in "Complication"

[3] Refers to findings of single pathogens within seven days of the index visit

[4] Before 2013, *S. dysgalactiae* ssp. *equisimilis* was reported as either group C or G streptococci, which is detailed in the Methods section

[5] To detect *F. necrophorum*, an extended culture was needed (see Methods section). In total, 625/1 370 (46%) of the patients had an extended culture

complication, 35% were cultured at least one day before the complication, and two were cultured later.

Antibiotics and outcomes

In the RADT cohort, in patients with a positive RADT pharyngotonsillitis within 30 days was less common in those who were prescribed antibiotics (8.7%; 95% CI 8.1–9.3%) than in those who were not prescribed antibiotics (12%; 95% CI 9.2–16%) (Table 2). In contrast, antibiotic prescription for patients with a negative RADT was associated with a higher proportion of pharyngotonsillitis within 30 days (9.7%; 95% CI 8.4–11%) compared to patients with no prescription (7.0%; 95% CI 6.1–8.0%). Antibiotic prescription was not associated with complication rates or tonsillectomy rates regardless of RADT result.

In the culture cohort, antibiotics were prescribed to 717 (52%) patients on the same day as the index visit and to another 159 (12%) patients during the following seven days (Additional file 1: Table S4). Only 106 (7.7%) patients were prescribed an antibiotic before a sample for culture was obtained. In patients with SDSE antibiotic prescription was associated with a lower proportion of pharyngotonsillitis within 30 days compared with no prescription (Table 3). In contrast, in patients with a negative culture antibiotic prescription was associated with a larger proportion of pharyngotonsillitis and peritonsillitis within 30 days, compared with no prescription.

In the RADT cohort, the proportion of peritonsillitis within 30 days differed with antibiotic chosen both in patients with a positive RADT and a negative RADT, with the lowest proportions among patients who were prescribed penicillin V (Table 4). In the culture cohort, antibiotic type was not associated with the outcomes.

Discussion

In this registry-based study of patients diagnosed with pharyngotonsillitis at a visit in primary healthcare, antibiotic prescription was associated with a lower proportion of return visits for pharyngotonsillitis in patients with a positive RADT for GAS but with a higher proportion of return visits in patients with a negative RADT for GAS. Regardless of test result, antibiotic prescription was not associated with a reduced incidence of purulent complications.

Meaning of the study

With RADTs being positive in 67% of tested patients (i.e. 47% of the whole population studied), GAS was the most common aetiology in our material. This proportion is much higher than expected from prevalence studies [3, 9, 22] and probably points to a classification bias, where the choice of diagnosis codes might have been affected by the test result. In throat cultures, all detected bacteria were equally common, but this finding is hard to interpret as the reason for obtaining a sample for culture (e.g. a more severe clinical presentation) is unknown. Moreover, a positive RADT should reduce the diagnostic necessity of a culture, so the true prevalence of GAS could be underestimated. The prevalence (15%) of *F. necrophorum* in prolonged anaerobic culture suggests that a similar proportion of routine cultures for streptococci might also harbour *F. necrophorum*. Certainly, the clinical presentation could have led to a selection bias of extended cultures; however, recent meta-analyses have reported a 18–19% prevalence of *F. necrophorum* in patients with a sore throat diagnosed in PHC [e, 12]. In our study, *F. necrophorum* and SDSE were most prevalent among patients aged 15–29. This finding is in line with previous studies: a low prevalence of *F. necrophorum* and SDSE in children and the highest prevalence in adolescents and young adults [8–11, 23]. Conversely, GAS was most prevalent in children, which probably reflects a large proportion of carriage in this age group [22, 24].

There was an association between antibiotic prescription and fewer return visits for pharyngotonsillitis in patients with a positive RADT, suggesting a protective role for antibiotics. This finding contrasts with previous findings of increased re-attendance in patients prescribed an immediate antibiotic due to changed expectations and behaviour (i.e., "medicalisation") [25–27]. On the other hand, antibiotics were associated with a higher rate of return visits for pharyngotonsillitis in patients with a negative RADT, which may suggest that the treatment was not effective for this group or that there was a medicalising effect.

Although the culture cohort only constituted a small proportion of all patients, there was an association between antibiotic prescription and fewer return visits for pharyngotonsillitis in patients with SDSE, suggesting a protective role of antibiotics in a subset of the patients with a negative RADT. Surprisingly, patients with negative cultures and antibiotic prescription had a higher incidence of all outcomes measured regardless of the antibiotic used for treatment. Our first thought was that most of these patients had initiated antibiotic treatment before being cultured, but this was only the case in 10% of the patients. Other explanations might be medicalisation, ineffective antibiotics, and confounding by indication (i.e., patients with more severe illness are more likely to receive antibiotics) [28].

Antibiotic prescription was not associated with fewer complications in any cohort. However, complications, especially peritonsillitis, are rare outcomes, and since 95% of the patients with a positive RADT were prescribed antibiotics, the comparison group was rather small. The actual numbers did point to a protective role for antibiotics for complications in patients with a positive RADT (Table 2), but there might have been too few cases to detect a significant difference. The small numbers were also evident in the culture cohort as almost none of the comparisons, no matter how large the difference, were statistically significant. The complication rate in this study was similar to a previous registry study in PHC [29] but lower than the average in randomised controlled trials [5]. Previous studies on the protective role of antibiotics are somewhat conflicting, with the limited trial evidence suggesting a lowered relative risk (RR = 0.10, 95% CI = 0.01–0.79, in studies conducted after the 1950s) [5], but large recent observational studies suggest either no protective role [17, 29] or a very small absolute risk reduction with a huge Number needed to treat (NNT) [30, 31].

Most patients who developed peritonsillitis were diagnosed within a few days after inclusion, with a median of three days in the RADT cohort and two days in the culture cohort. In the long-term follow-up, almost no new cases emerged between 30 and 60 days. These findings are consistent with previous reports of a very fast onset of peritonsillitis [17, 29, 32, 33], suggesting that some of the cases of peritonsillitis might already have been imminent or misdiagnosed as pharyngotonsillitis at inclusion.

Most treated patients received penicillin V, but the overall picture was that the antibiotic chosen was

unrelated to the outcomes, a finding in line with a previous meta-analysis [34]. The exception was peritonsillitis and tonsillectomy in the RADT cohort, where penicillin V was associated with fewer cases both in patients with a positive RADT and in patients with a negative RADT. In Sweden, patients with recurring pharyngotonsillitis are generally required to have tried three types of antibiotics before being eligible for tonsillectomy; therefore, clindamycin and cefadroxil, which are second-choice antibiotics, might be associated with complications and tonsillectomy more than penicillin V.

Strengths and weaknesses of the study

To our knowledge, this is the largest registry-based study investigating pharyngotonsillitis in PHC, with almost complete data on all recorded diagnoses of pharyngotonsillitis, complications and tonsillectomies for five years, from PHC (office hours and out-of-hours) and hospital clinics. In addition, this is the first study to present data on patients who had *F. necrophorum* detected in routine cultures. Unlike case–control studies and case reports, this study followed a cohort of patients with pharyngotonsillitis prospectively to estimate the incidence of outcomes. As no randomised controlled trial has been sufficiently sized to study the effect of antibiotics on non-group A streptococci and *F. necrophorum* in patients with pharyngotonsillitis, this study offers valuable observational data.

However, a registry study comes with inherent weaknesses. For example, we did not know the clinical circumstances of the patients (e.g. severity and duration of symptoms, patients' expectations, and physicians' intentions with tests and antibiotic prescription), differential diagnostic reasoning, and inter-rater reliability in terms of diagnostic skills and coding. Therefore, all results are based on the factual codes, test results, and prescriptions registered in the EMR system, a circumstance that calls for a cautious interpretation of the results. On the other hand, this study is based on a large quantity of real-life clinical data from PHC, mirroring both the disease panorama and the behaviour of physicians, nurses, and patients rather than on experimental trial data on a small, selected, and closely monitored population.

The definition of pharyngotonsillitis was confined to the applicable codes in ICD-10 (J02.x and J03.x) although we know from clinical experience and previous studies [35] that sore throats are sometimes coded as "upper respiratory infection" or "viral infection", especially if the patient has compelling viral symptoms. We made this choice because sore throat as a symptom does not lend itself to registry-based studies, and other codes encompass too many conditions to be useful. Narrowing in on ICD codes for pharyngotonsillitis, however, might have selected a population with a higher likelihood to benefit from antibiotics.

Excluding patients with a diagnosed complication on the same day might have underestimated the complication rate of certain bacteria. However, the primary aim was not to establish a link between aetiology and complications but to follow patients with a pharyngotonsillitis in PHC and study the effect of antibiotic prescription on different outcomes. Another study, focusing on complications, especially peritonsillitis, is nonetheless fully possible with this database and already in the planning.

Unanswered questions and future research

To better appreciate the effect of antibiotic treatment on resolution of symptoms, relapses, and complications in patients with non-group A streptococcal bacterial aetiology, a sufficiently sized randomised controlled trial is warranted. As regular penicillin V was found to be non-inferior to clindamycin and cefadroxil in this study, it might then be an interesting candidate to investigate further. The prevalence of throat cultures was low in our material, and any subsequent registry study on this topic will need to consider this in sizing calculations.

Conclusions

Antibiotic prescription was associated with a lower proportion of return visits for pharyngotonsillitis in patients with a positive RADT for GAS but with a higher proportion of return visits in patients with a negative RADT. Antibiotic prescription was not associated with a reduced incidence of purulent complications regardless of test result. Routine throat cultures were sparse in our setting (in line with national guidelines) and too few to draw any strong conclusions about the possible divergent outcomes in patients positive for SDSE and/or *F. necrophorum*.

Abbreviations

GAS: Group A streptococci; EMR: Electronic medical record; IQR: Interquartile range; PHC: Primary healthcare; PHCC: Primary healthcare centres; RADT: Rapid antigen detection test; SDSE: *Streptococcus dysgalactiae* Subspecies *equisimilis*.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-021-06511-y.

Additional file 1. Additional tables.

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Authors' contributions
JP: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Validation, Writing—original draft preparation, Writing—review & editing. KH: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing—review & editing. MR: Conceptualization, Writing—review & editing. MS: Conceptualization, Writing—review & editing. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study protocol was performed in accordance with relevant guidelines and regulations. Ethical approval was obtained from the Regional Ethics Review Board in Linköping, Sweden: 2016/529-31. All managers of the involved primary healthcare centres gave their permission to extract data, and these permissions were included in the application of ethical approval. As this study contains only retrospective anonymous patient data, the Regional Ethics Review Board in Linköping, Sweden, did not require informed consent from the patients. Confidentiality of the patients was ensured by using encrypted ID numbers.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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