Bronchoalveolar lavage findings in severe community-acquired pneumonia due to *Legionella pneumophila* serogroup 1

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Summary Background: No specific data are available in the literature on the bronchoalveolar lavage (BAL) findings of *Legionella pneumophila* pneumonia. We report on the cytological and immunophenotypical BAL data of three immunocompetent patients with severe community-acquired pneumonia due to *L. pneumophila* serogroup 1.

Methods: Retrospective chart review. The microbiological diagnosis was obtained by BAL culture or urinary antigen assay.

Results: All patients presented with high-grade fever, bilateral chest infiltrates and severe respiratory failure requiring ventilatory support. The cytological BAL pattern at presentation showed in all patients the association of a marked neutrophilia with a variable but remarkable percentage of lymphoblasts. Increased levels of activated T-lymphocytes (both HLA-DR and CD25 cells) and, in 2 out of 3 patients, of T-cells bearing the γδ T-cell receptor were the main immunophenotypical findings on flow cytometric analysis.

Conclusions: We suggest that the association of lymphoblasts with a marked neutrophilia in BAL fluid of patients with a clinical-radiological setting compatible with acute pneumonia should suggest *L. pneumophila* as a possible etiologic agent.

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Introduction

*Legionella pneumophila* is considered a common cause of severe pneumonia requiring admission to an intensive care unit (ICU), being second only to bacteremic pneumococcal pneumonia in several comprehensive studies. Unfortunately, the clinical findings have been shown not to be reliable in distinguishing *Legionella* infection from other bacterial pneumonias. Recently, the urinary antigen testing has been associated with good sensitivity (80–90%) and very high specificity (98–100%) in detecting *L. pneumophila* serogroup 1 infection, although concerns have...
been raised over the sensitivity of this test early in the course of the disease.\textsuperscript{6,7} In 17 patients with \emph{L. pneumophila} serogroup 1 pneumonia, 5 of 7 patients with a negative urinary antigen test during the first 5 days of illness had a subsequent positive test.\textsuperscript{7} To our knowledge, the literature contains no specific information on the bronchoalveolar lavage (BAL) findings in \emph{L. pneumophila} pneumonia. We report on the morphological and immunophenotypical BAL findings of 3 patients with severe community-acquired pneumonia (CAP) due to \emph{L. pneumophila} serogroup 1 both at onset and, in the 2 who survived the acute phase, after a 3-month follow-up.

Methods

The BAL findings of 3 patients diagnosed with severe CAP due to \emph{L. Pneumophila} serogroup 1 were collected and retrospectively reviewed together with the clinical, radiological, and laboratory data. For the etiologic diagnosis, either a positive urinary antigen test or/and a positive BAL culture for \emph{L. Pneumophila} serogroup 1 was required. BAL was performed both at onset and, in the 2 patients who survived the acute phase, at 3-month follow-up. The BAL protocol that we used matches the accepted technical recommendations and guidelines.\textsuperscript{8} Briefly, the fibroscope was wedged in a subsegmental bronchus of an affected lung area as showed by chest-CT and six 25 ml aliquots of sterile saline solution were instilled. Fluid was gently aspirated immediately after each aliquot was infused and collected in a sterile container; the first two aliquots of the recovered BAL were treated separately. After recovery, BAL fluid was filtered through a monolayer surgical gauze to remove mucus and centrifuged at 2000 revolutions/min for 15 min. Cytospin preparations were stained by both the May–Grunwald–Giemsa and the Papa-nicolau methods. An experienced pathologist performed the differential cell count (under light microscopy at $\times$1000 by counting 400 cells in random fields) and the evaluation of cytological cell characteristics. The total cell count was obtained by using a hemocytometer. The lymphocyte subpopulations were determined with a flow cytometer after staining with the following monoclonal antibodies: CD3 (pan-T-cells), CD4 (T-helper cells), CD8 (T-suppressor/cytotoxic cells), CD20 (B-cells), CD25 (lymphocytes expressing interleukin-2 receptors), CD57 (natural killer cells), HLA-DR (activated T-lymphocytes and B-cells), $\alpha/\beta$ TCR (T-lymphocytes bearing the $\gamma/\delta$ T-cell receptor). The microbiological studies on BAL included, in all patients, both cultures (for detection of common bacteria, mycobacteria, fungi, \emph{L. pneumophila}, adenovirus, cytomegalovirus, herpes simplex virus types I and II) and immunofluorescence tests (for detection of respiratory syncitial virus). Furthermore, an accurate search for both intracytoplasmatic or intranuclear viral inclusions and for \emph{P. Carinii} cysts was performed.

Results

The diagnosis of \emph{L. Pneumophila} pneumonia in the 3 immunocompetent patients of this series was obtained by urinary antigen assay in one (pt no. 2), and by both BAL culture and urinary antigen assay in the other two. All of them presented at the emergency room of Bologna Maggiore Hospital with high-grade fever, extensive bilateral chest infiltrates and respiratory failure. One patient (pt no. 1) was initially hospitalized in the internal medicine ward and then moved to the ICU on the second day of hospitalization because of rapidly progressive respiratory failure requiring ventilatory support. Patient no. 2, who presented with massive hemoperitoneum due to spleen rupture requiring emergency splenectomy, was moved to the ICU after surgery. The remaining patient (pt no. 3) was immediately admitted to the ICU and submitted to invasive mechanical ventilation.

The main clinical and outcome data are showed in Table 1. The only patient (pt no. 1) who is alive and well did receive anti-\emph{Legionella} drugs as part of the antibiotic regimen he received on hospital admission, before the etiologic diagnosis was obtained. Patient no. 2 presented with a massive hemoperitoneum—a finding that has been seldom described in the setting of legionellosis\textsuperscript{9}—and respiratory failure associated with bilateral infiltrates on admission chest X-ray. This patient survived the acute phase but developed ventilator-associated pneumonia due to \emph{P. aeruginosa} 1 month after the initial hospital admission and died after 5 months of hospitalization due to septic shock. The remaining patient (pt no. 3) died a few days after admission of pneumonia-induced refractory respiratory failure; this patients was the only current smoker and its initial antibiotic regimen did not contain anti-\emph{Legionella} drugs. Laboratory findings at presentation were remarkable, in all patients, for a marked increase of the white blood cells, the differential count being characterized by
a huge neutrophilia and a severe lymphopenia (Table 1).

All patients showed extensive, bilateral, alveolar consolidation with air bronchogram on chest-CT scan.

The BAL-fluid analysis is summarized in Table 2. The cytological BAL pattern at presentation was characterized by the association of a marked neutrophilia along with a variable percentage of large, activated lymphocytes with blastic features, exhibiting round or convoluted nuclei with multiple small nucleoli, and a basophilic cytoplasm (Fig. 1). Follow-up BAL after anti-Legionella antibiotic treatment, performed in the 2 patients who survived the acute phase, showed the reduction or even the disappearance of lymphoblasts and neutrophils (Fig. 2).

As far as the immunophenotypical BAL profile is concerned, a marked increase of activated T-lymphocytes (both HLA-DR+ and CD25+ cells) could be detected in all patients at presentation; curiously, only CD 25+ cells were found to be markedly reduced on follow-up BAL, whereas HLA-DR+ cells remained persistently increased.
Increased T-lymphocytes bearing the $\gamma/\delta$ T-cell receptor were detected, only at presentation, in 2 out of 3 patients (Table 2).

All the microbiological studies on the BAL fluid proved negative, except for the positive culture for L. Pneumophila serogroup 1 in 2 out of 3 patients (pt no. 1 and 3).

**Discussion**

We provide the first thorough description of the cytological and immunophenotypical BAL findings in three immunocompetent patients with severe pneumonia due to L. pneumophila, all of them infected by the serogroup 1 strain.

Early in the course of the disease, the presence of a variable percentage of lymphoblasts in association with a marked neutrophilia was the cytological hallmark and rendered the BAL profile of these patients different from the one usually observed in other bacterial pneumonias, the latter showing only a pronounced neutrophilia.\(^{10,11}\) It is of note that lymphoblasts should not be normally present in BAL fluid. To the best of our knowledge, a BAL cytology characterized by scattered blasts and a variable increase of neutrophils had been only attributed so far to opportunistic pathogens, especially P. carinii, in several settings of immunodepression.\(^{12,13}\) Interestingly, the presence of lymphoblasts in the setting of Legionella infection is frequently observed in the blood of both patients and experimental animals.\(^{14-16}\) Furthermore, a lymphocyte blastogenic response has been shown to occur even earlier than the production of measurable antibodies in 13 patients with acute legionellosis and has been suggested to be potentially useful to diagnosis.\(^{17}\)

The main immunophenotypical BAL findings at presentation included an increase of both activated T-lymphocytes (both HLA-DR+ and CD25+ cells) and, in 2 out of 3 patients, of $\gamma/\delta$ T-lymphocytes. Lymphocyte activation with lymphokines production is thought to be a key factor for the host resistance to Legionella infection and occurs in the blood in the first 2 weeks of infection as part of the cell-mediated immune response orchestrated by the host against the organism.\(^{14,18}\) The T-lymphocytes bearing the $\gamma/\delta$ receptor, on the other hand, are important in the host defence against intracellular pathogens, as members of the Legionellaceae family are.\(^{19,20}\) In 14 patients with Pontiac fever, a mild and transient form of intracellular bacterial disease caused by Legionella micdadei, Kroca and coworkers noticed an initial depletion of circulating $\gamma/\delta$ T-cells, followed by a pronounced and long-lasting expansion.\(^{21}\) The authors hypothesized that $\gamma/\delta$ T-cells are initially recruited at the primary site of infection before they expand in response to antigen and occur in high numbers in the blood.\(^{21}\)

Overall, the BAL findings at presentation from our patients suggest, also in the primary site of infection, the importance of cell-mediated immunity in the setting of Legionella pneumonia.\(^{14}\) Curiously, the patient (pt no. 3) whose BAL fluid had the lower number of lymphoblasts, the lower increase of activated T-lymphocytes, and the absence of $\gamma/\delta$ T-cells, died a few days after the onset of the disease. By considering these data, one...
could speculate that he developed a less effective cell-mediated immune response to the organism as compared to the other two. Nevertheless, the empiric antibiotic regimen he was submitted to before the etiologic diagnosis was obtained did not contain anti-Legionella drugs, a factor that is well known to negatively affect the outcome in the setting of Legionella pneumonia. Furthermore, he was a heavy smoker and his respiratory status could have been markedly impaired even before Legionella infection, leading to an earlier and more severe respiratory failure.

In the follow-up BAL, a common tendency towards the reduction of lymphoblasts, neutrophils, and CD25+ T-lymphocytes was evident, along with a persistent increase of the HLA-DR+ T-cells (Fig. 2). These similar changes in the BAL pattern are even more surprising if one considers the completely different clinical status of the 2 patients at the time they underwent follow-up bronchoscopy. In fact, one of them was an out-patient doing well and simply complaining of mild exertional dyspnea, whereas the other patient (pt no. 2) had never left the ICU and had developed a ventilator-associated pneumonia due to Pseudomonas Aeruginosa. Apparently, however, the only influence of the P. Aeruginosa infection on BAL in this patient was a less pronounced reduction in the neutrophils percentage.

In conclusion, our data suggest that a quite characteristic and homogeneous BAL profile can be observed in patients with severe pneumonia due to L. pneumophila serogroup 1. In the appropriate clinical setting, this data should prompt aggressive diagnostic search (culture and molecular tests on respiratory secretions, besides urinary antigen testing) and empiric anti-Legionella treatment. We look forward to verifying if the BAL findings that we observed will be confirmed in larger groups of patients with L. pneumophila pneumonia.

References