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ORIGINAL ARTICLE

Invasive *Fusobacterium necrophorum* infections and Lemièrre's syndrome: the role of thrombophilia and EBV

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Abstract The purpose of this investigation was to describe the clinical spectrum of invasive *Fusobacterium necrophorum* infections and Lemièrre's syndrome, to examine the role of underlying thrombophilia and concomitant mononucleosis in Lemièrre's syndrome, and to describe thromboembolic complications. Patients with invasive *F. necrophorum* infections were identified either prospectively or retrospectively through the regional database of clinical microbiology from 2000 to 2015. Patient records were reviewed and blood samples from patients with Lemièrre's syndrome were collected for Epstein–Barr virus (EBV) serology and screening for thrombophilia. Of the 65 patients included, 33 had Lemièrre's syndrome. Of the remaining 32 patients, other infections of the respiratory tract and abdominal or urogenital infections were most common. Patients with Lemièrre's syndrome or other tonsillar infections were younger than patients from the other groups. For Lemièrre's syndrome, the 26 patients with severe sepsis on admittance had longer duration of symptoms. Three of five patients who developed distant manifestations had more than 14 days of symptoms. Jugular vein thrombosis was verified in 14 patients, two of whom developed serious complications. Three of 26 patients tested had factor V Leiden mutation, corresponding to the background prevalence. One of 22 patients tested had a concomitant EBV infection. This study confirms earlier studies of the clinical spectrum caused by *F. necrophorum*. For Lemièrre's syndrome, the study adds to the knowledge on thromboembolic outcome, demonstrating that jugular vein thrombosis may cause severe complications. The time to treatment seems to be important for the risk of severe disease. In this study, concomitant EBV infection or underlying thrombophilia was uncommon.

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Introduction

Lemièrre's syndrome (LS) is a severe infection that typically starts with tonsillitis and progresses to involve the parapharyngeal space and the jugular vein, leading to thrombophlebitis and pulmonary septic emboli. *Fusobacterium necrophorum* is the causative pathogen in the majority of cases. Much of the knowledge on the natural course of LS stems from the original reports by Lemièrre [1]. In addition, numerous case reports have been published (for a summary, see [2]) and a few larger observational studies [3–8].

The pathogenesis of LS is largely unknown. Invasion and thrombus formation may be due to bacterial or host factors. We have previously shown that *F. necrophorum* binds to and activates components of the pro-coagulant and pro-inflammatory contact system [9], and can acquire plasmin activity [10]. An outer membrane protein of *F. necrophorum* that binds to endothelium was recently described for the animal pathogen *F. necrophorum* subspecies *necrophorum*, and could possibly direct the infection to involve the blood vessels [11]. Host factors could also play a role in *F. necrophorum* invasion and entry into the veins, and, subsequently, in thrombus formation. A few previous case reports or case series have reported thrombophilia in association with LS [12–15]. One

survey reported underlying thrombophilia in five of seven children with acute otitis media complicated by sinus thrombosis, but fusobacteria were not specifically reported in that survey [16]. Epstein–Barr virus (EBV) infection concomitant with LS has been described in several case reports (see [2] for references). In a series of six cases, three had evidence of acute EBV infection and the severity of LS seemed to be worse in patients with concomitant mononucleosis [17]. However, the true prevalence of EBV infection in patients with LS is unknown.

The aims of this study were to add to the previous knowledge of the clinical spectrum of LS and other invasive infections with *F. necrophorum* by describing 65 cases between 2000 and May 2015, to describe thromboembolic complications, and to investigate the frequency of underlying thrombophilia and concomitant EBV infection in LS.

Patients and methods

Identification of cases, case definition of LS, and analyses of patient blood samples

The study consists of a retrospective and a prospective part spanning the period from 2000 to May 2015 in the region of Skåne at the southern-most part of Sweden. The region has a population of approximately 1.2 million and ten hospitals from primary to tertiary level. The patients were identified by searching the databases of the Clinical Microbiology Laboratory (Skåne region, Sweden) for *F. necrophorum* isolated from blood or other normally sterile sites. In addition, we gained knowledge of three patients with culture-negative LS (or in one case with bacteria other than *F. necrophorum*) who were included by clinical criteria (see below). Informed consent to participate in the study was obtained from the patients. Diseased patients were included with the consent of the respective caregiver. Cases before 2008 were all included retrospectively. From 2008, the clinical microbiology department alerted us when a blood culture positive for *F. necrophorum* was encountered, and LS patients were included prospectively in our hospital from that time point, and from May 2012 from the whole region. In total, 14 LS patients were included prospectively from 2008 to May 2015. At a later follow-up search of the databases, ten additional LS cases between the years 2008 and 2015 were found and included retrospectively. The main reason for prospective inclusion of the patients was to collect pre-immune serum for EBV serology from LS patients. The medical records of both prospectively and retrospectively included patients were reviewed retrospectively.

Pre-immune serum was drawn within the first days of admission for the 14 prospectively included LS patients, and for immune serum at least two weeks later. Plasma for screening for thrombophilia was acquired after recovery for 12 prospectively included LS patients, and at the time of inclusion for 14 retrospectively included LS patients (median 7 years after the LS episode). The screening consisted of analyses for genetic (factor V Leiden and prothrombin 20210 G to A mutation, protein S, protein C, and antithrombin) and acquired (cardiolipin antibodies and lupus anticoagulants) thrombophilia at the Department of Laboratory Medicine, Skåne region, according to the standard methods of the laboratory. Analysis of EBV antibody titers (EBNA, anti-VCA IgG and IgM) from patient sera was performed at the Clinical Microbiology Laboratory, Skåne region, according to the standard methods of the laboratory.

Severe sepsis and septic shock were defined according to the guidelines of the Surviving Sepsis Campaign 2012 [18].

The case definition of LS was modified from the suggestion made by Riordan [2] and requires a history of anginal illness or other primary focus in the head and neck in the preceding 4 weeks or compatible clinical findings, and at least two of the following: (1) isolation of *F. necrophorum* from blood cultures or a normally sterile site; (2) evidence of internal jugular vein thrombophlebitis; (3) evidence of metastatic lesions in the lungs (multifocal pneumonia, or if only one lung infiltrate is present, a typical rounded consolidation, suggestive for septic embolization).

Microbiological methods

According to local blood culture routines, two blood cultures, each with one aerobic and one anaerobic blood culture bottle [BacT/ALERT FA/FN (bioMérieux, Marcy l'Etoile, France) before 2015 and BACTEC Plus Aerobic/BACTEC Lytic Anaerobic (BD, Sparks, MD) from 2015], are drawn. For small children, only aerobic blood cultures are routine [BacT/ALERT PF (bioMérieux) before 2015 and BACTEC Peds Plus (BD) from 2015]. Positive blood culture bottles were subcultured under anaerobic conditions on Fastidious Anaerobe Agar (Lab M, Heywood, UK) with 5 % horse blood and species determination was mostly following the guidelines of the "Anaerobic bacteriology manual" 2002 [19] and was sometimes complemented with sequencing of the 16S rRNA gene. Over the last several years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become the most common method for species determination (see below for details). Susceptibility testing by the Clinical Microbiology Laboratory was done with gradient strips (Etest, bioMérieux) on MH-F agar, or in case of insufficient growth, Fastidious Anaerobe Agar, using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for anaerobic Gram-negative bacteria. The detection of *F. necrophorum* DNA from throat swabs was done by real-time polymerase chain reaction (PCR) of the *rpoB* gene [20].

All bacterial isolates recovered from the microbiology department were analyzed using an ultrafleXtreme MALDI-TOF/TOF instrument with the Biotyper 3.1 software and the BDAL-5627 database (Bruker Daltonics; Bremen, Germany). The direct spotting method without formic acid extraction was used [21], and a score of at least 2.2 was achieved for all strains. The isolates were further characterized to the subspecies level by sequencing of the *gyrB* gene as previously described [9] using subspecies common primers [22].

Statistical analyses were performed using the GraphPad Prism version 6 software. The study was approved by the local research ethical committee (reference number 2011/709).

Results

All *F. necrophorum* infections and LS without verified *F. necrophorum*: epidemiology and microbiology

Sixty-eight patients were identified from the laboratory databases. Two patients declined to participate in the study, and four were lost to follow-up. Three additional LS patients were identified by clinical criteria. Altogether, 65 patients were included in the study.

The Clinical Microbiology Laboratory in the Skåne region serves a population of approximately 1.2 million. The incidence of invasive *F. necrophorum* infections was thus calculated to be 3.7 per million per year. Thirty-three of the included patients fulfilled our criteria for LS, resulting in a yearly incidence of 1.8 cases per million. Nine of the cases were diagnosed in the first 8 years of the study and 23 during the last 7.5 years. The demographic data, co-morbidities, laboratory results, and outcome are summarized in Table 1.

All 48 blood isolates of *F. necrophorum* that could be recovered belonged to the subspecies *funduliforme*. All isolates were deemed sensitive to penicillin, cephalosporin, clindamycin, and metronidazole by the Clinical Microbiology Laboratory.

Lemièrre's syndrome

Microbiology

Blood cultures were positive for 30 of 33 patients with LS. *F. necrophorum* was in monoculture in 20/30 blood cultures. Of the mixed cultures, three grew coagulase-negative staphylococci and one a *Micrococcus*, possibly representing contaminations. One culture grew a *Peptostreptococcus*, one *Veillonella*, and three viridans streptococci. In one patient, *F. necrophorum* was not detected, and blood cultures grew *Arcanobacterium haemolyticum* and viridans streptococci. Two patients had negative blood cultures but a positive PCR for *F. necrophorum* from pleural fluid or a throat swab. These patients who were culture-negative for *F. necrophorum* were all included based on the clinical criteria. In one patient, *F. necrophorum* was found in pulmonary tissue post-mortem. Since 2010 (the year *F. necrophorum* PCR analysis was introduced), all eight throat swabs analyzed from LS patients were positive for *F. necrophorum*. Of the 23 conventional throat swab cultures (which will not detect fusobacteria in part due to aerobic incubation), 19 were negative and four grew beta-hemolytic streptococci (two B, one C, and one G). The strepA antigen test was negative for 24 patients.

Symptoms and signs on admission

According to the medical records on admission, 22 of the 33 patients with LS had sought medical attention prior to admission, most often to a general practitioner. Twelve of these patients had a documented negative strepA test. Mononucleosis was suspected in nine patients, but the test for heterophilic antibodies was only documented in two cases, one positive (which later proved to be false-positive) and one negative. Antibiotics were prescribed for two patients (erythromycin and penicillin V). In one case, fusobacteria were suspected and the patient was told to come back in case of worsening symptoms to rule out LS. The median delay between the onset of symptoms and admission was 5 days (range 1–18 days).

The symptoms and signs on admission are summarized in Fig. 1. Thirty patients had a sore throat or a history of a sore throat within the proceeding weeks, of whom one also had a dental infection and one an otitis media. Two patients had no history of tonsillitis. One had a probable dental focus and one had a recent trauma to the outside of the neck, believed to be a locus minoris for the infection. In 18 patients, unilateral neck tenderness was documented upon admission, and in an additional three patients at a later examination. Whether this tenderness was due to lymphadenopathy or to thrombophlebitis could not be determined from the medical records. A peritonsillar abscess was found in one patient only.

At the time of admission, the majority of patients had laboratory signs of organ failure. Twenty-six patients fulfilled the criteria for severe sepsis on admission, and 29 over the first 24 h after admission. The most common abnormal parameters defining severe sepsis were low platelet counts ($<100 \times 10^9$/L in 14 patients), elevated bilirubin (>35 g/L in 14 patients), and elevated lactate (11 patients.). Six patients developed septic shock, all of whom received inotropic support. The median duration from the onset of symptoms to admittance was significantly longer in the groups with severe sepsis and septic shock (median 6 days, range 1–17 days) than in the group that did not fulfil the criteria for severe sepsis on admittance (median 3.5 days, range 2–5 days) (Mann–Whitney, $p=0.03$). Twelve patients required intensive care, most commonly due to respiratory failure, leading to ventilator assistance in six

Table 1 Summary of demographic data, clinical chemistry findings, severity and outcome for different groups of invasive *F. necrophorum* infections

	Lemièrre's syndrome	Tonsillitis, peritonsillar or retropharyngeal abscess	Other respiratory tract infections	Abdominal/urogenital infections	Other foci[a]
Number of patients	33	10	7	8	7
Age (years)****	19 (15–37)	22 (17–57)	63 (16–79)	61 (35–87)	60 (16–88)
Sex (% male)	55	60	86	75	50
Patients with co-morbidities, *n* (%)[b]	1 (3)	0	5 (71)***	4 (50)*	2 (28)
Days of symptoms prior to admission**	5 (1–18)	3 (1–5)	1 (1–45)	3 (1–28)	4 (1–6)
Platelets ($\times 10^9$/L) (normal >145)***	114 (10–304)	166 (121–292)	300 (207–476)	113 (89–153)	199 (55–268)
CRP (mg/L) (normal <3)	249 (81–444)	208 (119–583)	14 (7–329)	250 (185–338)	249 (32–377)
Bilirubin (μmol/L) (normal <25)	32 (9–172)	22 (17–57)	–	18 (7–33)	12 (7–35)
Creatinine (μmol/L) (normal <105)	113 (47–363)	89 (55–202)	77 (63–127)	143 (65–201)	112 (55–662)
Elevated lactate, *n* (% of patients)[c]	10 (30)	–	–	0	0
Severe sepsis, *n* (% of patients)	26 (79)	3 (30)	2 (29)	6 (75)	4 (57)
Septic shock, *n* (% of patients)	6 (18)	0	0	0	0
ICU, *n* (% of patients)	12 (36)	1 (10)	1 (14)	0	0
Duration of hospital stay (days)**	14 (5–37)	5 (0–36)	11 (3–25)	8 (4–16)	13 (5–51)
Duration of fever (days) after admission**	5 (1–16)	2 (0–5)	4 (1–5)	–	3 (1–21)
Duration of antibiotic treatment (days)*	31 (18–90)	14 (12–45)	17 (4–25)	17 (7–65)	29 (7–83)
Mortality within 4 weeks, *n* (% of patients)	1 (3)[d]	0	0	1 (13)	0

[a] Two DVT, one pericarditis, one septic arthritis, three unknown

[b] Charlson co-morbidity score equal to or greater than 1 [32]

[c] Due to different methods with different normal ranges (in arterial or venous blood), the exact value is not presented

[d] Died prior to admission

Numbers are median and range, unless otherwise stated. If 50 % or more of the data was missing in a group, the result is not shown (marked as –)

For dichotomous variables, all other groups were individually tested against the LS group using Fisher's exact test with a Bonferroni correction. For continuous variables, a Kruskal–Wallis test was used to compare medians of all groups. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$

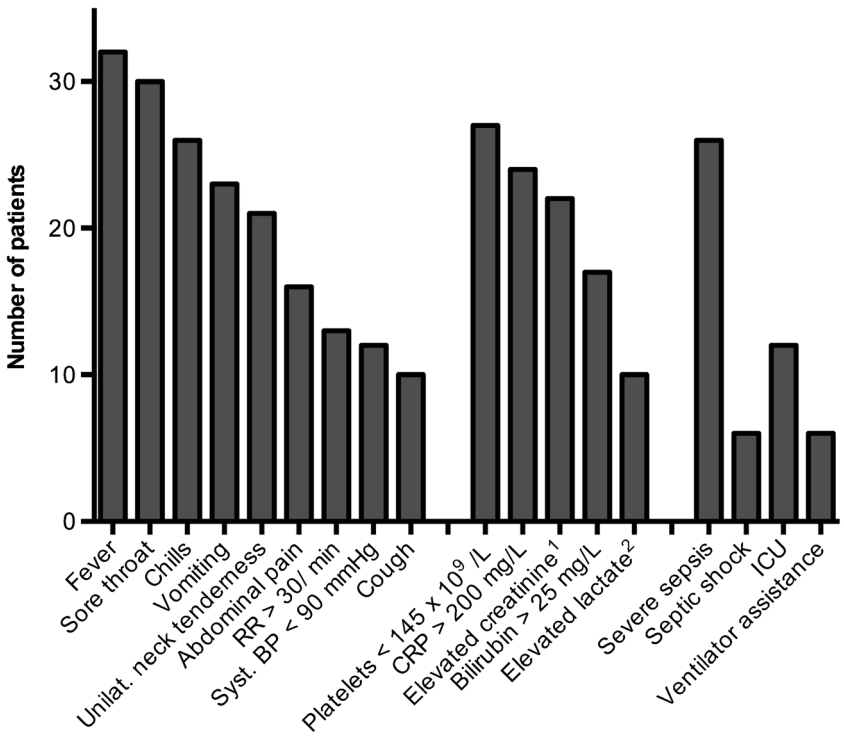

Fig. 1 Lemièrre's syndrome. Symptoms, signs, clinical chemistry findings and severity on admission, and early level of care ([1]>105 μmol/L or >50 % above the patient's habitual level; [2]>2.2 mmol/L for venous blood and >1.6 mmol/L for arterial blood)

patients and non-invasive ventilator assistance in an additional three patients. Dialysis was required for one patient.

Pulmonary involvement

All patients had pulmonary involvement. Seventeen patients had radiological evidence of pulmonary abscesses and 25 patients of pleural effusion. In ten patients, a chest drain was inserted and, most commonly, serous pleural fluid was drained. Empyema, defined as pus or low pH of pleural fluid, was only verified in three patients (but the pH was analyzed for one patient only), two of whom had more than two weeks of symptoms prior to treatment. One patient first received a pleural drainage and later developed a large interlobar abscess, despite on-going antibiotic therapy, necessitating thoracotomy to drain the abscess.

Manifestations outside the neck and lungs

Five patients developed manifestations outside the neck and lungs: two cases of meningitis (one also with multiple brain abscesses), one thrombosis of the cavernous sinus, one osteitis of the iliacal bone with local spread to the iliopsoas muscle, and one mediastinitis. In the two cases of meningitis, diagnosis was verified by elevated cells counts and low glucose of the cerebrospinal fluid (CSF). Culture of the CSF was negative in both patients but, in these cases, anaerobic cultures were not performed. It is notable that, of the five patients who had been ill for more than 14 days before admission, three developed distant manifestations. Splenomegaly or hepatomegaly was noticed in 12 of 18 patients who were examined with either computed tomography (CT) scan or ultrasound.

Thromboembolic complications

Jugular vein thrombosis was verified in 14 of the 23 patients who were examined by either ultrasound, magnetic resonance imaging (MRI), or CT. Six patients with thrombosis were treated immediately after diagnosis with full-dose low molecular weight heparin (LMWH), but one of them presented very late, on day 14 after the onset of fever, and had already, by the time the jugular vein thrombosis was diagnosed, developed a thrombosis of the cavernous sinus and abducens nerve palsy. There was no sign of local abscess formation in connection to the cavernous thrombosis. In one of the patients with full-dose anticoagulation, the thrombosis progressed from 2 to 7 cm, reaching the jugular foramen during the first 3 days of treatment. Two of the patients with jugular vein thrombosis were treated with prophylactic dose LMWH only, one of whom had a very high thrombosis reaching the sigmoid sinus, which comprised the drainage of the central nervous system (CNS), and later resulted in a one-sided palsy of the hypoglossal and glossopharyngeal nerves. Six patients with jugular vein thrombosis did not receive any or a few doses only of anticoagulants and did not develop any complications. The duration of anticoagulant therapy differed between the patients. LMWH was given initially to all anticoagulated patients. Three patients subsequently received warfarin for 6 months, and three received LMWH for the entire course of treatment, which varied between 19 days to 3 months.

Screening for thrombophilia

Plasma from 26 patients with LS was collected and analyzed for thrombophilia as described above. A previous study of 287 healthy blood donors from the same region was used as a control for fV mutation [23]. Three (12 %) of the patients and 29 (10.1 %) of the healthy blood donor controls had fV mutation. There was no significant difference between the groups using Fisher's exact test (p=0.7). One of the patients had both fV mutation and lupus anticoagulants. No other thrombophilia was detected. All the three patients with underlying thrombophilia had a CT scan of the neck at the time of admission and only one had a verified jugular vein thrombosis. The two patients with complicated thrombosis (thrombosis of the cavernous sinus and high jugular vein thrombosis with hypoglossal paresis) had normal coagulation screening.

EBV

In total, 23 patients with LS were tested for EBV. Fourteen patients had already been tested as part of the clinical work-up. In addition, eight more paired sera collected prospectively between the years 2008 and 2015 were analyzed for EBV antibodies. Only one patient was positive for VCA-IgM, indicating a concomitant mononucleosis. One patient died from LS prior to admission, and the forensic testing revealed high numbers of EBV copies in the CSF (26,000/mL) and serum (500 copies/mL), but anti-EBV antibodies could, unfortunately, not be analyzed.

Antibiotic therapy and outcome

All patients were treated with a β-lactam antibiotic on admission, most commonly a cephalosporin. Six patients received a combination of a β-lactam and clindamycin or metronidazole. On day 5, when the etiology was known in most cases, 28 patients received a combination of a β-lactam (most commonly a penicillin) and either metronidazole or clindamycin. At discharge, single therapy with either metronidazole or clindamycin was the most common treatment (Fig. 2). The median total duration of antibiotic therapy was 31 days (range 18–90 days).

The median time to defervescence (defined as the day after the last time a temperature of 38 °C or more was measured) was 5 days (range 1–16 days) after admission and start of antibiotic therapy. In many cases, the patient seemed to improve initially,

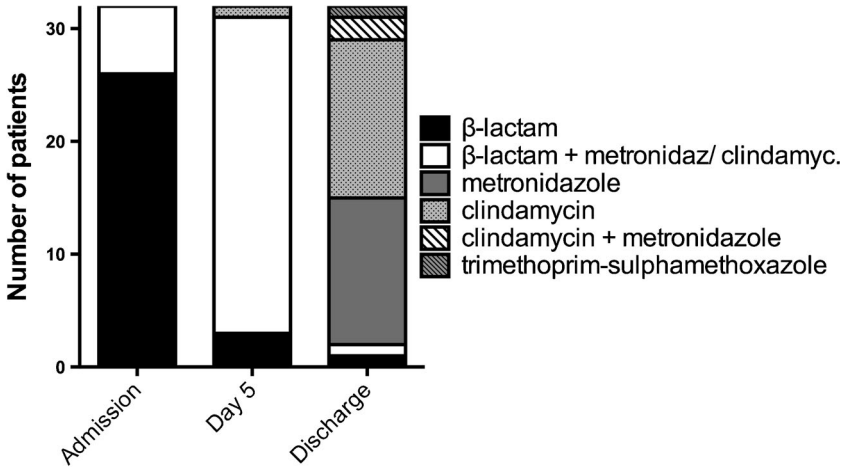

Fig. 2 Lemièrre's syndrome. Antibiotic therapy per patient on admission, on day 5, and at discharge [Antibiotics that were changed after less than 24 h were excluded. Levofloxacin or a macrolide was often added to the initial therapy to cover legionellosis (not shown)]

but after a few days, the fever reoccurred. No conclusion on the type of antibiotic treatment and outcome could be drawn. Pulmonary abscesses were seen in 17 patients and not associated with longer duration of fever, whereas in seven of the ten patients who received a pleural drainage, the duration of fever was longer than 10 days. There was no in-hospital mortality, but one patient was never admitted and died at home, and was diagnosed on forensic examination. Neurological sequelae were documented in one patient with a hypoglossal paresis. Respiratory sequelae were not documented, but pulmonary function was generally not examined on follow-up.

Non-Lemièrre invasive infections

Infections of the respiratory tract

Seventeen patients had *F. necrophorum* infections of the respiratory tract but did not fulfil criteria for LS. Four patients with a positive blood culture for *F. necrophorum* had a peritonsillar abscess, without any signs of further invasion or involvement of the jugular vein or lungs. They generally had mild disease. One patient had a retropharyngeal abscess that had progressed per continuum to the mediastinum and pleura, from which culture yielded *F. necrophorum* together with viridans streptococci and mixed anaerobic bacteria. Five patients had tonsillitis only. Of these, three had Lemièrre-like conditions with cough and unilateral tenderness of the neck, two of whom had severe sepsis, and one of whom had a single pulmonary infiltrate, but not fulfilling our case definition for LS.

Two patients had a diagnosis of a probable aspiration pneumonia, of whom one was a young woman who was found unconscious after intoxication. Two patients had lobar pneumonia and two patients had pneumonia with empyema. One patient developed high fever after a dental extraction, with no signs of dissemination other than a positive blood culture. Three of the six patients with non-Lemièrre pleuropulmonary infections had serious underlying chronic diseases.

Abdominal and urogenital infections

Of the eight patients with bacteremia originating from the urogenital or abdominal tract, three had an infection of the liver or a manifestation adjacent to the liver: one had a portal vein thrombosis diagnosed after the third reoccurrence of fever and two had liver abscesses (one a week after extirpation of anal polyp and one of whom also developed septic arthritis of the acromioclavicular joint). These three patients had no serious underlying diseases.

One patient had a tubo-ovarial abscess and, in one patient, diverticulosis was believed to be the origin. Three patients had advanced cancer of the urogenital or gastrointestinal tract and the *F. necrophorum* bacteremia was believed to originate from damaged barriers in the gastrointestinal tract due to the tumor invasion.

Other primary foci and unknown origin

Of the remaining seven patients, three patients had a positive blood culture for *F. necrophorum* as the only sign of dissemination and no primary focus was found. One patient had septic arthritis of the shoulder without any other apparent focus. One patient with lung cancer and invasion of the mediastinum developed purulent pericarditis a few days after bronchoscopy. She was also undergoing dental treatment for severe periodontitis. One very unusual case was a woman who was admitted with fever and complaints of pain proximal to the knee after a minor trauma. She was found to have multiple septic pulmonary emboli, but no symptoms from the upper respiratory tract. Later during the course of disease, she was diagnosed with a deep vein thrombosis (DVT) in the same leg as the original trauma. Another patient was also diagnosed with a DVT. He developed fever over the next couple of days, and, after one week, blood cultures grew *F. necrophorum* and a CT scan showed a large abscess of the calf.

Discussion

This study describes the spectrum of invasive infections with *F. necrophorum* and LS. Whereas the groups of tonsillitis, peritonsillar abscess, and LS have much in common, mainly affecting teenagers and young adults, the other invasive infections with *F. necrophorum* are very diverse, mainly affecting chronically ill patients with predisposing diseases such as metastatic cancer. In our survey and others', there are cases with evidence of direct spread of a retropharyngeal/parapharyngeal abscess to the mediastinum and secondary to the lungs. To avoid inclusion of these patients in the LS group, we have specified the requirements of the radiological findings defining LS. We agree that a wider definition as suggested by Hagelskjaer Kristensen and Prag [7], including all invasive *F. necrophorum* infections with a primary focus in the head and neck (even when a positive blood culture is the only sign of dissemination), may give clinicians a simple way of paying attention to the potentially serious condition, but in order to pinpoint the particular syndrome involving the jugular vein that is clearly confined to a certain young age group, we decided to use a more narrow definition. We believe this will help in finding clues to the pathogenesis and possibly in obtaining a better understanding of treatment options and outcome. Only children above 13 years of age were included in the study, which may explain why we did not observe otitis as a primary focus in our study. However, there were no blood cultures positive for *F. necrophorum* from children under the age of 13 years, but this may be partly explained by the frequent routine of only aerobic blood cultures in small children. Also, anaerobic culturing from the middle ear or at surgical treatment for mastoiditis is not routinely performed unless there is an abscess formation.

Since there is no ICD10 code for LS, we could not search the diagnosis database for culture-negative LS cases, but we gained knowledge of three additional cases of LS without positive blood cultures for *F. necrophorum*. We cannot exclude the possibility that there are more clinical cases, but we believe that the number is very limited when a diagnosis of LS has been made, since infectious disease physicians are always involved in the care of LS patients. A larger problem is that there was a sharp rise in the incidence of LS over the study period, with 23 of the cases diagnosed during the last 7.5 years of the study. There have not been any changes in the national treatment guidelines for tonsillitis that should have an impact on non-streptococcal tonsillitis. We believe that improved microbiological methods and higher awareness of LS are more likely reasons for the increased incidence. At the end of the study period, we saw more mild cases (data not shown), which would probably have been overlooked or misdiagnosed during the earlier years.

Using a narrow definition of LS, we found that all patients were between 15 and 37 years old, and most patients were below 25 years old. The reason why LS is restricted to this age group is not clear. One study using PCR from throat swabs of healthy recruits and students found *F. necrophorum* in about 20 % of the individuals [22], and *F. necrophorum* is an important pathogen causing tonsillitis in the same age group [24–26]. In contrast, study of the microbiota of the tonsils of 20 patients after tonsillectomy showed that *F. necrophorum* was almost absent in children and healthy young adults, but prevalent in young adults with recurrent tonsillitis. However, the number of patients in each group was small [27]. It is possible that a recent acquisition of *F. necrophorum* in combination with a lack of immunity could be important for the development of LS.

We observed tonsillitis caused by *F. necrophorum* accompanied by bacteremia, but not fulfilling our criteria for LS, in five patients. It is reasonable to believe that LS originates in a *F. necrophorum* tonsillitis that progresses to the parapharyngeal space. However, there is yet no evidence that the treatment of *F. necrophorum* tonsillitis can prevent LS or peritonsillar abscess or even reduce the duration of symptoms. Knowing that LS is very rare, the challenge is to identify the patients who are at risk of developing LS, in the same young age group in which *F. necrophorum* tonsillitis seems to be a common, and probably often uncomplicated, condition. In the present study, a majority of the patients had already sought medical attention prior to admittance. On admittance, most patients fulfilled the criteria for severe sepsis. However, the duration of symptoms was significantly longer in the group with severe sepsis compared to the patients who did not fulfil the criteria. Therefore, it is likely that many of the signs and symptoms on admission had not developed at the time of the first medical attention. Knowledge about LS among general practitioners, and close follow-up of patients with strepA-negative tonsillitis without spontaneous improvement should at least make it possible to avoid the longest delays (in our study up to 18 days).

F. necrophorum has been reported to be the most common pathogen found in peritonsillar abscesses [28], and a rise in antibody titers against *F. necrophorum* was demonstrated in sera from patients with peritonsillar abscess [29]. In our survey, we found five patients with a peritonsillar abscess and a positive blood culture for *F. necrophorum*, which seems low considering that peritonsillar abscess is a common diagnosis. The reason for this is probably that culturing from blood is not normally performed in this uncomplicated condition, and the prevalence of positive blood cultures in patients with peritonsillar abscesses is, thus, not known. However, only one of the patients with LS had a peritonsillar abscess, which is in line with the findings of Hagelskjaer Kristensen et al. [7], but contrasting to the original finding by Lemièrre, who stated that a peritonsillar abscess was a common precursor of LS [1, 2]. In the pre-antibiotic era, this may have been the case, whereas nowadays, patients with a peritonsillar abscess are almost always receiving antibiotics in combination with drainage, possibly hindering the development of LS. Less apparent infections of the parapharyngeal space, leading to invasion of the jugular vein, may be more likely to be left untreated.

Dissemination to the lungs is caused by septic embolization, whereas distant manifestations such as bone and joint infections must arise from generalized bacteremia. Dissemination to distant sites and abscess formation surrounding the jugular vein were uncommon in the present study. There was a tendency that such patients had delayed diagnosis. Lemièrre reported very frequent distant manifestations, and also local suppuration surrounding the jugular vein [1]. In our survey, earlier recognition and treatment likely prevented distant or severe local complications for most of the patients.

Jugular vein thrombosis could be visualized in 14 patients, but involvement of the vein lies within the definition, whether directly visualized or not. Most patients with a thrombosis did not undergo repeated radiology or ultrasound, and the development of the thrombosis is, thus, unclear. However, one patient with an occluding thrombus progressed during the first 3 days of full-dose anticoagulation, but she did not develop any serious complications more than local swelling and tenderness. In contrast, the jugular vein thrombosis resulted in serious complications in two cases. None of them had received full-dose anticoagulant therapy prior to the complication (one had received prophylaxis dose only and later developed ipsilateral hypoglossal and glossopharyngeal paresis, and one presented on day 14 of fever with a cavernous sinus thrombosis already on admission). Whether full-dose anticoagulation could have prevented these complications is not clear, and in the case of the cavernous sinus thrombosis, the delay in diagnosis and antibiotic treatment may have been more important than the lack of antithrombotic treatment. Still, it is notable that both serious complications developed before full dose antithrombotic treatment was given. The incidence of serious complications of the jugular vein thrombosis is not known. We found two cases of complications, whereas Hagelskjaer Kristensen and Prag et al. did not find any sinus thrombosis or other complications due to the thrombosis in their study from Denmark [7]. Larger retrospective studies to assess the risk of sinus thrombosis are warranted, which could help in the decision-making on whether or not to anticoagulate.

Our study shows that the majority of patients with LS do not have thrombophilia as defined above. Moreover, there was no apparent correlation between thromboembolic events and underlying thrombophilia. The study is underpowered to detect small differences, but suggests that routine screening for thrombophilia may not be indicated in patients with thrombosis due to LS. Screening for thrombophilia was done according to the local guidelines for coagulation screening of patients with unusual thrombosis. Other, more unusual causes of thrombophilia can not, of course, be ruled out, but are unlikely. For retrospectively included patients, the testing was sometimes done many years after recovery (mean 7 years), which is of no importance for genetic thrombophilia but could theoretically affect the acquired thrombophilia. Lupus anticoagulants and cardiolipin antibodies are, in most cases, permanent; however, they can fluctuate in titer over time. In some rare cases, they could also disappear [30, 31].

Serological evidence for a concomitant EBV infection was only present in one of 22 patients tested, and elevated EBV virus copies were found in one patient on forensic examination. Whether this represented a re-activation or a primary infection is not clear. Thus, EBV infection seems to be less important than expected from previous case reports and case series, which probably reflects a tendency to publish positive findings. However, a concomitant infection due to any respiratory bacterium or virus, including EBV, may still be important in facilitating for *F. necrophorum* invasion.

This study confirms the findings of earlier studies [3–8] that *F. necrophorum* seems to act as a primary pathogen in a younger age group causing infections mainly originating at the tonsils, and as an opportunist in older age groups, causing infections due to factors like aspiration, or barrier leakage in patients with intra-abdominal cancers. For LS, the study adds to the knowledge about thromboembolic outcome, although no conclusions can be drawn on the risk of severe complications and treatment options. We also find that the time to appropriate treatment seems to be an important factor for the risk of developing severe complications and distant manifestations. Among the patients with LS, concomitant EBV infection or underlying thrombophilia appears to be uncommon. To facilitate further studies, and to increase the awareness of LS, an ICD code for LS would be very useful.

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References

1. Lemièrre A (1936) On certain septicaemias due to anaerobic organisms. Lancet 227(5874):701–703
2. Riordan T (2007) Human infection with *Fusobacterium necrophorum* (Necrobacillosis), with a focus on Lemierre's syndrome. Clin Microbiol Rev 20(4):622–659
3. Nohrström E, Mattila T, Pettilä V, Kuusela P, Carlson P, Kentala E, Mattila PS (2011) Clinical spectrum of bacteraemic *Fusobacterium* infections: from septic shock to nosocomial bacteraemia. Scand J Infect Dis 43(6–7):463–470. doi:10.3109/00365548.2011.565071

4. Brazier JS, Hall V, Yusuf E, Duerden BI (2002) *Fusobacterium necrophorum* infections in England and Wales 1990–2000. J Med Microbiol 51(3):269–272
5. Huggan PJ, Murdoch DR (2008) Fusobacterial infections: clinical spectrum and incidence of invasive disease. J Infect 57(4):283–289. doi:10.1016/j.jinf.2008.07.016
6. Afra K, Laupland K, Leal J, Lloyd T, Gregson D (2013) Incidence, risk factors, and outcomes of *Fusobacterium* species bacteremia. BMC Infect Dis 13:264. doi:10.1186/1471-2334-13-264
7. Hagelskjaer Kristensen L, Prag J (2008) Lemierre's syndrome and other disseminated *Fusobacterium necrophorum* infections in Denmark: a prospective epidemiological and clinical survey. Eur J Clin Microbiol Infect Dis 27(9):779–789
8. Hagelskjaer LH, Prag J, Malczynski J, Kristensen JH (1998) Incidence and clinical epidemiology of necrobacillosis, including Lemierre's syndrome, in Denmark 1990–1995. Eur J Clin Microbiol Infect Dis 17(8):561–565
9. Holm K, Frick IM, Björck L, Rasmussen M (2011) Activation of the contact system at the surface of *Fusobacterium necrophorum* represents a possible virulence mechanism in Lemièrre's syndrome. Infect Immun 79(8):3284–3290. doi:10.1128/iai.05264-11
10. Holm K, Rasmussen M (2013) Binding and activation of plasminogen at the surface of *Fusobacterium necrophorum*. Microb Pathog 59–60:29–32. doi:10.1016/j.micpath.2013.04.004
11. Kumar A, Menon S, Nagaraja TG, Narayanan S (2015) Identification of an outer membrane protein of *Fusobacterium necrophorum* subsp. *necrophorum* that binds with high affinity to bovine endothelial cells. Vet Microbiol 176(1–2):196–201. doi:10.1016/j.vetmic.2014.12.015
12. Goldenberg NA, Knapp-Clevenger R, Hays T, Manco-Johnson MJ (2005) Lemierre's and Lemierre's-like syndromes in children: survival and thromboembolic outcomes. Pediatrics 116(4):e543–e548
13. Cho YP, Choi SJ, Jung BH, Hwang JW, Han MS, Kim YH, Kwon TW, Lee SG (2006) Lemierre's syndrome in a patient with antiphospholipid syndrome. Ann Vasc Surg 20(2):274–277. doi:10.1007/s10016-006-9004-4
14. Hope A, Bleach N, Ghiacy S (2002) Lemierre's syndrome as a consequence of acute supraglottitis. J Laryngol Otol 116(3):216–218
15. Klinge L, Vester U, Schaper J, Hoyer PF (2002) Severe Fusobacteria infections (Lemierre syndrome) in two boys. Eur J Pediatr 161(11):616–618. doi:10.1007/s00431-002-1026-5
16. Oestreicher-Kedem Y, Raveh E, Kornreich L, Yaniv I, Tamary H (2004) Prothrombotic factors in children with otitis media and sinus thrombosis. Laryngoscope 114(1):90–95. doi:10.1097/00005537-200401000-00015
17. Chacko EM, Krilov LR, Patten W, Lee PJ (2010) Lemierre's and Lemierre's-like syndromes in association with infectious mononucleosis. J Laryngol Otol 124(12):1257–1262. doi:10.1017/s0022215110001568
18. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R; Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup (2013) Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 39(2):165–228. doi:10.1007/s00134-012-2769-8
19. Jousimies-Somer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM (2002) Anaerobic bacteriology manual, 6th edn. Star Publishing Company, Belmont
20. Aliyu SH, Yong PF, Newport MJ, Zhang H, Marriott RK, Curran MD, Ludlam H (2005) Molecular diagnosis of *Fusobacterium necrophorum* infection (Lemierre's syndrome). Eur J Clin Microbiol Infect Dis 24(3):226–229. doi:10.1007/s10096-005-1298-6
21. Veloo AC, Elgersma PE, Friedrich AW, Nagy E, van Winkelhoff AJ (2014) The influence of incubation time, sample preparation and exposure to oxygen on the quality of the MALDI-TOF MS spectrum of anaerobic bacteria. Clin Microbiol Infect 20(12):O1091–O1097. doi:10.1111/1469-0691.12644
22. Jensen A, Hagelskjaer Kristensen L, Prag J (2007) Detection of *Fusobacterium necrophorum* subsp. *funduliforme* in tonsillitis in young adults by real-time PCR. Clin Microbiol Infect 13(7):695–701
23. Svensson PJ, Zöller B, Dahlbäck B (1997) Evaluation of original and modified APC-resistance tests in unselected outpatients with clinically suspected thrombosis and in healthy controls. Thromb Haemost 77(2):332–335
24. Hedin K, Bieber L, Lindh M, Sundqvist M (2015) The aetiology of pharyngotonsillitis in adolescents and adults—*Fusobacterium necrophorum* is commonly found. Clin Microbiol Infect 21(3):263.e1–263.e7. doi:10.1016/j.cmi.2014.08.020
25. Jensen A, Hansen TM, Bank S, Kristensen LH, Prag J (2015) *Fusobacterium necrophorum* tonsillitis: an important cause of tonsillitis in adolescents and young adults. Clin Microbiol Infect 21(3):266.e1–266.e3. doi:10.1016/j.cmi.2014.09.020
26. Batty A, Wren MW, Gal M (2005) *Fusobacterium necrophorum* as the cause of recurrent sore throat: comparison of isolates from persistent sore throat syndrome and Lemierre's disease. J Infect 51(4):299–306. doi:10.1016/j.jinf.2004.09.013
27. Jensen A, Fagö-Olsen H, Sørensen CH, Kilian M (2013) Molecular mapping to species level of the tonsillar crypt microbiota associated with health and recurrent tonsillitis. PLoS One 8(2):e56418. doi:10.1371/journal.pone.0056418
28. Ehlers Klug T, Rusan M, Fuursted K, Ovesen T (2009) *Fusobacterium necrophorum*: most prevalent pathogen in peritonsillar abscess in Denmark. Clin Infect Dis 49(10):1467–1472. doi:10.1086/644616
29. Klug TE, Henriksen JJ, Rusan M, Fuursted K, Krogfelt KA, Ovesen T, Struve C (2014) Antibody development to *Fusobacterium necrophorum* in patients with peritonsillar abscess. Eur J Clin Microbiol Infect Dis 33(10):1733–1739. doi:10.1007/s10096-014-2130-y
30. Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA (2010) Antiphospholipid syndrome. Lancet 376(9751):1498–1509. doi:10.1016/s0140-6736(10)60709-x
31. Gebhart J, Posch F, Koder S, Perkmann T, Quehenberger P, Zoghlami C, Ay C, Pabinger I (2015) Increased mortality in patients with the lupus anticoagulant: the Vienna Lupus Anticoagulant and Thrombosis Study (LATS). Blood 125(22):3477–3483. doi:10.1182/blood-2014-11-611129
32. Charlson ME, Pompei P, Ales KL, MacKenzie CR (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 40(5):373–383