5 Baird MF, Graham SM, Baker JS, Bickerstaff GF. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. *J Nutr Metab* 2012; **2012**: 960363. doi: 10.1155/2012/960363.
6 Fraser AD. Chemotherapy as a cause of low serum creatine kinase activity (Letter). *Clin Chem* 1980; **26** (11): 1629–30.
7 Gunst JJ, Langlois MR, Delanghe JR, De Buyzere ML, Leroux-Roels GG. Serum creatine kinase activity is not a reliable marker for muscle damage in conditions associated with low extracellular glutathione concentration. *Clin Chem* 1998; **44** (5): 939–43
8 Mueller K, Swanson R. Possible captopril inhibition of creatine kinase: a case report (Abstract). *Clin Chem* 1985; **31**: 941.
9 Bruns DE, Morgan WS, Davis JE, Ladenson JH. Low apparent creatine kinase activity and prolonged lag phases in serum of patients with metastatic disease: elimination by treatment of sera with sulfhydryl agents. *Clin Chem* 1976; **22** (11): 1889–95.
10 Wei N, Pavlidis N, Tsokos G, Elin RT, Plotz PH. Clinical significance of low creatine phosphokinase values in patients with connective tissue diseases. *JAMA* 1981; **246** (17): 1921–3.
11 De Scheerder IK, Delanghe JR, De Buyzere ML, Hollanders G, Clement DL, Leroux-Roels GG. Low serum creatine kinase in patients with infective endocarditis. *Clin Chim Acta* 1991; **197**: 117–22.
12 Delanghe JR, De Buyzere ML. Low serum creatine kinase activity in rheumatoid arthritis. *J Rheumatol* 1997; **24** (1): 231–2.
13 Van de Moortel L, Speeckaert MM, Fiers T, Oeyen S, Decruyenaere J, Delanghe J. Low creatine kinase activity is associated with worse outcome in critically ill patients. *J Crit Care* 2014; **29** (5): 786–90.
14 Delanghe J, De Buyzere M, De Scheerder I, Van Rostenberghe H, Rodenbach J, Faust U. Post-transcriptional modification of creatine kinase in intensive care patients. *Clin Chim Acta* 1990; **187** (2): 115–24.
15 Rosalki SB. Low serum creatine kinase activity. *Clin Chem* 1998; **44** (5): 905.

Bacteraemia caused by *Fusobacterium necrophorum*: a clinical approach to Lemierre's syndrome

J-H. LEE*, T. U. YANG†, H. W. LEE‡, S. Y. YOON‡ and C. S. LIM‡
Department of Laboratory Medicine, Osan Hankook General Hospital, Osan-si, Gyeonggido; †Korea Centers for Disease Control and Prevention; and ‡Department of Laboratory Medicine, Korea University College of Medicine, Seoul, Korea

Differential diagnosis can be established by a primary physician through assessment of patient information and presentation. However, it can be hard to discriminate disease entities when symptoms overlap. For example, the differential diagnosis of infective colitis versus other abdominal problems can be complicated. Here, we reported a case of Lemierre's syndrome caused by *Fusobacterium necrophorum*. Characteristically, this case was initially thought to be a common cold with infective viral or bacterial colitis due to diarrhoea symptom. Follow-up study revealed bacteraemia caused by *F. necrophorum*. Lemierre's syndrome can represent septic thrombophlebitis of the internal jugular vein that typically presents as an oropharyngeal infection.[1] This case will help to understand an effective approach for the evaluation of patients with Lemierre's syndrome caused by *F. necrophorum*.

Correspondence to: Dr Chae Seung Lim
Department of Laboratory Medicine, Korea University Guro Hospital 148, Gurodong-ro, Guro-gu, Seoul 152-703, Korea
Email: malarim@korea.ac.kr

A 21-year-old Korean man with no history of serious illness was admitted to the emergency room with fever and a sore throat. The fever had begun five days prior and watery diarrhoea three days prior. The patient experienced a high fever (40.5°C), tachycardia (132 beats/min), and tachypnoea (24 breaths/min), and his blood pressure was reported to be 60 to 100 mmHg. Laboratory data at the time of admission were as follows: peripheral white blood cell count was 5900/μL with 97.5% of cells being neutrophils; total bilirubin was increased (2.13 mg/dL); sodium, potassium, and chloride (Na-K-Cl) levels were 127 mmol/L, 3.3 mmol/L, 92 mmol/L, indicating hyponatraemia, hypokalaemia, and hypochloraemia; and erythrocyte sedimentation rate (ESR) was elevated (19 mm/h). Peripheral blood smear analysis revealed relative neutrophilia, toxic change in neutrophilic series, and normocytic normochromic anaemia. Plasma fibrinogen, fibrin degradation product, and D-dimer levels were increased (489 mg/dL, 15.22 μg/mL, and 3.29 μg/mL, respectively). Negative results were reported for the Widal test, hepatitis B surface antigen, hepatitis B surface antibody, anti-hepatitis C virus antibody, human immunodeficiency antigen and antibody, stool white blood cell, *Clostridium difficile* toxin assay, polymerase chain reaction (PCR) for astrovirus, rotavirus, adenovirus, and norovirus from stool specimens, and PCR for influenza A, influenza B, RSV A, RSV B, parainfluenza, coronavirus, rhinovirus, enterovirus, adenovirus, bocavirus, and metapneumovirus from nasal swab specimens. Acute phase indicators, including C-reactive protein, procalcitonin, and ferritin levels, were markedly increased (245.68 mg/L, >100 ng/mL, and 492.20 ng/mL, respectively). Preliminary analysis suggested infective colitis or sepsis. The patient was administered intravenous ceftriaxone (2 g/day for 4 days) and intravenous metronidazole (500 mg*3/day for three days) was added three days after admission. Four days after admission, ciprofloxacin (400 mg/200 mL*2/day) was administered and clindamycin phosphate (600 mg/4 mL*3/day) was added to the regimen six days after admission. Fever subsided to below 38°C. The patient was discharged without critical sequelae 13 days after admission with an eight-day prescription of post-prophylactic amoxicillin plus potassium clavulanate (375 mg*3/day).

Initial chest radiography did not show active lesions in the lungs. However, patchy opacities in the lower part of the lungs indicated the possibility of pneumonia one day after admission. Hepatosplenomegaly and moderate fatty liver were noted on abdominal computed tomography (CT) on the day of admission. Sinus tachycardia and borderline resting pulmonary hypertension were diagnosed using 2D echocardiography and Doppler ultrasound. Three days after admission, chest CT revealed multiple variable-sized nodules with ground-glass halo in both lungs, subsegmental atelectasis in both basal-dependent lungs, and small pleural effusions in both dependent hemithoraces (Fig. 1). Septic

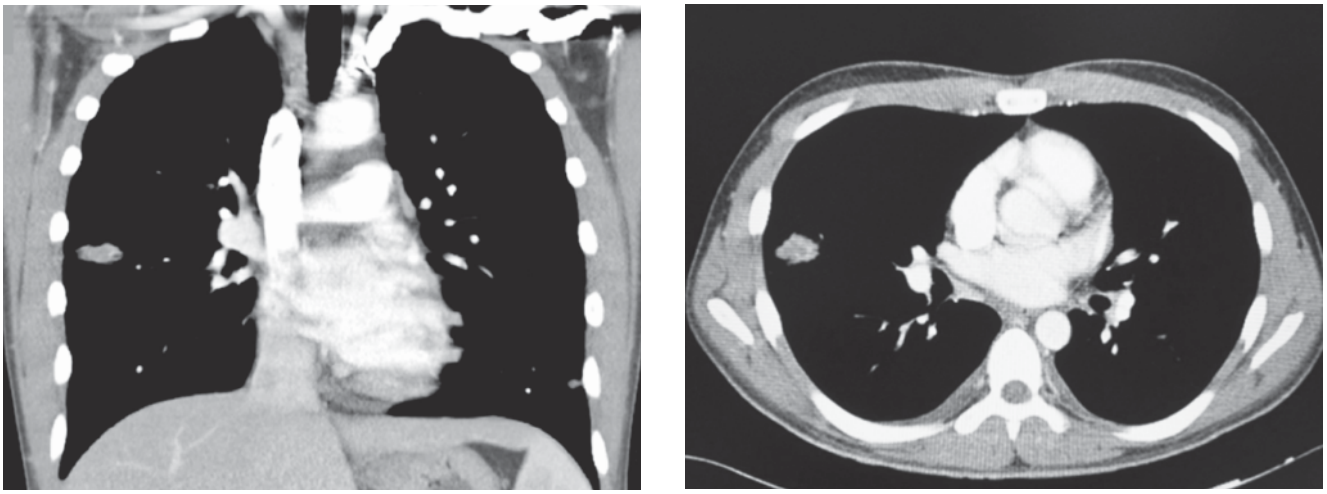

Fig. 1. Computed tomography (CT) images of multiple, variable-sized nodules with ground-glass halo in the lung (images obtained three days after admission).

thrombophlebitis involving the left internal jugular vein (IJV) was suspected owing to the presence of a 2.2-cm elongated abscess (Fig. 2), multiple lymphadenitis in the bilateral neck, diffuse bilateral parotid gland enhancement, and a small consolidative nodule in the right upper lung on a neck CT image obtained nine days after admission.

Blood, sputum, urine, and stool samples were cultured for diagnostic purposes. The automated blood culture system BacT/ALERT 3D (bioMérieux, Durham, NC) was used to determine whether samples were positive for the presence of anaerobic microorganisms after overnight culture in anaerobic blood bottles. The subculture from the centrifuged anaerobic bottle was streaked on blood agar, chocolate agar, and *Brucella* agar, and then incubated in a 5% CO_2 anaerobic jar at 37°C for 48 hours. Growth of small, colourless colonies was observed on the plates following incubation. These colonies were used for biochemical identification using Vitek 2 anaerobe and a *Corynebacterium* identification card (bioMérieux), Rapid ID 32 A (bioMérieux, Marcy-l'Etoile, France), and API 20 A (bioMérieux). Vitek 2 indicated a 95% probability that the colonies were *F. necrophorum*, Rapid ID 32 A showed positive results for indole production and alkaline phosphatase, and API 20 A demonstrated only indole production.

To confirm the subculture results, 16S rRNA gene amplification and sequencing were carried out. 16S sequencing is generally accepted as a standard identification method.[2] Briefly, the culture colony specimen was boiled with 100 μL DNA extraction buffer at 100°C for 20 minutes. Extracted DNA 3.0 μL, 10X buffer 2.0 μL, 10 mmol/L dNTP 0.5 μL, 16S rRNA-F 10 pmol, 16S rRNA-R 10 pmol, and *Taq* polymerase 1 unit were mixed and incubated at 95°C for 20 seconds, 64°C for 30 seconds, and 72°C for 30 seconds for 35 cycles. The PCR end product was loaded for electrophoresis on a 2% agarose gel. The following primer sets were used in PCR reactions: 4F: 5′-TTGGAGAGTTTGATCCTGGCTC-3′, 27F: 5′-AGAGTTTGATCMTGGCTCAG-3′, 534R: 5′-TACCGC GGCTGCTGGCAC-3 801R: 5 -GGCGTGGACTTCCAGGGT ATCT-3 . The BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3130 automated sequencer (Applied Biosystems, Foster City, CA) were used for 16S rRNA sequencing. The sequenced samples were analysed using a BLAST search of the GenBank public database.[3]

The patient was admitted to the emergency room after experiencing fever and sore throat for five days and watery diarrhoea for three days. An initial diagnosis of infective colitis or sepsis was made and the patient was treated with empirical antibiotic therapy under close observation. Three days after admission, Gram-negative rod-shaped bacteria were isolated from patient blood using an anaerobic blood culture protocol. The organism was identified as *F. necrophorum* six days after admission. The initial identification was confirmed using 16S rRNA gene sequencing, in accordance with the criteria of the U.S. Clinical and Laboratory Standards Institute.[4] The patient

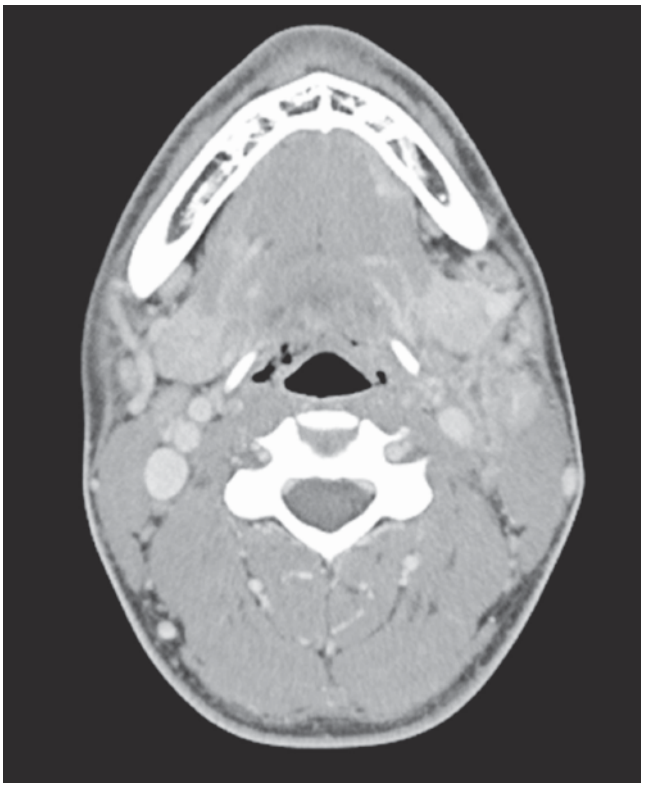

Fig. 2. Septic thrombophlebitis involving the left internal jugular vein with a 2.2 cm (black arrow width) elongated abscess cavity (image obtained nine days after admission).

was treated with a modified antibiotic therapy of ciprofloxacin and clindamycin without anticoagulant treatment. The patient's symptoms improved and he was discharged 13 days after admission and received further out-patient care. *Fusobacterium necrophorum* is an anaerobic, non-mobile, filamentous, non-spore forming Gram-negative bacillus found in normal oral flora[5] and is the causative agent of Lemierre's syndrome.[6,7] Lemierre's syndrome can be presented with septic thrombophlebitis of the IJV that typically presents as an oropharyngeal infection.[1] This syndrome, a severe and life-threatening infection, has been estimated to have higher incidence, mortality, and morbidity than rheumatic fever in adolescents and young adults,[8] and is most commonly reported in otherwise healthy young adults.[9] Characteristically, the patient presenting with watery diarrhoea described in this case report was diagnosed with thrombophlebitis of the left IJV with lung abscess. Watery diarrhoea symptoms can mask the initial diagnosis of Lemierre's syndrome as demonstrated by our initial diagnosis of infective colitis. A diagnosis of Lemierre's syndrome is often not considered until causative (for example, anaerobic, Gram-negative rod-shaped) bacteria are isolated from blood culture, at which point, clinicians will begin testing for septic thrombophlebitis.[6,10] Although Lemierre's syndrome is a rare disease, it should be considered in healthy adolescents presenting with fever and neck pain after minor oropharyngeal infections.[11] Patients suspected of having Lemierre's syndrome should be treated aggressively owing to the high morbidity and mortality associated with the syndrome, even if blood culture tests are negative.[12]

One report of a multicentre study of antimicrobial susceptibility of anaerobic bacteria in Korea in 2012 suggested that piperacillin-tazobactam, cefoxitin, imipenem, meropenem, metronidazole and chloramphenicol showed antimicrobial sensitivity against most anaerobic isolates, but *Fusobacterium* species were not adequately evaluated.[13]

Herein we report a case involving a 21-year-old Korean male presenting with watery diarrhoea who was successfully treated for Lemierre's syndrome caused by *F. necrophorum*. Patients presenting with neck or chest symptoms, fever, sore throat, and with watery diarrhoea should be evaluated for Lemierre's syndrome. It is important for primary care physicians to be aware of the initial characteristic signs of this disease and to be capable of differentiating these symptoms from those caused by other infections, in order to provide prompt and appropriate antibiotic treatment. Radiologic and microbiologic assays are useful tools in the diagnosis of Lemierre's syndrome. Appropriate antibiotic management will help mitigate the rapid and critical symptoms associated with this disease, and thus reduce the need for surgical or other invasive treatment strategies. □

References

1 Kuppalli K, Livorsi D, Talati NJ, Osborn M. Lemierre's syndrome due to *Fusobacterium necrophorum*. *Lancet Infect Dis* 2012; **12** (10): 808–15.

2 Rennie RP, Brosnikoff C, Turnbull L *et al*. Multicenter evaluation of the Vitek 2 anaerobe and *Corynebacterium* identification card. *J Clin Microbiol* 2008; **46** (8): 2646–51.

3 Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. GenBank. *Nucleic Acids Res* 2007; **35** (Database issue): D21–5.

4 Petti CA Bosshard PP, Brandt ME *et al*. Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.

5 Dimitropoulou D, Lagadinou M, Papayiannis T, Siabi V, Gogos CA, Marangos M. Septic thrombophlebitis caused by *Fusobacterium necrophorum* in an intravenous drug user. *Case Rep Infect Dis* 2013; **2013**: 870846.

6 Armstrong AW, Spooner K, Sanders JW. Lemierre's syndrome. *Curr Infect Dis Rep* 2000; **2** (2): 168–73.

7 Golpe R, Marin B, Alonso M. Lemierre's syndrome (necrobacillosis). *Postgrad Med J* 1999; **75** (881): 141–4.

8 Centor RM. Expand the pharyngitis paradigm for adolescents and young adults. *Ann Intern Med* 2009; **151** (11): 812–5.

9 Hagelskjaer Kristensen L, Prag J. Lemierre's syndrome and other disseminated *Fusobacterium necrophorum* infections in Denmark: a prospective epidemiological and clinical survey. *Eur J Clin Microbiol Infect Dis* 2008; **27** (9): 779–89.

10 Leugers CM, Clover R. Lemierre syndrome: postanginal sepsis. *J Am Board Fam Pract* 1995; **8** (5): 384–91.

11 Figueras Nadal C, Creus A, Beatobe S, Moraga F, Pujol M, Vazquez E. Lemierre syndrome in a previously healthy young girl. *Acta Paediatr* 2003; **92** (5): 631–3.

12 Bang YY, Kim JT, Chang WH, Oh TY, Kong JH. Lemierre syndrome. *Korean J Thorac Cardiovasc Surg* 2011; **44** (6): 437–9.

13 Lee Y, Park YJ, Kim MN, Uh Y, Kim MS, Lee K. Multicenter study of antimicrobial susceptibility of anaerobic bacteria in Korea in 2012. *Ann Lab Med* 2015; **35** (5): 479–86.