Legionella pneumophila serogroup 3 pneumonia in a patient with low-grade 4 non-Hodgkin lymphoma: a case report

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Abstract

Introduction: Nosocomial legionellosis has generally been described in immunodepressed patients, but Legionella pneumophila serogroup 3 has rarely been identified as the causative agent.

Case presentation: We report the case of nosocomial L. pneumophila serogroup 3 pneumonia in a 70-year-old Caucasian man with non-Hodgkin lymphoma. Diagnosis was carried out by culture and real-time polymerase chain reaction of bronchoalveolar lavage fluid. The results of a urinary antigen test were negative. A hospital environmental investigation revealed that the hospital water system was highly colonized by L. pneumophila serogroups 3, 4, and 8. The hospital team involved in the prevention of infections was informed, long-term control measures to reduce the environmental bacterial load were adopted, and clinical monitoring of legionellosis occurrence in high-risk patients was performed. No further cases of Legionella pneumonia have been observed so far.

Conclusions: In this report, we describe a case of legionellosis caused by L. pneumophila serogroup 3, which is not usually a causative agent of nosocomial infection. Our research confirms the importance of carrying out cultures of respiratory secretions to diagnose legionellosis and highlights the limited value of the urinary antigen test for hospital infections, especially in immunocompromised patients. It also indicates that, to reduce the bacterial load and prevent nosocomial legionellosis, appropriate control measures should be implemented with systematic monitoring of hospital water systems.

Introduction

Legionnaires’ disease is often a hospital-acquired infection. In Italy, 9.2% of nosocomial cases were recorded in 2009 with a fatality rate (34%) significantly higher than community-acquired cases (12%) [1]. This difference is due to the patients’ state of immunodeficiency, which is known to be an important risk factor in contracting Legionella pneumonia. Often, hospital water systems are colonized by Legionella and this contamination is responsible for most cases of hospital-acquired legionellosis [2]. A link between the presence of the bacterium in hospital water systems and nosocomial legionellosis has been reported [3], suggesting that it is necessary to sample hospital water routinely for Legionella and make sure that this microorganism is not present in transplant units or other wards with significantly immunosuppressed patients [4]. Sixteen serogroups of Legionella pneumophila are known. Serogroup 1 is the most common in clinical and environmental isolates [5], whereas L. pneumophila serogroup 3 has rarely been isolated in immunocompromised patients [6].

Case presentation

In this report, a case of hospital-acquired pulmonary legionellosis in an immunocompromised patient - a 70-year-old Caucasian man - is described. Sixteen years before he presented to us, he had a condition that was diagnosed as low-grade non-Hodgkin lymphoma of the B-cell small lymphocytic type (stage IV with mediastinal,
abdominal, bone marrow, and superficial node site involvement), and nine courses of combined polychemo-
therapy were administered with cyclophosphamide, doxorubicin, vincristine, and prednisone. Four years
before he presented to us, lymphoma chemotherapy was
started again with cyclophosphamide and fludarabine
because of a progression of mesenteric involvement of
the disease. This regimen was interrupted four months
later because of toxicity, and 11 courses of alemtuzumab
were administered thereafter in the Oncology and
Hematology Clinic of our division.

Three years before he presented to us, he was dis-
charged from our ward after a two-week hospitalization
for fever and pancytopenia (first hospital admission of
this report). After seven days, he was hospitalized again,
in another ward of the same hospital, because of fever,
dyspnea, and bowel movements with loose stools (sec-
ond admission). A chest X-ray showed an inflammatory
infiltrate at the base of his right lung, and another slight
infiltration seemed to be spreading in the upper field of
his left lung. The results of laboratory tests were unre-
markable except for significant increases of erythrocyte
sedimentation rate and C-reactive protein, minimal
increases of aspartate aminotransferase and alanine ami-
notransferase, and mild anemia. The results of blood
cultures, a cytomegalovirus antigen test in serum, and a
Clostridium difficile toxin stool test were negative. A
Course of empirical antibiotic therapy with piperacillin/
tazobactam plus ciprofloxacin was started. A rapid
defervescence ensued, and our patient was discharged
after an eight-day course of antibiotics. However, two
weeks after discharge, fever recurred and he was hospi-
talized again in our ward to investigate a possible oppor-
tuntistic respiratory infection (third admission). He
appeared mildly ill and complained of pleuritic pain at
the base of his right lung when breathing deeply; a low-
grade fever was present, but his respiratory rate (20
breaths per minute), heart rate (88 beats per minute),
and blood pressure (120/70 mm Hg) were normal; his
arterial blood oxygen saturation was 96% while he was
breathing room air. Inspiratory crackles were evident
over his right lower lung field, but the results of the
physical examination were otherwise normal. Blood cul-
tures were requested in order to check for common patho-
ogens as an empiric antibiotic therapy was started
with piperacillin/tazobactam. However, several hours
after admission, his fever increased to over 38°C and
persisted for five days. Therefore, a chest computed
tomography (CT) scan and a bronchoscopy test with
bronchoalveolar lavage (BAL) were performed and,
although our patient’s general clinical condition
remained stable and blood culture results were negative,
beta-lactams were replaced with clarithromycin to pro-
vide antibacterial coverage against possible atypical
respiratory pathogens. The chest CT scan showed evi-
dence of an area of lung parenchymal consolidation in
his right lower lobe with adjacent ground-glass opacities
and micronodules. No lymphadenopathy was evident.
A microbiological examination of BAL fluid was negative
for common Gram-negative and Gram-positive patho-
gens, fungi, Nocardia spp., Mycobacterium tuberculosis,
Pneumocystis jiroveci, cytomegalovirus, adeno virus, and
respiratory syncytial virus, but Legionella was detected
after four days of incubation. For culture, BAL fluid,
dundiluted or diluted (1:10) in trypticase soy broth, was
plated on a buffered charcoal yeast extract (BCYE) med-
ium with 0.1% alpha-ketoglutaric acid and on selective
BCYE agar medium supplemented with glycine, vanco-
mycin, polymyxin B, and cycloheximide (GVPC) (Becton
Dickinson, Milan, Italy). Colonies were identified as L.
pneumophila serogroup 3 by using the Dresden mono-
clonal antibody panel [7]. The results of real-time poly-
merase chain reaction (PCR) assays performed on DNA
extracted from BAL fluid were also positive for Legio-
ella spp. (Nanogen Advanced Diagnostic, Torino,
Italy). The results of urinary antigen tests for L. pneu-
ophila serogroup 1 (Binax, Portland, ME, USA) and
non-serogroup 1 (Biotec, Dreieich, Germany) were
negative.

A prompt clinical response was observed after clari-
thromycin treatment. Two days after the start of macro-
clide treatment, our patient was afebrile and had no
chest pain and his condition was generally improving
and remained so thereafter. A chest X-ray performed
two weeks later showed a slight reduction of right lung
opacity, and he was discharged after 20 days of hospital
stay while on clarithromycin therapy. This treatment
was continued for seven more days. A follow-up chest
CT scan obtained five weeks later revealed an almost
complete resolution of the previously documented right
lung consolidation and ground-glass areas.

An environmental investigation was carried out after
the isolation of the infectious agent. First of all, our
patient’s home water system was investigated for the
presence of L. pneumophila, but this pathogen was
found in neither the cold nor the hot water system (data
not shown). Given that the incubation time for L. pneu-
ophila is generally two to 10 days and that, six days
after being discharged from his first hospitalization (for
pancytopenia), our patient was admitted again with
respiratory symptoms, we suspected that he had been
exposed to the microorganism during the period spent
in the hospital. To verify this hypothesis, an intensive
environmental investigation was carried out on the hos-
pital’s hot and cold water systems. A microbiological
analysis of water samples, collected from the sink and
shower of our patient’s room from the first hospital
admission, revealed that the hot water sample was
contaminated by *L. pneumophila* serogroup 3 (Table 1). The concentration of 8000 colony-forming units per liter (CFU/L) exceeded the 1000 CFU/L of the European guidelines for hotels and the 1000 CFU/L of the Italian guidelines for hospitals [8]. Also, a lot of other sites of the hospital’s hot water system were highly colonized by *L. pneumophila*, including serogroups 3, 4, and 8 (Table 2). Legionella was not isolated from the cold water system. Molecular typing of *L. pneumophila* clinical and environmental isolates was carried out by using the amplified fragment length polymorphism method [9], and the genomic profile of the *L. pneumophila* clinical strain matched with that of the *L. pneumophila* serogroup 3 strain (data not shown).

On the basis of these results, an extensive program of microbiological controls in the entire hospital water system was implemented. In accordance with the European guidelines, shock hyperchlorination was applied to the water distribution system [10] with a single addition of chlorine to the water to obtain concentrations of free residual chlorine of 20 to 50 mg/L throughout the water system, including the distal point. After this treatment, cultures of water samples collected one day after hyperchlorination showed a decrease in *L. pneumophila* concentration. However, a considerable increase of CFU was observed one month later (Table 3). Therefore, continuous hyperchlorination treatment (free residual chlorine of 1 to 3 mg/L) of the hospital water system is now routinely applied and monitoring is performed every six months. In addition, periodic monitoring of the *Legionella* CFU in the water system is carried out along with careful clinical surveillance of legionellosis cases in low- and high-risk patients with pneumonia. No other nosocomial *Legionella* pneumonia cases have been observed so far.

**Discussion**

Owing to the difficulty of distinguishing legionellosis from other forms of pneumonia by clinical and roentgenographic analysis, specific laboratory diagnostics should be enforced in order to increase the detection rate of nosocomial Legionnaires’ disease. Culture remains the most specific diagnostic procedure for legionellosis [5]. The usefulness of urinary antigen detection for the diagnosis of Legionnaires’ disease is well documented. However, because it is fast and easy to perform, this test has caused a decrease in the use of cultures to detect infection, resulting in incomplete surveillance for legionellosis [5]. The Binax urinary antigen kit detects *L. pneumophila* serogroup 1 antigen [11], whereas the Biotest urine antigen enzyme immunoassay has a wide range of cross-reactivity to the other serogroups and species. Benson and colleagues [12] reported that both Binax and Biotest urinary antigen kits were capable of detecting multiple serogroups of *L. pneumophila*, including serogroup 3. However, in this case and in other legionellosis cases [6], the urine antigen test was negative when the above-mentioned kits were used and the correct diagnosis was made only when BAL fluid-specific culture and real-time PCR methods were used. This demonstrates the importance of using at least one of these methods as part of the routine microbiological testing of BAL fluid, especially in immunocompromised patients with pulmonary contamination.

| Sampling site                        | Sampling mode | Legionella pneumophila load, CFU/L |
|--------------------------------------|---------------|-----------------------------------|
| Water mains                          | Pre-flushing  | Not detected                       |
|                                       | Post-flushing | Not detected                       |
| Hospital water harvesting tanks       | Pre-flushing  | Not detected                       |
|                                       | Post-flushing | Not detected                       |
| Collector pipes                      | Pre-flushing  | Not detected                       |
|                                       | Post-flushing | Not detected                       |
| Boiler                               | Pre-flushing  | $5 \times 10^3$                    |
|                                       | Post-flushing | $4.5 \times 10^3$                  |
| Room closest to the boiler (sink)     | Pre-flushing  | $6.5 \times 10^3$                  |
|                                       | Post-flushing | $7 \times 10^3$                    |
| Room farthest from the boiler (sink)  | Pre-flushing  | $4 \times 10^3$                    |
|                                       | Post-flushing | $5 \times 10^2$                    |

CFU/L: colony-forming units per liter.
infiltrates. In addition, every time that the clinical features are equivocal, a negative result in urinary antigen tests should not be a reason for ruling out the disease.

Our patient was first empirically treated with clarithromycin to cover possible intracellular atypical respiratory pathogens [13]. After the isolation of the infectious agent, this treatment was not replaced with other antibiotics such as levofloxacin [14], because of the prompt clinical response to this macrolide and because of the previous poor response to a regimen with an antibiotic containing fluoroquinolone (ciprofloxacin). Indeed, the use of a fluoroquinole (ciprofloxacin) not specific for pulmonary infections and the short course (eight days) of treatment in a severely immunosuppressed patient could concur with the recurrence of respiratory symptoms and could have induced the third hospitalization.

Colonization of water systems by Legionella spp. occurs in hospitals throughout the world [15]. In hospital wards where immunosuppressed patients are subjected to chemotherapy drugs, hot water systems should be free of Legionella contamination.

The water system of our hospital was colonized by L. pneumophila of different serogroups. Although various methods to control Legionella in water distribution systems have been described in the literature (for example, methods based on physical or chemical treatments or both), none of these treatments is able to eradicate the bacterium permanently, and re-colonization occurs as soon as the treatments are interrupted [16]. Also, in our experience, the ineffectiveness of hyperchlorination treatments in eradicating L. pneumophila was demonstrated by the re-growth of Legionella only one month later to levels similar to those observed before treatment. This suggests a need for continuous monitoring and a maintenance regime for the hospital plumbing system, particularly in wards accommodating high-risk patients.

Conclusions

This case report demonstrates that (a) culture of respiratory secretions and real-time PCR for L. pneumophila should be part of the routine microbiological testing of BAL fluid from immunocompromised patients with pulmonary infiltrates, (b) a negative result from a urinary antigen test does not rule out the presence of legionellosis, and (c) the monitoring of the Legionella species in hospital water systems can help to bring in control measures to reduce the bacterial load and is therefore a proactive strategy for the control of the Legionella infection in hospitalized patients.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal. The admitting hospital approved the use of patient samples and data.

Abbreviations

BAL: bronchoalveolar lavage; BCYE: buffered charcoal yeast extract; CFU: colony-forming units; CT: computed tomography; PCR: polymerase chain reaction.

Acknowledgements

This work was funded by Fondazione Cassa di Risparmio di Perugia, "Progetto Ricerca di Base". We are grateful to Maria Luisa Ricci, of the Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy, for critical comments and suggestions and to Catherine Macpherson for editorial assistance.

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Authors’ contributions

CC carried out the environmental investigation. AM performed the microbiological diagnosis and drafted the manuscript. AC and PF were responsible for the clinical management and therapy. AV drafted the

| Table 3 Results of microbiological analysis from the hospital's hot water samples one day and one month after hyperchlorination |
|---------------------------------------------------------------|
| **Legionella pneumophila load, CFU/L**                        |
| **Sampling site**                                             |
| **Sampling mode**                                             |
| **Before treatment**                                          |
| **One day after treatment**                                  |
| **One month after treatment**                                |
| Boiler                                                       |
| Pre-flushing                                                 |
| 5 x 10^3                                                     |
| 1.5 x 10^2                                                   |
| 3.5 x 10^3                                                   |
| Post-flushing                                                |
| 4.5 x 10^3                                                   |
| 2 x 10^2                                                     |
| 5 x 10^4                                                     |
| Room closest to the boiler (sink)                            |
| Pre-flushing                                                 |
| 6.5 x 10^3                                                   |
| 4 x 10^2                                                     |
| 8.5 x 10^3                                                   |
| Post-flushing                                                |
| 7 x 10^3                                                     |
| 2.5 x 10^3                                                   |
| 6 x 10^4                                                     |
| Room farthest from the boiler (sink)                         |
| Pre-flushing                                                 |
| 4 x 10^4                                                     |
| 2.5 x 10^3                                                   |
| 2.5 x 10^3                                                   |
| Post-flushing                                                |
| 5 x 10^3                                                     |
| 1.5 x 10^2                                                   |
| 3.5 x 10^2                                                   |
| Room farthest from the boiler (sink)                         |
| Pre-flushing                                                 |
| 4 x 10^4                                                     |
| 2 x 10^2                                                     |
| 2.5 x 10^3                                                   |
| Post-flushing                                                |
| 4.5 x 10^3                                                   |
| 4 x 10^2                                                     |
| 3 x 10^3                                                     |

CFU/L: colony-forming units per liter.
Competing interests
The authors declare that they have no competing interests.

Received: 23 February 2011 Accepted: 17 August 2011
Published: 17 August 2011

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http://www.jmedicalcasereports.com/content/5/1/387

Cite this article as: Mencacci et al. Legionella pneumophila serogroup 3 pneumonia in a patient with low-grade 4 non-Hodgkin lymphoma: a case report. Journal of Medical Case Reports 2011 5:387.