Case Report

Community-acquired Legionnaires' disease caused by *Legionella pneumophila* serogroup 10 linked to the private homePaul Christian Lück,¹ Thomas Schneider,² Jutta Wagner,³ Ilona Walther,⁴ Ursula Reif,⁵ Stefan Weber⁶ and Klaus Weist⁷

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We describe the case of a 66-year-old man with a culture-proven *Legionella* pneumonia after kidney transplantation. The patient developed the infection 15 days after discharge from a university hospital. *Legionella* pneumonia caused by *Legionella pneumophila* serogroup 5/10 was established by positive direct fluorescence assay, positive urinary-antigen detection and isolation of the causative agent. The infection was successfully treated by giving appropriate antibiotics, but the further course was complicated by invasive aspergillosis, cytomegalovirus pneumonia, failure of the transplanted kidney and development of septic anaemia. Four months after the diagnosis of *Legionella* pneumonia the patient died of multi-organ failure. The microbiological and epidemiological investigation revealed that strains from the water supply of the patient's private home were indistinguishable from the patient's isolate by amplified fragment length polymorphism analysis and sequence-based typing (SBT). Unrelated strains of serogroups 4, 5, 8 and 10 from the Dresden strain collection were of different SBT types. Thus, SBT is a very useful tool for epidemiological investigation of infections by *L. pneumophila* serogroups other than serogroup 1.

Received 6 May 2007

Accepted 22 October 2007

Introduction

Legionnaires' disease, the pneumonic form of legionellosis, usually is acquired by inhalation or aspiration of legionellae from contaminated environmental sources. Potable water is an important source of both nosocomial and community-acquired *Legionella* infections (Fields *et al.*, 2002). Since 2001, legionellosis is a notifiable disease in Germany. Around 500 cases are reported through the public health system each year, which is considered to be the tip of the iceberg (Robert Koch Institut, 2006). Based on an ongoing

study on the aetiology and outcome of community-acquired pneumonia in Germany (www.capnetz.de), the frequency of *Legionella* pneumonia is estimated to be 15 000 to 30 000 cases per annum (Lück *et al.*, 2006). The majority of community-acquired cases are caused by strains belonging to *Legionella pneumophila* serogroup 1 (Helbig *et al.*, 2002, Yu *et al.*, 2002). Among the 1300 clinical isolates from Europe, serogroup 10 is the fourth most common cause of *Legionella* pneumonia (Helbig *et al.*, 2002).

Due to the ubiquitous prevalence of legionellae in water supply systems, strains isolated from patients and environmental sources must be compared by molecular typing techniques to confirm or exclude a suspected

Abbreviations: AFLP, amplified fragment length polymorphism; EWGLI, European Working Group for Legionella Infections; SBT, sequence-based typing.

environmental reservoir as the source of the infection. These techniques include mAb typing, various DNA restriction patterns [amplified fragment length polymorphism (AFLP) or PFGE] (Fry *et al.*, 1999; Lück *et al.*, 1994; Sax *et al.*, 2002) and more recently sequence-based typing (SBT) (Gaia *et al.*, 2005). This method is in fact a variation of multilocus sequence typing and uses sequence variations in five virulence-associated *L. pneumophila* genes (*flaA*, *pilE*, *mip*, *mompS*, *proA*) and one house-keeping gene (*asd*). It has been successfully used to type *L. pneumophila* serogroup 1 strains, and a few strains belonging to serogroup 6, 8 and 10 (Gaia *et al.*, 2005). It is now established as the standard subtyping technique within the European Working Group for Legionella Infections (EWGLI).

In this study the SBT method was used to compare the allelic profiles of *L. pneumophila* serogroup 4, 5, 8 and 10 isolates from patients and environmental sources. These strains were selected because they are cross-reacting and might be considered a group of antigenically related strains.

Case report

A 66-year-old man was hospitalized at the University Hospital of Berlin from August 23rd to September 8th 2004. Eighteen days later, he was readmitted to the intensive care unit of the same hospital with a 3 day history of fever. The patient has received a cadaveric kidney transplant 4 months earlier and was now on cortisone therapy. On admission, his vital signs were as follows: core body temperature 32.8 °C, pulse 92 beats min⁻¹, blood pressure 90/65 mmHg, respiratory rate 28 breaths min⁻¹ and his chest radiograph showed bilateral consolidation. He was intubated, given ventilatory assistance and empiric antibiotic therapy with macrolides. His clinical status improved slowly and the X-ray showed a resolution of the

pneumonia. The further course was complicated by invasive aspergillosis, cytomegalovirus pneumonia, failure of the transplanted kidney and septic anaemia. Four months after developing *Legionella* pneumonia he died of multi-organ failure. At the time of death no radiological signs of pneumonia remained. However, it must be emphasized that the *Legionella* infection was the onset of the chain of events leading to the fatal outcome.

A bronchoalveolar lavage fluid collected on day 2 after admission was positive for *L. pneumophila*-species antigen by direct immunofluorescence mAb staining (Bio-Rad). Subsequently, *Legionella* colonies grow on buffered-charcoal yeast extract agar. By using mAbs the strain was typed as serogroup 5/10, since both serogroup-specific mAbs reacted brightly with this strain (Helbig *et al.*, 2002). A urine sample taken on the same day gave a positive result using the Biotest *Legionella* urinary antigen test.

Environmental samples were taken from hospital and home water supplies according to standard protocols (ISO, 2004). Water samples from the hospital where the patient stayed yielded *L. pneumophila* serogroups 1 and 9. The concentrations ranged from 10 000 to 20 000 c.f.u. l⁻¹. *L. pneumophila* serogroup 5/10 was not detected. However, water samples from the private home of the patient collected 3 and 5 weeks after the onset of the clinical illness yielded 30 and 280 c.f.u. l⁻¹ of *L. pneumophila* serogroup 5/10, respectively. An environmental isolate from the hospital in the patient's town was also serogroup 10. All isolates were then referred to the German Legionella Reference Laboratory in Dresden and typed by using the Dresden panel of mAbs (Table 1). Epidemiologically unrelated strains of antigenically related serogroups 4, 5, 8 and 10 were taken from the strain collection in Dresden.

To obtain an initial indication of the genetic relatedness of the *L. pneumophila* isolates from the patient, the hospital water system and from tap water at his home, the isolates

Table 1. Allelic profiles of *L. pneumophila* isolates belonging to serogroups 4, 5, 8 and 10 from the patient, his private home, and nine unrelated strains

Isolate	Serogroup	<i>flaA</i>	<i>pilE</i>	<i>asd</i>	<i>mip</i>	<i>mompS</i>	<i>proA</i>	Origin
L04-564	5/10	2	10	3	13	9	4	Patient, CAP
L04-565	5/10	2	10	3	13	9	4	Patient, CAP
W04-989	5/10	2	10	3	13	9	4	Patient, home water supply
W04-990	5/10	2	10	3	13	9	4	Patient, home water supply
Hoch 89	10	2	10	17	28	9	4	Unrelated, patient NAP
W05-191	10	2	10	3	28	9	4	Unrelated, Hospital H water
Concord-3	8	8	1	22	22	6	10	Unrelated, ATCC 35 096
Leiden 1	10	2	10	3	28	9	4	Unrelated, ATCC 43 283
L05-129	10	1	4	3	5	9	1	Unrelated, patient CAP
L01-388	10	1	4	3	5	10	1	Unrelated, patient NAP
L96-176/2	5	1	10	3	13	9	4	Unrelated, patient NAP
Eu 315	10	3	10	1	28	14	9	Unrelated, patient NAP
L05-431	4	3	13	1	1	14	9	Unrelated, patient CAP

CAP, Community-acquired pneumonia; NAP, nosocomial pneumonia.

were genotyped by the AFLP method as described by Fry *et al.* (1999). Subsequently, SBT was used to genotype all strains listed in Table 1 as described previously (Gaia *et al.*, 2005). The allele numbers of the isolates were determined by using the EWGLI software tool (available online at http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php). The results of AFLP and SBT typing confirmed that the clinical strain and environmental isolates from the patient's home were indistinguishable. This is a strong argument for the transmission from the patient's home water supply to the patient. All unrelated strains were of different SBT patterns (Fig. 1, Table 1). To date, the SBT profile of the patient's isolate has only been recorded once before from an isolate from Tokyo, Japan (http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php).

Discussion

The frequency of community-acquired legionellosis is often under-reported. This is mainly due to the fact that specific laboratory tests are required to diagnose a *Legionella* infection. The patient reported here had undergone kidney transplantation 4 months previously and therefore belonged to the high risk group for legionellosis. He presented with the clinical signs compatible with *Legionella* pneumonia. This aetiology was clinically suspected and confirmed by three laboratory methods. In our case the Biotest urine antigen assay detected the infection caused by serogroup 5/10. Currently, the vast majority of legionellosis cases are detected by urinary-antigen detection (DenBoer &

Yzerman, 2004). However, the urinary-antigen assay does not reliably detect non-serogroup 1 infections (Benson *et al.*, 2000; Horn 2001). The direct fluorescence antibody test uses a mAb that detects all serogroups of *L. pneumophila* and thus has a broader spectrum (Fields *et al.*, 2002). Culturing *Legionella* strains is still the gold standard method but suffers from only moderate sensitivity (DenBoer & Yzerman, 2004; Fields *et al.*, 2002; Lück *et al.*, 2006). However, it is still the prerequisite for epidemiological molecular typing studies to confirm or exclude a given environmental reservoir as the source of infection. According to data from the Robert Koch Institute (2006), in only half of the urinary-antigen-positive cases is an attempt made to cultivate legionellae from clinical samples, which significantly hampers the epidemiological investigation.

Genotyping by PFGE, AFLP or SBT is an important epidemiological tool, and many studies have demonstrated the usefulness of differentiating isolates of *L. pneumophila* in order to confirm or refute epidemiological associations (Fields *et al.*, 2002; Fry *et al.*, 1999; Gaia *et al.*, 2005). All band-based techniques suffer from reduced inter-laboratory reproducibility. However, they are excellent when all strains are compared in one run as shown here. Recently, EWGLI adopted SBT as a simple, rapid and reproducible method for genotyping *L. pneumophila* serogroup 1 (Gaia *et al.*, 2005). In the same study the applicability of SBT for epidemiologically related strains belonging to serogroup 6, 8 and 10 was demonstrated. To further test the utility of this method we applied SBT to strains belonging to serogroups 4, 5, 8 and 10. The present study was initiated as *L. pneumophila* serogroup 5/10 isolates became available from a patient and environmental samples, i.e. from the private home where the patient had spent the time after discharge from the hospital during the suspected incubation period (2 to 10 days). In our study, all strains belonging to serogroups 4, 5, 8 and 10 were typable. Since the number of strains belonging to these serogroups investigated is rather small, a final assessment of the index of discrimination is difficult to perform. Interestingly, strains with the allelic profiles 2, 10, 3, 28, 9 and 4 were found in this study as well as in a previous publication (Gaia *et al.*, 2005). Taking together SBT represents a valuable tool for molecular epidemiological studies on *L. pneumophila* strains belonging to serogroups 2–15.

The patient had stayed in hospital in Berlin during the summertime. He felt ill 15 days later, thus exceeding the usual incubation period of 2 to 10 days, which is assumed for nosocomially acquired cases. However, it has been reported that *Legionella* can colonise the respiratory tract for a certain time and cause pneumonia later (Marrie *et al.*, 1992). Occasionally, the incubation time might be longer than 10 days (DenBoer *et al.*, 2002). Thus the hospital could not be ruled out as the source of the infection. Since the private home of the patient was also considered to be a possible source of the infection, it was inspected by the local health department and water samples were collected. The colony counts were 30 c.f.u. l⁻¹ on October 18th 2004

Fig. 1. AFLP patterns of *L. pneumophila* isolates from the patient. Lane 1, 2, *L. pneumophila* serogroup 5/10 isolate from the patient, isolates correspond to L04-564 and L04-565 of Table 1; lane 3, unrelated isolate of *L. pneumophila* serogroup 1 from a patient of the same hospital suffering from nosocomial *Legionella* infection; lane 4, unrelated isolate from the water system of the University Hospital of Berlin, where the patient had been treated (*L. pneumophila* serogroup 1); lanes 5–7, three isolates from tap water outlets of the patient's home (*L. pneumophila* serogroup 5/10, isolates of lane 5 and 6 correspond to isolates W04-989 and W04-990 of Table 1); lane 8, *L. pneumophila* serogroup 1, Knoxville-1 (ATCC 33153).

and 280 c.f.u. l⁻¹ 3 weeks later. The results from serotyping and SBT methods revealed the potable water supply to be the source of infection (Table 1).

An association between the degree of *Legionella* contamination and the occurrence of legionellosis has been described (Best *et al.*, 1983; Stout *et al.*, 1992). However, fluctuations in the amount of legionellae in a give water supply system, as well as differences in the virulence properties, make it extremely difficult to predict a correlation between the number of legionellae in the environmental samples and the risk for humans. In the present case the number of *Legionella* colonies detected 3 and 5 weeks after the onset of the illness in the home of the patient were below the level considered to merit immediate action (<1000 c.f.u. l⁻¹) (EWGLI, 2005). On this basis, no remedial action and disinfection of the water supply was initiated. We do not know the concentration of the causative strain in the water supply at the moment of the infection. It might be speculated that the number of legionellae were higher during the hot summer. We also have no data concerning the virulence of the given serogroup 5/10 strain. Since the patient belonged to the high-risk group of transplant patients, a *Legionella* contamination <1000 c.f.u. l⁻¹ might be relevant, whereas such concentrations possess little or no risk for immunocompetent patients. Keeping in mind that patients with T-cell affecting immunosuppressive therapy have an increased risk of legionellosis, one might consider supplying these patients with *Legionella*-free water, either by decontaminating the home water system or by using terminal tap water filters.

The results of this investigation emphasize the importance of collaboration and exchange of data between local health authorities, clinics and the reference laboratory. Furthermore, it demonstrates the importance of isolating and typing *Legionella*, for further strengthening the epidemiological link of a specific environment to a patient.

Acknowledgements

We are grateful to Carolin Dix, Jutta Paasche and Kerstin Seeliger for technical assistance in culturing water samples and typing of legionellae. This study was supported by the Federal Ministry of Education and Research of Germany, Network of Competence in Medicine CAPNetz.

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