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The aetiology of pharyngotonsillitis in adolescents and adults – *Fusobacterium necrophorum* is commonly found

K. Hedin[1,2], L. Bieber[3], M. Lindh[4] and M. Sundqvist[5]

1) *Department of Clinical Sciences, Family Medicine, Lund University, Malmö,* 2) *Unit for Research and Development, Kronoberg County Council,* 3) *Department of Clinical Microbiology, Central Hospital, Växjö, Sweden,* 4) *Department of Clinical Virology, Sahlgrenska University Hospital, Gothenburg* and 5) *Department of Laboratory Medicine, Clinical Microbiology, University Hospital, Örebro, Sweden*

Abstract

Sore throat is common in primary healthcare. Aetiological studies have focused on the presence of a limited number of pathogens. The aim of the present study was to investigate the presence of a wide range of bacteria and viruses, including *Fusobacterium necrophorum*, in patients with pharyngotonsillitis and in asymptomatic controls. A prospective case control study was performed in primary healthcare in Kronoberg County, Sweden. Patients ($n = 220$) aged 15 to 45 years with a suspected acute pharyngotonsillitis, and controls ($n = 128$), were included. Nasopharyngeal and throat swabs were analysed for β-hemolytic streptococci, *F. necrophorum*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*, and 13 respiratory viruses. Serum samples were analysed for antibodies to Epstein-Barr virus. The patient history and symptoms, including Centor score, were analysed in relation to pathogens. In 155/220 (70.5%) of the patients, as compared to 26/128 (20.3%) of the controls (p <0.001), at least one microorganism was found. Group A streptococci, *F. necrophorum*, and influenza B virus were the three most common findings, and all significantly more common in patients than in controls (p <0.001, p 0.001, and p 0.002, respectively). Patients with *F. necrophorum* only ($n = 14$) displayed a lower Centor score than patients with Group A streptococcus only ($n = 46$), but a higher score than patients with influenza B, other viruses, or no potential pathogen (Kruskal-Wallis p <0.001). A pathogen was detected in 70% of the patients, displaying a wide range of pathogens contributing to the aetiology of pharyngotonsillitis. This study supports *F. necrophorum* as one of the pathogens to be considered in the aetiology of pharyngotonsillitis.

Corresponding author: K. Hedin, Unit for Research and Development, Box 1223, S-351 12 Växjö, Sweden
E-mail: katarina.hedin@ltkronoberg.se

Introduction

A sore throat is a common complaint in primary healthcare (PHC) [1]. The condition can be caused by a wide variety of infectious agents [2], where the most common is *Streptococcus pyogenes* (Group A streptococci, GAS) [3–5]. Also, large colony variants of Streptococcus group C and G [6], *Mycoplasma pneumoniae* [3], *Arcanobacterium haemolyticum* [7], and several viruses [3,5,8] have been associated with a sore throat.

The aetiology has mainly been studied in children [9,10], and most studies have been performed with a focus on the presence of a limited number of pathogens [3,5], and studies covering both viral and bacterial aetiology are lacking [5]. The criteria presented by Centor *et al.* 30 years ago were developed to predict the presence of GAS as the cause of the patients' symptoms, and thus predict the patients who were considered in need of antibiotic therapy [11].

In recent years, the anaerobic Gram-negative bacterium *Fusobacterium necrophorum* has been suggested as an important cause of acute pharyngotonsillitis in adolescents and young

adults [12–14]. At the same time, some authors have noticed an increase of severe infections caused by *F. necrophorum* (Lemièrre syndrome) [15,16]. This conclusion is not, however, generally accepted [17], as the increase may be due to publication bias [18,19]. The studies focusing on *F. necrophorum* as a throat pathogen have, so far, been based on routine clinical microbiology data and have focused on the presence of this bacterium only [12,14,15].

The aim of the present study was to investigate the presence of *F. necrophorum* together with β-hemolytic streptococci, *Mycoplasma pneumoniae, Chlamydophila pneumoniae*, and 14 respiratory viruses in adults attending PHC with the symptoms of pharyngotonsillitis, compared to asymptomatic controls. The second aim was to investigate the association of these pathogens to the patient's history and symptoms and signs, and in relation to the Centor score in particular.

Material and methods

Setting
A prospective case control study was conducted in five primary healthcare centres (PHCC) in Kronoberg County, Sweden. Data were collected between January 15 and April 30, 2011 and from October 1, 2011 to April 15, 2012.

Study population
Patients. Patients aged 15 to 45 years with a suspected acute pharyngotonsillitis who contacted the PHCC and were considered in need of a consultation by the physician according to the national Swedish guidelines [20], were recruited. According to standard procedures, all patients contacted the PHCC via telephone, and a nurse primarily assessed if the patient's symptoms called for a visit to the physician. Informed consent was obtained and the physician made the study assessment during the consultation. This included the Centor criteria, i.e. absence of cough, fever >38.5°C, tender lymphadenitis, and tonsillar coating, as well as potential confounders such as smoking habits, recent antibiotic treatment, history of sore throat, and the duration of symptoms prior to the consultation. Since the study did not interfere with the clinical management, the risk for bias was minimal.

Controls. Patients 15 to 45 years old visiting the PHCC for any medical reason other than an infection were recruited as a control group during the same time periods. Informed consent was obtained before sampling was performed. A questionnaire including questions about potential confounders such as recurrent sore throat, smoking habits, and antibiotic treatment in the last month was completed.

Sampling
All sampling was performed by staff at the PHCCs. A swab for the culture of β-hemolytic streptococci and *F. necrophorum* was obtained by rolling the swab on the tonsils at both sides and placing it in Amies medium with charcoal (Copan, Brescia, Italy). Two samples for the analyses of respiratory viruses *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* were obtained using flocked swabs transported in 3 mL of Universal Transport Medium (UTM; Copan), one from the pharynx and one from the nasopharynx. In the patient group, a capillary blood sample was obtained for the analysis of Epstein-Barr virus (EBV)-specific antibodies. According to the procedures used in the county at the time of the study, the result of the culture for β-hemolytic streptococci was reported to the physician.

Microbiological procedures
All samples were stored in a refrigerator and transported to the Department of Clinical Microbiology, Central Hospital, Växjö. Throat swabs were cultured for the recovery of β-hemolytic streptococci (Lancefield group A, C, and G), using a double-layered agar (Columbia agar (Oxoid, Basingstokes, UK) covered with sheep blood agar, blood agar base (Merck, Darmstadt, Germany) with 5% sheep blood (SVA, Uppsala, Sweden), and 5 mg/mL methyl violet (Merck, Darmstadt, Germany) with a bacitracin disc (0.2 IU)). For *F. necrophorum*, a selective anaerobic plate (fastidious anaerobe agar (Lab M) with 5% horse blood (SVA) supplemented with vancomycin 2.5 mg/L (ICN Biomedicals, Irvine, CA, USA) and nalidixic acid 5.0 mg/L (MP Biomedicals, Santa Ana, CA, USA)), and with a kanamycin tablet 500 μg (Rosco, Taastrup, Denmark) was used [21]. The agar plates were incubated under anaerobic conditions in 35–37°C for 1 and 4 days, respectively. For species identification, Streptex (Remel Europe Ltd., Dartford, England) and MALDI-TOF (Microflex mass-spectrometer and Biotyper 3.1 software, Bruker Daltonics, Bremen, Germany) were used.

All samples transported in UTM were stored in −80°C until shipped on dry ice to the Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, Sweden for further analyses. All samples were analysed using multiplexed PCRs for 13 respiratory viruses and the two intracellular bacteria *M. pneumoniae* and *C. pneumoniae* as previously described [22]. Any detected DNA or RNA was considered a positive result.

The blood samples were centrifuged at 3000 rpm for 10 minutes, and the sera were stored in −20°C until analyses were performed. All samples were screened for the presence of Epstein-Barr virus nucleic antigen – antibodies (EBNA) (Novagnost EBV-EBNA IgG; Siemens, Tarrytown, NY, USA) on a BEP2000 (Siemens). Samples negative for EBNA were analysed with EBV IgM (Enzygnost Anti-EBV/IgM II; Siemens) and

EBV-Viral Capsid Antigen IgG (VCA IgG, (Enzygnost Anti-EBV/IgG; Siemens)). Patients with positive EBV IgM with or without VCA IgG were considered to have a primary EBV infection.

Routine microbiology data

Sampling strategies and detection techniques for the routine diagnostics of β-hemolytic streptococci (culture), influenza A and B (PCR), and *M. pneumoniae* (PCR) were not changed during the study period. All samples analysed for these pathogens were retrieved from the LIS system (ADBakt, Autonik AB, Sköldinge, Sweden) at the Department of Clinical Microbiology, Växjö from August 2010 to July 2012, and the proportion of positive samples were analysed on a monthly basis and compared to the study data.

Statistical analyses

To be able to detect a 10% difference in the presence of *F. necrophorum* between the patients and the controls, 180 patients had to be included given a power of 0.80 and an α value of 0.05. The statistical analyses were performed using the SPSS 20.0 software (IBM, Armonk, NY, USA). For descriptive statistics, median values and proportions were used. Comparisons between proportions of categorical variables in two independent groups were performed using the χ^2 test or Fisher's exact test, when expected frequencies were small. Multiple logistic regressions were used to model the relationship between the outcome variable and several independent variables. The Kruskal-Wallis test was used when comparing medians in more than two groups. Missing data were <2% for all variables among the patients except for tonsillar coating, where 6% (14/220) was missing. Among the controls, background data were missing for 3% (4/120) of the individuals.

Ethical considerations

The study was approved by the regional ethics board in Linköping, Sweden, Drn: 2010/267-31. All patients and controls received written information about the study before seeing the physician. The patients had the opportunity to ask questions about the study before informed consent was obtained. The sampling methods used in this study were well established and familiar to the personnel performing the sampling, and thus were regarded as safe for the included patients and controls.

Results

Characteristics of the patients and controls

In total, 220 patients and 128 controls were recruited. The median age was 33 years (range 15–48 years) in the patient group and 31 years (range 16–46 years) in the control group.

TABLE 1. Characteristics of the study population

	Percent		
	Patients (n = 220)	Control group (n = 128)	χ^2 p
Female	64.1	75.8	0.02
Smoker	14.0	8.1	0.10
A history of often having a sore throat	33.3	7.3	<0.001
Previous tonsillectomy	13.2	12.1	0.76
Antibiotic treatment in the last month	7.9	5.6	0.44

Characteristics of the study population are presented in Table 1. All patients were evaluated according to the symptoms and signs building up the Centor score. The median Centor score was 2 (14.1% presented with Centor score 4, 24.5 % with a score of 3, 31.4% score 2, 22.7% score 1, and 7.3 % with score 0).

Aetiology

The detection of bacteria and viruses in patients and controls. In 155/220 (70.5%) of the patients, as compared to 26/128 (20.3%) of the controls, (p <0.001), at least one bacterium or virus was found. The corresponding figures for viruses, independent of the presence of bacteria, were 70 (31.8%) and 11 (8.6%) (p <0.001), respectively, while at least one bacterium, with or without the presence of viruses, was found in 103 (46.8%) patients and in 17 (13.3%) controls (p <0.001). In 58 (26.4%) patients, more than one microorganism was found. Two bacteria or viruses were found in 20.9%, three in 4.1% and four in 1.4% of the patients. The most common combination was GAS and *F. necrophorum* (n = 11). Six of the controls were positive for more than one microorganism.

The distribution of the bacteria and viruses found is presented (see Results section). GAS (p <0.001), *F. necrophorum* (p 0.001), and influenza B (p <0.002) were significantly more commonly found among the patients than among controls, and represented the three most common findings in the patient group.

Bacteria and viruses found in patients with the suspicion of pharyngotonsillitis. Except for *C. pneumoniae*, all tested bacteria and viruses were found in at least one patient. Of the 103 (46.8%) patients positive for bacteria, 15 (14.6%) were infected with more than one bacterial species, and 16 (15.5%) had a concomitant finding of at least one virus. GAS was the most common finding (n = 66), followed by *F. necrophorum* (n = 33), influenza B virus (n = 16), and rhinoviruses (n = 14) (Table 2). EBNA IgG was found in 197/220 (89.6%) of the patients, indicating previous primary infection. Of the EBNA-negative patients, five were deemed to have a present EBV infection. The full pattern of coexistence of the analysed bacteria and viruses is shown in Supplementary Table 1.

TABLE 2. Bacteria and viruses detected through culture, multiplexed PCRs, or serology in patients with a sore throat and in controls

	Percent (number of patients)		
	Patients	**Controls**	**Fisher or χ^2 test[a]**
	(*n* = 220)	(*n* = 128)	p
GAS	30 (66)	2.3 (3)	<0.001[a]
GCS	3.6 (8)	0.8 (1)	0.16
GGS	3.2 (7)	7.0 (9)	0.10[a]
Fusobacterium necrophorum	15.0 (33)	3.1 (4)	0.001[a]
Chlamydophila pneumoniae	0	0	—
Mycoplasma pneumoniae	1.8 (4)	0.8 (1)	0.66
Adenovirus	4.1 (9)	1.6 (2)	0.34
Bocavirus	0.5 (1)	0.8 (1)	1.0
Coronavirus NL63	1.8 (4)	0	0.30
Coronavirus OC43	1.8 (4)	0	0.30
Coronavirus HKU1	0.9 (2)	1.6 (2)	0.63
Coronavirus 229E	1.4 (3)	0.8 (1)	1.0
EBV	5 (2.3)	NA	—
Enterovirus	0.5 (1)	0.8 (1)	1.0
Influenza A virus	1.8 (4)	0	0.30
Influenza B virus	7.3 (16)	0	0.002[a]
Metapneumovirus	1.8 (4)	0	0.30
Parainfluenzavirus	0.5 (1)	0	1.0
Rhinovirus	6.4 (14)	2.3 (3)	0.09[a]
RSV	1.8 (4)	1.6 (2)	1.0

GAS, Group A streptococci; GCS, Group C streptococci; GGS, Group G streptococci; EBV, Epstein-Barr virus; RSV, respiratory syncytial virus.
Statistical analyses were calculated using χ^2 test or Fisher's exact test.
[a]χ^2 test was used.

Importance of the sampling site for the detection of viruses. Most viruses were found either in the throat sample or in the nasopharyngeal sample (Supplementary Table 2). There was no significant difference in the cycle threshold values (± 1 cycle threshold) between the different sampling sites, or between patients with only a virus found or in combination with bacteria, thus indicating similar amounts of viruses in the samples.

Temporal variations

For several pathogens, the detection rate in the patient group was unevenly distributed during the study. Adenoviruses (first inclusion period), rhinoviruses (second period), and influenza B (first period) were found during separate time frames of the study, while GAS and *F. necrophorum* were isolated throughout the inclusion periods. For influenza B and GAS, the relative prevalence in the study population at a given time was in relation to the detection rate in clinical samples (data not shown).

Microorganisms in relation to symptoms, signs, and Centor score

The association of bacteria and viruses significantly associated with disease (i.e. only bacteria, only viruses, GAS, *F. necrophorum*, and influenza B virus) to background characteristics, the specific symptoms and signs and Centor score are presented in Tables 3 and 4. Patients with only one or several bacteria found had a higher median Centor score (56.4% with Centor score ≥3, median 3) than patients with viruses only (25.0% with Centor score ≥3, median 2) (Mann-Whitney *U* test p <0.0001). In patients with no bacteria or viruses detected, 24.6% had a Centor score ≥3 with a median score value of 2.

Patients with *F. necrophorum* only (*n* = 14) displayed a lower Centor score than GAS only (*n* = 46), but higher than patients with influenza B, other viruses, or no potential pathogen found (Kruskal-Wallis p <0.001). The multiple regression analysis revealed "being a smoker" and the presence of tonsillar coatings to be of importance for the likelihood of finding *F. necrophorum* (Table 5).

Discussion

This prospective case control study, analysing 20 potential causative agents of sore throat (6 bacteria and 14 viruses), could detect at least one of these in 70.5% of patients 15–45 years of age attending PHC with the suspicion of pharyngotonsillitis, compared to 20.3% in asymptomatic controls of the same age. GAS, *F. necrophorum*, and influenza B were significantly more common in the patients, and the highest level of Centor score was found in patients with GAS. Several viruses (influenza B, adeno- and rhinovirus) and GAS showed a marked seasonal variation and thus contributed differently to the aetiology of pharyngotonsillitis at a given time point.

As far as we know, this is the first prospective study with a broad diagnostic approach, including both bacteria and viruses that relate to the reported signs and symptoms in patients aged 15–45 years with the suspicion of pharyngotonsillitis. The high quality of clinical data allowed us to perform analyses on the relationship between specific pathogens and patient history, symptoms, and signs. The summer was considered a low season of pharyngotonsillitis and therefore not included, the overall Centor score was relatively low, and the clinical assessment of the patients was not guided, all of which can be considered limitations of the study. This, however, reflects the patient group and practice in Swedish PHCs and is therefore considered relevant. The study was powered to be able to detect a significant difference in the presence of *F. necrophorum* in pharyngotonsillitis patients, compared to a control group. Since the power was not calculated on the associations between bacteria and viruses and signs or symptoms, we may have missed some associations. This should encourage further studies.

We could not identify a potential pathogen in 30% of the patients. This could be due to sampling, or the detection methods used [2,3,7]. However, the methods for sampling and analyses of the samples used here are the same that may be

TABLE 3. Aetiologic agents in relation to the background characteristics of the patients

	Patients *n*	Background data of the patients (percent)					
		Woman	Smoker	Often has a sore throat	Prolonged sore throat	Previous tonsillectomy	Treated with antibiotics the last 3 months
No pathogen	65	63	16	29	44	12	6
Only viruses	52	65	14	48	29	16	12
Only bacteria	85	68	13	28	26	13	9
GAS (only)	46	70	4	22	30	17	7
Fusobacterium necrophorum (only)	14	74	46	43	36	7	23
Influenza B (only)	13	69	0	50	15	17	8

GAS, Group A streptococci.

used in the clinical setting and thus relevant. Non-infectious causes of sore throat have also been reported by other authors [23], but were not included in this study. Interestingly, the median Centor score in the group of patients with no bacterium or virus found (Centor score 2) were similar to the group with viruses found, indicating that there may be more infections due to viruses rather than bacteria being undetected in this group.

As expected, GAS was the most common pathogen [24]. The Centor score for these patients was higher than for patients with non-GAS infections, supporting the continuous use of this score to predict the presence of GAS [5]. There was a large diversity of the viruses contributing to the aetiology, and the temporal variation resulted in influenza B (only period 1) being the most common viral cause of pharyngotonsillitis in this age group. Respiratory viruses were also detected in the control group. This might be seen as over-reporting of the presence of these viruses due to the high sensitivity of PCR. However, we would argue, rather, that it reflects asymptomatic infections or prolonged shedding of viruses. Despite the unbiased inclusion, as many as 15% of the patients were culture-positive for *F. necrophorum,* while only 3% of the healthy controls were positive for this bacterium. Previously, Aliyu *et al.* described patients, aged 5 months to 79 years, with a sore throat where a routine culture was obtained, and found

F. necrophorum in 10% of these samples using PCR, but did not find any *F. necrophorum* among 100 healthy controls [12]. Amess reported 4.9% of routine throat cultures in the age group <1–88 years to be positive for *F. necrophorum*, with the highest prevalence in patients ages 11–25 years (9.8%) [14]. In patients 18–32 years of age with "non-streptococcal tonsillitis," Jensen *et al.* identified *F. necrophorum* with PCR in almost half of the samples, and in lower amounts among 21% of the controls [13]. The use of samples from routine microbiology and "non-streptococcal" samples will tend, contrary to the prospective open inclusion used in the present study, to over-report the pathogen studied. *Fusobacterium necrophorum* was the only microorganism found in 14 patients. These patients were not necessarily less ill than the patients with GAS only, but received a lower Centor score due to a higher presence of cough. Although the power of the study was not calculated to rule out associations between signs and symptoms, this study could not find any association between age [14], recent primary EBV infection [25], or recurrent sore throat [26] and the detection of *F. necrophorum*, as suggested by others. However, smoking and coating of the tonsils increased the likelihood of detecting *F. necrophorum*. Importantly, some of the controls were positive for *F. necrophorum*, and the presence of this bacterium is thus not strictly associated with symptoms. Among nearly two-thirds of patients with a presence of *F. necrophorum*, another

TABLE 4. The potential pathogens significantly associated with disease (i.e. only bacteria, only viruses, GAS, *Fusobacterium necrophorum* and influenza B virus) in relation to Centor score, symptoms and signs

	Patients *n*	Symptoms and signs in the patients (%)				Centor score (%)				
		No cough	Fever	Lymphadenitis	Tonsillar coating	0	1	2	3	4
No pathogen	65	59	43	52	28	11	37	28	14	11
Only viruses	52	33	64	62	30	10	27	38	19	6
Only bacteria	85	79	67	69	57	4	9	31	33	24
GAS (only)	46	87	76	73	60	2	9	22	37	30
Fusobacterium necrophorum (only)	14	64	50	57	74	0	7	57	21	14
Influenza B (only)	13	8	92	69	33	8	15	54	23	0

GAS, Group A streptococci.

TABLE 5. Presence of any *Fusobacterium necrophorum* among patients with pharyngotonsillitis in primary healthcare

Presence of	Presence of any *Fusobacterium necrophorum* in the samples		
	Crude model	Adjusted models	
	OR (95% CI)	Model 1[a] aOR (95%CI)	Model 2[b] aOR (95%CI)
No cough	1.22 (0.56–2.63)		
Lymphadenitis	1.26 (0.57–2.77)		
Fever	0.97 (0.45–2.08)		
Tonsillar coating[c]	2.87 (1.31–6.24)[c]	2.66 (1.21–5.85)[c]	2.75 (1.19–6.33)[c]
Runny nose	0.16 (0.65–3.06)		
Younger than 26 years	0.22 (0.33–1.55)		
Smoker	1.37 (0.64–2.93)		3.66 (1.41–9.49)[c]
Often has a sore throat	0.38 (0.09–1.68)		
Tonsillectomised	0.38 (0.09–1.68)		

Crude (univariate) odds ratio (OR) and adjusted ORs (aOR) from the logistic regressions are presented with 95% confidence intervals (95% CI). aORs were calculated using multiple logistic regressions with backward elimination of all variables. The variables in the last step are shown in the adjusted models.
[a]Model 1 Adjusting for no cough, lymphadenitis, fever, tonsillar coating.
[b]Model 2 Adjusting for the variable in model 1 and runny nose, younger than 26 years, smoker, often has a sore throat.
[c]Significant figures.

microorganism was found (both GAS and viruses). The growth, and possibly also the pathogenic potential of *F. necrophorum*, may thus be facilitated by an underlying inflammation.

In conclusion, this study showed that GAS and several respiratory viruses, together with *F. necrophorum*, are of importance in the aetiology of pharyngotonsillitis. The exact distribution of each specific pathogen will, at a given time, be subjected to local and temporal variations. Both Centor and Bank *et al.* have suggested that a broad antibiotic treatment of tonsillitis could prevent the few cases of severe infection (i.e. Lemièrre syndrome) [27,28]. We consider this suggestion premature, as the finding of *F. necrophorum* in a predisposing tonsillitis has not been shown to be of importance for the development of Lemièrre syndrome, and as the best treatment option of *F. necrophorum* has not yet been established. More studies are warranted to further define the importance of *F. necrophorum* in tonsillitis, establish a standard for how to handle the finding of *F. necrophorum* in pharyngotonsillitis, and determine whether antibiotic treatment is needed. We urge diagnostic laboratories to consider the methods needed to find this bacterium in throat swabs (i.e. PCR-based methodology or anaerobic culture for 4 days) [13].

Transparency declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2014.08.020

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