Liver cirrhosis as a predisposing condition for Legionnaires’ disease: a report of four laboratory-confirmed cases from China

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Here, we describe four cases of laboratory-confirmed Legionella infection. Case 1 was a culture-confirmed case of Legionella infection in a patient with liver cirrhosis. Following this, three other liver cirrhosis cases (cases 2–4) were diagnosed with Legionella infection as confirmed by quantitative real-time PCR. The cause of the pneumonia was determined as Legionella pneumophila by positive direct fluorescence assay and isolation of the causative agent. The infections were successfully treated by administering appropriate antibiotics. These cases highlight the importance of considering Legionella as a cause of pneumonia in patients with liver disease and lung infections. The strain of L. pneumophila isolated from Case 1 was characterized as being closely related to strain Philadelphia-1 (ATCC 33152), which is the type strain of the species, belonging to serogroup 1 and sequence type 36 (ST36), and is known to be distributed worldwide. To our knowledge, this is the first report of Legionella infection on the Chinese mainland for a decade and highlights the need to raise awareness of diagnostic methods for Legionnaires’ disease in China and the requirement for further epidemiological surveillance strategies to monitor this disease.

Introduction

Legionnaires’ disease (LD) is a systemic infection caused by the Gram-negative bacteria Legionella pneumophila. The primary presentation is pneumonia. Members of the genus Legionella are typically found in both the natural environment, such as hot springs and soil, as well as artificially hydrated environments, such as water supply facilities. In studies undertaken between 1994 and 2003, Legionella species accounted for 0.6–3.9% of causative pathogens for all community-acquired pneumonia cases (Miyashita et al., 2006). Legionella pneumophila is responsible for >90% of cases and the most prevalent disease-causing variant of this species is serogroup 1 (Yu et al., 2002). The clinical features of pneumonia caused by Legionella are diverse. Early symptoms include fever, headache, diarrhoea and coughing. During the course of the illness, stupor, respiratory- and even multiorgan failure may develop. Elevation of creatinine kinase has also been reported as a disease-specific symptom of LD (Sopena et al., 1998).

Transmission of bacteria from the environment to humans occurs via the inhalation or aspiration of Legionella-containing aerosols (Blatt et al., 1993; Breiman et al., 1990). Epidemiological analyses based on the molecular typing of clinical isolates of Legionella have detected sporadic, epidemic, and endemic strains (Bernander et al., 2003). Strain differentiation is necessary to identify the source of contamination and determine the route of transmission. Risk factors associated with the occurrence of LD include old age, being male, smoking, having chronic heart and lung diseases, renal or hepatic failure, recent travel with an overnight stay outside of the home, recent changes in domestic plumbing, a history of cancer or haematological malignancies, steroid therapy or other immunosuppressive treatments, and diabetes mellitus (Che et al., 2008; Fields et al., 2002; Miyashita et al., 2006). In addition, numerous case reports suggest that there is a higher rate of Legionella infection among immunocompromised and splenectomized patients compared with immunocompetent patients (Jaeger et al., 1988; Jernigan et al., 1994; Kümper et al., 2008; McClelland et al., 2004; Singh et al., 2002; Lück et al., 2008).
A variety of subtyping techniques have been used to identify and characterize Legionella strains, including monoclonal antibody (mAb) analysis (Joly et al., 1986), amplified fragment length polymorphism (AFLP) analysis (Fry et al., 2002), pulsed-field gel electrophoresis (PFGE) (Casini et al., 2008; Zhou et al., 2010), and, more recently, sequence-based typing (SBT) (Gaia et al., 2005; Ratzow et al., 2007) and nested SBT (Ginevra et al., 2009). The SBT methodology is, in fact, a variation of multilocus sequence typing (MLST) which considers sequence variations not only in housekeeping genes but also in virulence-associated L. pneumophila genes. This method was established by the European Working Group for Legionella Infections and is now used internationally as the standard method for typing L. pneumophila.

Here, we report a L. pneumophila strain that was isolated from bronchoalveolar lavage (BAL) fluid of a liver cirrhosis patient; subsequently L. pneumophila genomic DNA was detected from BAL fluid samples from three other patients with liver diseases. Genotypic characteristics of the first strain and the three strains, identified by quantitative real-time PCR (qPCR) using DNA from positive BAL fluid samples, were determined by PFGE and/or SBT analyses. To our knowledge, this is the first report of LD in a patient suffering from cirrhosis of the liver, and is also the first case of Legionella infection reported on the Chinese mainland for a decade. Thanks to a correct diagnosis, all the patients were successfully treated and fully recovered from LD.

Case report

A 42-year-old male, diagnosed with liver cirrhosis in early 2010, was admitted to an intensive care unit in May 2011 with signs of a respiratory infection. At admission, his blood pressure was 108/69 mmHg, temperature was 38.3°C, pulse rate was 100 min⁻¹ and respiratory rate was 20 min⁻¹. Pulmonary inflammation was diagnosed by computed tomography (CT) (Fig. 1). A sputum sample was collected on day 2 post-admission and yielded Candida albicans growth, following inoculation onto Candida chromogenic agar and incubation at 28°C for 2 days. Five days after admission, the patient’s condition worsened dramatically and a further chest CT scan showed a bilateral spread of the infection in the lungs (Fig. 1). The patient developed shortness of breath, chest tightness, laboured breathing and a worsened cough. A BAL sample was collected for culture and molecular investigations. Genomic DNA was extracted from the BAL fluid. The BAL samples were centrifuged at 6000 g for 10 min and the pellets were resuspended in 2 ml sterile-distilled water (Invitrogen). Genomic DNA was extracted from 0.2 ml resuspended liquid using the QIAamp DNA Mini kit (Qiagen), according to the manufacturer’s instructions. PCR analyses were performed with primers that targeted the Legionella 16S rRNA gene (for the detection of members of the genus Legionella; Yamamoto et al., 1993) and the L. pneumophila mip gene (for the detection of L. pneumophila; Mahbubani et al., 1990), and qPCR analyses were performed with primers that targeted the Legionella 55 rRNA gene (for the detection of members of the genus Legionella). qPCR mixtures (20 μl) contained 2 μl genomic DNA from each sample and fluorescent probes to detect the products. Quantification was performed using the primers L5SL9 (‘-ACTATAGCGATTTGGAAACCA-3’) and L5SR93 (‘-GCGATGACCTTTCCGAT-3’), which were designed to amplify a fragment of the Legionella 55 rRNA gene (Mahbubani et al., 1990), and the molecular beacon HEX-CCGCGCAATGTAGTGTGAGGC-BHQ (Diederen et al., 2006). qPCRs were conducted using a quantitative PCR instrument (Stratagene) with the following reaction conditions: an initial denaturing step of 95°C for 2 min, followed by 40 cycles of 95°C for 10 s and 60°C for 20 s. All the results of the PCRs and qPCRs were positive. Subsequently, Legionella colonies were grown by spreading 0.1 ml of appropriate dilutions of each sample onto buffered charcoal–yeast extract agar (Oxoid) and incubating at 37°C for 10 days. The colonies were identified as L. pneumophila serogroup 1 by using monoclonal antisera (Denka Seiken). On the basis of these results, we made a definitive diagnosis of LD. The patient was treated with clarithromycin and, after 18 days of antibiotic therapy, the patient’s condition improved. Subsequently, signs of lung infection on subsequent CT scans disappeared (Fig. 1).

PFGE was used (Zhou et al., 2010) to characterize this strain of L. pneumophila (named ICDC-LP001). The PFGE pattern of this strain was compared to those present in the reference database for L. pneumophila in China. At the time of comparison, the database contained 516 isolates of environmental L. pneumophila, isolated between 2009 and 2011, and 162 PFGE patterns, including 122 strains isolated from the city our patient originated from. This current strain showed the same pattern as the L. pneumophila reference strain Philadelphia-1 (ATCC 33152T) but was different to all other strains in the database (Fig. 2). Furthermore, we analysed the genomic DNA of this L. pneumophila strain, which had been extracted from BAL fluid, using SBT (Gaia et al., 2005; Ratzow et al., 2007). SBT indicated that the allele numbers for flaA, pilE, asd, mip, monopS, proA and neuA were 3, 4, 1, 1, 14, 9 and 1, respectively. This strain was assigned the sequence type (ST) number ST36, which was the same ST as that of the type strain of L. pneumophila, Philadelphia-1 (ATCC 33152T). By querying the L. pneumophila SBT database (available at http://www.ewgli.org), we found that ST36 was prevalent both in clinical and environmental strains distributed over 25 countries between 1982 and 2011, including one environmental strain that was isolated in Shanghai, China, in 2009.

Subsequently, 10 additional BAL fluid samples, each from separate patients with a liver disease, were collected in order to investigate possible L. pneumophila infection (Table 1). All the patients had a lung infection with high fever, cough and sputum but had not been given a diagnosis. Each sample was cultured and DNA was extracted for molecular analyses. Unfortunately, no Legionella strain was isolated from these
Liver cirrhosis patient infected by *L. pneumophila*

**Fig. 1.** Chest CT scans of patient 1. The date when the patient was examined is indicated.

**Fig. 2.** PFGE patterns and clustering results of *L. pneumophila* clinical strain ICDC-LP001 compared with reference strain Philadelphia-1 and environmental isolates from the city of residence of the patient, obtained from the PFGE database. The PFGE patterns were analysed using the BioNumerics software package (version 5.1; Applied Maths). Clustering was created using the unweighted pair group method with arithmetic mean (UPGMA).
samples and urinary antigen tests were not performed. However, three samples were 5S rRNA qPCR-positive for \textit{Legionella}. The \textsc{nested} Sequence-Based Typing protocol for epidemiological typing of \textit{Legionella pneumophila} directly from clinical samples (Version 1.0, available at http://www. ewgl.org) was used to genotype the three qPCR-positive samples. Seven loci were amplified and sequenced successfully for two of the samples, and only three loci were sequenced for the third sample (Table 2). The sample from patient 4 was assigned ST36, the same ST as the sample from patient 1. The sample from patient 3 was assigned ST346, which has a one-locus difference to ST36 [\textit{mip}(1) for ST36 and \textit{mip}(28) for ST346]. The three sequenced loci from the sample from patient 2 were \textit{pilE}(4), \textit{asd}(1) and \textit{mompS}(14), the same loci as ST36 and ST346. Clarithromycin was given to the three qPCR-positive patients and the patients recovered.

**Discussion**

In the early stages of \textit{Legionella} pneumonia, some patients present various symptoms, such as high fever, cough, sputum, diarrhoea and impaired level of consciousness, without having any breathing difficulties (Yagyu et al., 2003). These cases are often ignored, especially among patients with other diseases that also cause some of these symptoms. In the present case, the patient was a middle-aged man with severe liver disease. Since the patient had fever, cough and a lung infection on first presentation and \textit{Candida albicans} was isolated from the patient’s sputum, we suspected he was suffering from a fungal infection. However, the patient’s clinical symptoms did not improve after 5 days of hospitalization and a chest CT scan demonstrated significantly more pulmonary infiltration. We then decided to reconsider the cause of his symptoms and \textit{Legionella} infection was confirmed. Clinical practice guidelines recommend testing for \textit{Legionella} in selected patients, including seriously ill patients without an alternative diagnosis, older and immunocompromised patients and patients non-responsive to \textit{β}-lactam antibiotics (British Thoracic Society Standards of Care Committee, 2001; Mandell et al., 2007). However, in the Chinese mainland, Legionnaires’ disease is usually ignored in the differential diagnosis of pneumonia as most physicians lack experience with this disease. Based on the cases reported here, we suggest that patients with liver cirrhosis suffering from lung disease should also be tested for possible \textit{Legionella} infection.

As the clinical signs of Legionnaires’ disease are usually not specific, a prompt and accurate laboratory diagnosis is crucial. In our lab, diagnostic methods were established to detect \textit{Legionella} in both clinical and environmental samples, including culture, serology and conventional PCR and qPCR, as well as genotyping methods, such as PFGE, SBT and nested modified SBT. The urinary antigen test is useful for early diagnosis of \textit{Legionella} infections; however, this method was not performed on any cases in this study. The sensitivity of this method is limited because the commercially available urinary antigen test detects only serogroup 1 \textit{L. pneumophila}. Culture-confirmed diagnosis of \textit{Legionella} infections is often hampered by early antibiotic treatment and demanding culture requirements. \textit{Legionella} species do not grow on standard microbiology media, thus they are usually not detected from blood or sputum cultures. Culture isolation from BAL fluid is considered the gold standard for diagnosis, but \textit{Legionella} requires 4–10 days to grow and this method is incapable of detecting ‘viable but not culturable’ (VBNC) cells (Bej et al., 1991; Allegra et al., 2008). The shortcomings of culture have prompted the development and routine application of

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**Table 1. Details of the patients tested in this study and results obtained from culture and qPCR analysis**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (y)/gender</th>
<th>Date of admission</th>
<th>Comorbidity</th>
<th>Detection of \textit{Legionella} in BAL fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Culture</td>
</tr>
<tr>
<td>Patient 1</td>
<td>42/M</td>
<td>16/05/2011</td>
<td>Liver cirrhosis, lung infection, cholecystitis</td>
<td>+</td>
</tr>
<tr>
<td>Patient 2</td>
<td>57/M</td>
<td>16/05/2011</td>
<td>Obstructive jaundice, diabetes, emphysema, lung infection</td>
<td>–</td>
</tr>
<tr>
<td>Patient 3</td>
<td>60/M</td>
<td>08/03/2011</td>
<td>Liver cirrhosis, pneumonia, splenectomy, cholecystectomy</td>
<td>–</td>
</tr>
<tr>
<td>Patient 4</td>
<td>33/M</td>
<td>15/06/2011</td>
<td>Liver cirrhosis, lung infection</td>
<td>–</td>
</tr>
<tr>
<td>Patient 5</td>
<td>48/M</td>
<td>14/07/2011</td>
<td>Liver cirrhosis, peritonitis, lung infection</td>
<td>–</td>
</tr>
<tr>
<td>Patient 6</td>
<td>20/F</td>
<td>13/07/2011</td>
<td>Acute liver failure, hepatic encephalopathy, lung infection</td>
<td>–</td>
</tr>
<tr>
<td>Patient 7</td>
<td>46/F</td>
<td>07/06/2011</td>
<td>Drug-induced liver injury, hepatitis, lung infection</td>
<td>–</td>
</tr>
<tr>
<td>Patient 8</td>
<td>37/M</td>
<td>03/07/2011</td>
<td>Liver cirrhosis, hepatic encephalopathy, hepatitis, renal insufficiency, lung infection</td>
<td>–</td>
</tr>
<tr>
<td>Patient 9</td>
<td>41/F</td>
<td>08/04/2011</td>
<td>Liver failure, hepatic encephalopathy, acute respiratory distress syndrome, lung infection</td>
<td>–</td>
</tr>
<tr>
<td>Patient 10</td>
<td>49/M</td>
<td>08/07/2011</td>
<td>Liver cirrhosis, meningitis, lung infection</td>
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</tr>
<tr>
<td>Patient 11</td>
<td>27/F</td>
<td>05/08/2011</td>
<td>Drug-induced liver injury, cerebral oedema, hepatic encephalopathy, adult Still’s disease, lung infection</td>
<td>–</td>
</tr>
</tbody>
</table>
Table 2. Sequence-based typing of *Legionella* strains identified in this study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Origin of DNA</th>
<th>Loci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>flaA</em> <em>pilE</em> <em>asd</em> <em>mip</em> <em>mompS</em> <em>proA</em> <em>neuA</em></td>
</tr>
<tr>
<td>1</td>
<td>BAL fluid from patient 1</td>
<td>3 4 1 1 14 9 1</td>
</tr>
<tr>
<td>2</td>
<td>BAL fluid from patient 2</td>
<td>– 4 1 – 14 –</td>
</tr>
<tr>
<td>3</td>
<td>BAL fluid from patient 3</td>
<td>3 4 1 28 14 9 1</td>
</tr>
<tr>
<td>4</td>
<td>BAL fluid from patient 4</td>
<td>3 4 1 1 14 9 1</td>
</tr>
<tr>
<td>5</td>
<td>Strain from patient 1</td>
<td>3 4 1 1 14 9 1</td>
</tr>
</tbody>
</table>

Acknowledgements

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References


