Fusobacterium necrophorum: Most Prevalent Pathogen in Peritonsillar Abscess in Denmark

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Background. Group A streptococci are commonly regarded as the most prevalent cause of acute bacterial tonsillitis and peritonsillar abscess (PTA). However, the majority of PTA aspirates also contain strains of anaerobes, and accumulating evidence indicates that *Fusobacterium necrophorum* (FN) could be involved in acute tonsillitis. The purpose of the present study was to describe the epidemiology and bacteriology of PTA in Denmark, with particular emphasis on correlations between microbiological, clinical, and laboratory data.

Methods. A retrospective study on all patients with PTA admitted to the ear, nose, and throat department at Aarhus University Hospitals from January 2001 through December 2006 was conducted.

Results. In total, 847 patients were included in the study. The mean annual incidence of PTA was 41 cases/100,000 population. FN was the most frequently detected bacteria (in 23% of cultures), followed by group A streptococci (in 17%) and groups C and G streptococci (counted together, in 5%). Of the 191 FN isolates detected, 155 (81%) grew as pure culture. Patients infected with FN were significantly younger than patients infected with other strains of bacteria ($P < .001$). Patients with FN exhibited significantly higher neutrophil counts ($P < .001$) and C-reactive protein values ($P = .01$) than did patients infected with other bacteria.

Conclusions. To our knowledge, this study is the first report of FN being the most prevalent pathogen in PTA patients. The significantly higher neutrophil counts and C-reactive protein values strongly indicate the pathogenic importance of FN in PTA. The widespread reliance on rapid streptococcal antigen test in general practice to appoint patients for antibiotics and the highest PTA incidence ever reported raise concern that highly virulent bacteria may be left initially untreated.

A peritonsillar abscess (PTA) is defined as a collection of pus between the tonsillar capsule and the pharyngeal constrictor muscle. It is the most frequent complication of tonsillitis and the prevailing cause of acute admission to the ear, nose, and throat department at Aarhus University Hospitals (Aarhus County, Denmark). Management requires surgical drainage and antimicrobial therapy. Because of the commonly held view that group A streptococci (GAS) are the most prevalent cause of bacterial tonsillitis, the standard diagnostic test used by Danish general practitioners is the rapid streptococcal antigen test. Upon detection of GAS antigen, antibiotic treatment is routinely initiated. At present, penicillin is the primary drug of choice in Denmark.

However, accumulating evidence suggests that alternative bacteria such as *Fusobacterium necrophorum* (FN) and group C streptococci (GCS) could be involved in tonsillitis and the pathogenesis of PTA [1–6]. Indeed, it was recently demonstrated that FN is present in 10% of all throat swabs and that a clinical diagnosis of tonsillitis was equally likely to be caused by FN as by GAS [1]. In a Danish polymerase chain reaction (PCR) study, FN was detected in 15% of throat swab samples from tonsillitis patients aged 18 to 32 years, and the investigators concluded that FN could be a cause of acute tonsillitis and may account for some of the cases previously assumed to be of viral etiology [2]. By means of real-time PCR, Aliyu et al [3] identified FN in 10% of throat swab samples from pharyngitis patients who presented to general practitioners, whereas FN was never seen in throat swab samples from healthy control subjects. GCS was found to be the responsible pathogen in 4%–11% of patients with acute sore throat [4–6].

In PTA aspirates, a mixed aerobic and anaerobic flora

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is most commonly detected. GAS is isolated in 10%–45% of patients, FN in 0%–38%, and GCS in 4%–18% [7–15].

On the basis of the reviewed literature and our observation that (1) FN-positive PTA cultures are observed with increasing frequency at the ear, nose, and throat department of Aarhus University Hospitals and (2) patients infected with FN seem to be more severely affected, both clinically and with regard to biochemical findings, than patients infected with other bacteria, we hypothesized that FN might play a pathogenic role in acute tonsillar infection. In the present study, we obtained cultures from a large number of patients with clinical PTA. We aimed to describe the epidemiology and bacteriology of PTA in Denmark and to study correlations between microbiological, clinical, and laboratory data.

MATERIALS AND METHODS

All medical records of patients with PTA who were admitted to the ear, nose, and throat department of Aarhus University Hospitals from January 2001 through December 2006 were reviewed. Clinical diagnosis of PTA was based on the visual detection of peritonsillar pus. Cultures were made from pus aspirates or pus swab samples.

The following clinical and demographic data were obtained: age, sex, date of admission, duration of symptoms, duration of hospitalization, type of antimicrobial therapy before admission, temperature, C-reactive protein level, leukocyte count, and neutrophil count. Diagnosis of mononucleosis was based upon identification of atypical lymphocytes. All blood samples and temperature measurements were obtained at admission.

Apart from Aarhus University Hospitals, treatment in Aarhus County of patients with PTA was performed in Randers Hospital and in private ear, nose, and throat practices. Information concerning the number of patients treated for PTA from 2001 through 2006 at these locations was acquired to determine the incidence of PTA in Aarhus County.

Culture and identification of bacteria from tonsil specimens (pus aspirates and swab samples) was performed as part of the routine diagnostic procedures. Pus aspirates and pus swab samples were transported to the Department of Clinical Microbiology in sterile and sealed syringes and Stuart medium, respectively. Briefly, blood agar, chocolate agar, anaerobic plates, and thioglycolate broth (SSI Diagnostic) were used to culture the specimens. The media were incubated at 35°C, either in a carbon dioxide–enriched atmosphere or anaerobically, for ⩽3 days. Speciation for microorganisms was performed by standard methods [16].

Light to moderate growth of viridans streptococci, *Neisseria* species, *Lactobacillus* species, coagulase-negative staphylococci, *Prevotella* species, and *Fusobacterium nonnecrophorum* alone or in mixture were reported as "mixed oral flora."

Statistical analyses were performed using analysis of variance (ANOVA), the Student *t* test, and the Kruskal-Wallis test for nonparametric data. The normality of the data was assessed using quantile quantile (QQ) plots. Statistical significance was defined as $P < .025$.

RESULTS

In total, 847 patients (448 males), aged 0–91 years, were included. Six hundred twenty (73%) of the patients were aged 8–30 years. Tonsillectomy was performed in 726 patients, 110 patients were treated with aspiration and incision, in 7 patients tonsillectomy was performed subsequent to initial insufficient aspiration and incision, and 4 patients experienced spontaneous perforation. Culture of samples from the abscess cavity was performed for 760 patients.

The median duration of symptoms was 5 days (range, 1–60 days), and the median duration of hospitalization was 2 days (range, 1–10 days). The mean temperature of patients at admission was 37.4°C (95% CI, 37.1°C–37.7°C). Other demographic and clinical details, stratified by bacterial diagnosis, are summarized in Table 1. The following bacteria, listed by decreasing frequency, were detected: FN (191 isolates), GAS (141 isolates), GCS and group G streptococci (GGS) (together, 39 isolates), *Staphylococcus aureus,* mixed anaerobes, and *Haemophilus influenzae* (Table 2). Pure cultures were grown from 155 (81%) of 191 FN isolates, 127 (90%) of 141 GAS isolates, and 21 (54%) of 39 GCS/GGS isolates. All FN, GAS, and GCS/GGS isolates were susceptible to penicillin.

Patients infected with FN were significantly younger than patients infected with other strains of bacteria, including GAS, GCS, and GGS ($P < .001$; Kruskal-Wallis). Patients with FN exhibited significantly higher neutrophil counts than did patients who were infected with other bacteria ($P < .001$; t, 3.67). PTA patients diagnosed with simultaneous mononucleosis displayed significantly lower neutrophil counts than did PTA patients not diagnosed with simultaneous mononucleosis ($P < .001$; t, 4.68). FN-positive patients had significantly higher C-reactive protein values than did patients infected with other bacteria ($P = .01$; t, 2.59). An age-stratified analysis yielded the same results with regard to significant differences in the neutrophil counts and C-reactive protein values of FN-positive patients, compared with those of other PTA patients. No other significant findings were observed in the microbiological, clinical, and laboratory data. Mononucleosis was detected among 26 (3.1%) of the 847 patients with PTA.

Information about preadmission use of antibiotics was available for 776 (92%) of 847 patients. A total of 293 (38%) of the 776 patients had taken antibiotics before admission. A penicillin-type antibiotic was administered to 274 (94%) of these 293 patients.

No significant seasonal variation was found with regard to the number of patients or bacterial findings. Cultures were

Table 1. Clinical and Laboratory Data Stratified by Microbiologic Findings

Variable	All	FN	GAS	GCS / GGS	FN + BHS[a]	Other bacteria	Mixed oral flora[b]	No culture	Mononucleosis
No. of patients	847	167	133	23	24	58	355	87	26
No. of males	448	90	76	18	13	28	182	41	17
Age,[c] median years	21	18[d]	23	19	21	24	23	21	19
C-reactive protein, nmol/L	1080 (1027–1132)	1218[d] (1089–1347)	1061 (933–1189)	1061 (895–1227)	1157 (783–1530)	975 (795–1154)	1036 (931–1142)	1009 (844–1174)	870 (610–1129)
Leukocytes (1×10^9 cells/L)	14.3 (13.9–14.6)	15.1(14.5–15.7)	15.1 (14.7–15.4)	14.3 (12.8–15.8)	13.8 (12.0–15.6)	12.5 (11.5–13.5)	13.7 (13.0–14.3)	14.9 (12.9–17.0)	12.5 (10.6–14.3)
Neutrophils (1×10^9 cells/L)	11.06 (10.77–11.35)	12.06[d] (11.46–12.65)	11.81 (11.16–12.46)	10.88 (9.23–12.53)	11.43 (9.82–13.03)	9.42 (8.45–10.38)	10.60 (9.97–11.23)	10.82 (9.83–11.80)	6.82[e] (5.08–8.57)

NOTE. Data are mean (95% confidence interval) unless otherwise indicated. Patients with mononucleosis are also included according to the bacterial findings. BHS, β-hemolytic groups A, C, and G streptococci; FN, *Fusobacterium necrophorum;* GAS, group A streptococci; GCS/GGS, groups C and G streptococci.

[a] Concerning the category FN + BHS, FN + GAS and FN + GCS/GGS are added because the number of such isolates was low and no clinical or laboratory differences were detected between the 2 groups.

[b] Mixed oral flora was defined as light to moderate growth of viridans streptococci, *Neisseria* species, *Lactobacillus* species, coagulase-negative staphylococci, *Prevotella* species, and *Fusobacterium nonnecrophorum* alone or in mixture.

[c] Age was a non-Gaussian distribution.

[d] Significantly different from FN-negative patients (neutrophil count, $P < .001$; C-reactive protein level, $P = .01$).

[e] Significantly different from mononucleosis-negative patients ($P < .001$).

Table 2. Influence of Preadmission Antibiotic Treatment on Culture

Variable	Patient receipt of preadmission antibiotics			
	No (n = 483 patients)	Yes (n = 293 patients)	Unknown (n = 71 patients)	All (n = 847 patients)
No. of cultures	436	260	64	760
Mixed oral flora[a]	180	143	32	355
Positive cultures	256	117	32	405
No. of isolates	287	138	36	461
No. of organisms (% of isolates)				
Fusobacterium necrophorum	127 (44%)	49 (36%)	15 (42%)	191 (41%)
Group A streptococci	95 (33%)	36 (26%)	10 (28%)	141 (31%)
Groups C or G streptococci	25 (9%)	12 (9%)	2 (6%)	39 (8%)
Staphylococcus aureus	4 (1%)	8 (6%)	3 (8%)	15 (3%)
Haemophilus influenzae	6 (2%)	3 (2%)	0 (0%)	9 (2%)
Other aerobes	17 (6%)	17 (12%)	5 (14%)	39 (8%)
Other anaerobes	13 (5%)	12 (9%)	1 (3%)	26 (6%)
Yeast	0 (0%)	1 (1%)	0 (0%)	1 (0%)

[a] Mixed oral flora was defined as light to moderate growth of viridans streptococci, *Neisseria* species, *Lactobacillus* species, coagulase-negative staphylococci, *Prevotella* species, and *Fusobacterium nonnecrophorum* alone or in mixture.

obtained from 760 patients, of which 355 (47%) cultures contained only mixed oral flora. Of the 405 (53%) positive cultures, 461 isolates were found. In 436 cultures of samples recovered from patients without preceding antibiotic treatment, 256 (59%) had positive results; in 260 cultures of samples recovered from patients treated with antibiotics before admission, 117 (45%) had positive results.

The proportion of patients who received preadmission antibiotics remained stable from 2001 through 2006. Of 141 GAS-positive patients, 36 (26%) had taken antibiotics before admission; of 206 patients infected with GCS/GGS or FN but in whom no GAS was detected, 52 (25%) had taken antibiotics before admission. No significant differences were observed in clinical or laboratory data between the patient groups, with or without antibiotic treatment preceding admission.

Besides the 847 patients hospitalized in Aarhus University Hospitals, 224 patients were treated for PTA as outpatients at Aarhus University Hospitals, 46 patients were treated at Randers Hospital, and 484 patients were treated in private ear, nose, and throat practices in Aarhus County. In total, 1601 patients were diagnosed with PTA in Aarhus County (catchment population of 650,000) during our observation period of 6 years. Thus, the mean annual incidence of PTA was 41 cases/100,000 population.

DISCUSSION

FN is a gram-negative, obligate anaerobic, pleomorphic rod. It is the pathogen most commonly associated with Lemierre Syndrome.

However, unlike in Lemierre Syndrome, most FN infections remain localized. In Denmark, FN is most frequently isolated in PTA, tonsillitis, subcutaneous wounds, cervical lymphadenitis with a solitary abscess, and otitis media [17]. Haegelskaer Kristensen and Prag [17] documented all FN isolates in Denmark from 1998 through 2001 to be subspecies *Funduliforme* (biotype B). In contrast to the infectivity of FN subspecies *necrophorum* (biotype A) isolated in animals, the infectivity of biotype B strains is typically not enhanced by other bacteria [18, 19]. FN cannot be considered part of the normal tonsillar flora, because no convincing confirming culture evidence exists.

A mixed aerobe and anaerobe culture is the most common finding in PTA aspirates [8, 9, 20]. GAS is consistently the most prevalent pathogen, and FN is scarcely reported outside Finland [7–11, 14, 15, 21]. In a study by Jousimies-Somer et al [8], 38% of PTA aspirates were positive for FN, and in 14 of 15 cultures that had only one anaerobe bacteria, FN was isolated. Mitchelmore et al [10] detected FN in 8% of PTA aspirates, and FN was the only anaerobe to occur as a pure growth.

Several studies suggest that FN and GCS/GGS are common pathogens in acute tonsillitis [2–6]. The present finding that FN was more prevalent than GAS in patients with PTA raises the suspicion that GAS could play a less dominant role in acute bacterial tonsillar disease than hitherto believed. Furthermore, 155 (81%) of the 191 FN isolates detected grew as pure culture, which is an additional indication of pathogenetic significance. Still, it cannot be ruled out that GAS is the dominant pathogen in acute bacterial tonsillitis and that other bacterial species, in particular FN, are a subsequent overgrowth phenomenon that develops after an abscess has formed. However, to fully elucidate this possibility, immune response studies would likely be required. Interestingly, patients infected with FN had significantly higher neutrophil counts and C-reactive protein val-

ues, compared with those of other PTA patients, further indicating the pathogenic importance of this bacteria.

Divergent bacterial cultures have been detected in the tonsil core and on the surface swab samples from patients with tonsillar hyperplasia and/or chronic recurrent tonsillitis [22–25]. Importantly, GAS was concomitantly present both in the tonsil core and on the surface in only ~60% of patients. In the case of anaerobic bacteria, including FN, the concordance was even lower [24, 25]. There are no studies concerning this dissimilarity in patients with acute infection. For these reasons, it remains uncertain whether quick diagnostic tests based on the detection of FN and GCS antigens in a surface swab sample could reliably contribute to the diagnosis of bacterial causes of tonsillitis.

In the present study, we report an annual PTA incidence of 41 cases/100,000 population. To our knowledge, this is the highest incidence of PTA in the literature. Previously reported annual incidences were in the range of 10 to 37 cases/100,000 population [15, 26–28]. In previous studies, 31%–46% of diagnosed PTAs were preceded by symptoms of sore throat for which patients sought medical attention [15, 29]. In our study, 38% of the patients had taken antibiotics before admission, and it seems reasonable to assume that an even larger number had consulted their general practitioner with symptoms of sore throat. Previous studies suggest that there could be a protective effect of appropriate antibiotics administration to patients with bacterial tonsillitis to avoid suppurative complications [30, 31]. With the caveat that the only systematic review [30] primarily contains very old data from a period when the prevalence of PTA in untreated patients was much higher than it is today, it is possible to speculate that the high incidence of PTA in Aarhus County could be a consequence of the relatively restrictive antibiotics policy. Furthermore, the reliance on the GAS antigen throat swab for the diagnosis of bacterial tonsillitis likely leads to false negative results that could contribute to subsequent development of PTA in tonsillitis cases that were initially left untreated.

To our knowledge, the present study is the first report in which FN was the most prevalent bacterial pathogen in patients with PTA. This study strongly implies that FN is of pathogenic importance in PTA, because patients infected with this bacteria had significantly larger immune responses than other PTA patients had and because 81% of the FN detected grew as pure culture. We also document the highest incidence of PTA reported in recent literature. Taken together, these observations challenge the conventional wisdom that acute bacterial tonsillar infection is caused primarily by GAS and raise concern that highly virulent bacteria are left initially untreated. Finally, reliance solely on the rapid streptococcal antigen tests for determining the appropriateness of antibiotics treatment in tonsillitis seems inadequate, because it carries a significant risk of undertreatment.

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