

Prevalence of Pulmonary Infections Caused by Atypical Pathogens in non-HIV Immunocompromised Patients

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Abstract

Although atypical bacteria are important causes of lower airway infections, data on their role in immunocompromised patients are scarce. The aim of the study was to evaluate the prevalence of atypical pulmonary infections in patients with various types of immunosuppression, and to analyze clinical characteristics of these infections. Eighty non-HIV immunocompromised patients with different underlying diseases and clinical and radiological signs of pulmonary infection were enrolled. Due to incomplete data on eight patients, 72 patients were eligible for final analysis (median age 58 years). All patients underwent fiberoptic bronchoscopy and bronchoalveolar lavage. Bronchoalveolar lavage fluid (BALF) fluid samples were sent for direct microscopy, cultures, and fungal antigen detection, when appropriate. Commercial qualitative amplification assay (PNEUMOTRIS oligomix Alert Kit[®]), based on nested PCR method, was used to detect specific DNA sequences of *L. pneumophila*, *C. pneumoniae*, and *M. pneumoniae* in BALF. There were 61 (84.7 %) patients with hematologic diseases, 3 (4.2 %) after solid organ transplantation, and 8 (11.1 %) with miscellaneous diseases affecting immune status. Specific sequences of *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* DNA were found in 7 (9.7 %), 2 (2.8 %), and 0 patients, respectively. In 8 of these patients co-infections with different microorganisms were demonstrated. Co-infection with *A. baumannii* and *P. aeruginosa* was diagnosed in three patients who died. We conclude that

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atypical lower airway infections are uncommon in immunocompromised patients. The majority of these infections are co-infections rather than single pathogen infections.

Keywords

Atypical bacteria • Bronchoalveolar lavage fluid • *Chlamydophila pneumoniae* • *Legionella pneumophila* • *Mycoplasma pneumoniae* • Immunodeficiency • Respiratory infections

1 Introduction

The incidence of lower airway infections in immunocompromised patients is high and the course of a disease is usually more severe than that in immunocompetent hosts (Sousa et al. 2013; Bonatti et al. 2009). Mortality rate largely depends on the type and severity of immunosuppression, with the highest rate reported after hematopoietic stem cell transplantation (HSCT) and somewhat lower in solid organ transplant (SOT) recipients and patients with hematologic malignancies (HM) (Cervera et al. 2006; Rañó et al. 2001; 2002). It has also been shown that the outcome of pulmonary infections is significantly affected by a delay in diagnosis of specific etiology. An increase in mortality rate from 29 to 71 % has been reported in patients in whom the etiology of infection was ascertained within the first 7 days of onset of symptoms compared with those with later diagnosis (Rañó et al. 2001). The etiology of lower respiratory tract infections in immunocompromised patients is diverse. It includes common bacteria, uncommon bacterial agents, and opportunistic pathogens such as various fungal species and viruses. Although atypical bacteria are important causes of pulmonary infections in the general population, data on the role of these pathogens in immunocompromised patients are relatively scarce. In the immunocompetent hosts *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* are responsible for 1–36 % and 3–22 % of community acquired pneumonia (CAP) cases, respectively (Singanayagam et al. 2014; Masiá et al. 2007; Gleason 2002). The majority of these infections affect children and young adults and present as mild, self-

limiting disease (Capelastegui et al. 2012). However, even 26 % of patients may require hospital admission and in-hospital death rate may be as high as 5 %. The prevalence of *Legionella pneumophila* pneumonia in the general population is slightly lower (2–16 %), but this infection is usually more severe. In two studies, *L. pneumophila* was responsible for 2–9 % of CAP that required hospitalization (Yu and Stout 2008; Gleason 2002). On the other hand, recent data do not confirm the relation between *L. pneumophila* infection and increased in-hospital mortality rate (Capelastegui et al. 2012).

It might be hypothesized that the course of atypical pulmonary infections in immunocompromised patients can be more severe than that in the general population and that the co-infection with atypical pathogens can aggravate the course of pulmonary disease caused by typical bacteria or fungi. Surprisingly, there are little data on the incidence and clinical features of atypical pulmonary infections in immunocompromised patients. According to the available publications, the incidence of these infections is quite low (Corti et al. 2009; Jain et al. 2004; Perez and Leigh 1991). However, a few cases of life threatening pneumonia caused by *C. pneumoniae* and *L. pneumophila* have been described (Di Stefano et al. 2007; Heinemann et al. 2000). Whether the true prevalence of atypical pathogen infections in immunocompromised hosts is low or it is underestimated due to low sensitivity of the diagnostic methods seems to be an interesting issue. It must be realized that the culture of atypical bacteria is difficult and demanding and can be offered by few

laboratories only. Serological methods, including specific IgM and IgG antibodies detection in the serum, have limited clinical application due to a delay in the diagnosis and suboptimal sensitivity in patients with immunoglobulin deficiency (false negative results) (Bartlett 2008; Hammerschlag 2000; Welti et al. 2003). Likewise, *L. pneumophila* antigen detection in the urine has limited sensitivity as a negative result of this test does not exclude infection with other than serotype 1 *L. pneumophila* strains. The introduction of polymerase chain reaction (PCR)-based methods that can identify specific genetic material in different biological samples, including bronchoalveolar lavage fluid (BALF), throat swabs, and nasopharyngeal samples, enables a rapid, sensitive, and specific diagnosis of atypical pathogen infection even if patients are already treated with an antibiotic (Murdoch 2004; Welti et al. 2003; Murdoch 2003). Therefore, the aims of this study were to evaluate the prevalence of atypical lower airway infections using nested PCR (nPCR) method in patients with various types of immunosuppression and to analyze clinical characteristics of these infections.

2 Methods

2.1 Patients

The study protocol was approved by an Institutional Bioethics Committee. The study group consisted of 80 non-HIV immunocompromised patients with different underlying diseases and clinical and radiological signs of pulmonary infection. Due to incomplete data on eight patients, 72 patients were eligible for final analysis (median age 58; range 16–79 years; F/M – 21/51). The patients were treated in a large multidisciplinary university hospital and in a specialized center for hematology and hematologic oncology in Warsaw, Poland. All met the following inclusion criteria: (1) known immunosuppression; (2) clinical or radiological signs and symptoms of pulmonary infection; and (3) signed informed consent for diagnostic bronchoscopy.

Immunosuppression was defined as: (1) hematologic diseases or malignancies (HDM); or (2) immunosuppressive chemotherapy due to any malignant disease; or (3) immunosuppressive treatment due to solid organ or hematologic stem cell transplantation (SOTR); or (4) immunosuppressive therapy due to autoimmune or other diseases; or (5) miscellaneous chronic diseases that could affect the immune state (MISC group).

Clinical signs and symptoms suggestive of lower airway infection included recent cough, fever, dyspnea, or auscultatory findings. Radiological findings consistent with pulmonary infection were defined as the presence of the following pulmonary abnormalities: single or multifocal consolidations, areas of ground glass opacity, pulmonary nodules, interstitial pattern which could not have been explained by other causes, such as e.g. progression of lung tumors or new lung metastases. Exclusion criteria were the following: (1) known AIDS or positive result of HIV test; (2) contraindications to diagnostic bronchoscopy, i.e., unstable hemodynamic status, gas exchange abnormalities resulting in hypoxemia (SaO₂ below 92 %) despite low flow oxygen therapy; and (3) respiratory failure requiring mechanical ventilation.

2.2 Bronchoscopy Procedure

All patients underwent fiberoptic bronchoscopy under local anesthesia. The insertion of a bronchoscope (Olympus BF 1 T180 or Pentax EB 1970 K; Tokyo, Japan) was preceded by premedication with atropine sulphate 0.5 mg s.c. and midazolam 7.5 mg p.o., and by local anesthesia of the upper airways with 2 % lidocaine. Suction was avoided in the upper airways and trachea to minimize contamination of the working channel of the bronchoscope. Additional portions of lidocaine were applied to the lower airways when necessary. After visual inspection of the lower airways, bronchoscope was wedged in segmental or sub-segmental bronchus in accordance with the localization of radiological abnormalities. In case of no relevant radiological abnormalities, bronchoscope was

wedged in the medial or lateral segment of the right middle lobe (RB4 or RB5). Two hundred milliliters of sterile, pre-warmed (37 °C) 0.9 % saline solution were instilled either in ten 20 ml portions or four 50 ml portions and withdrawn by gentle suction. Bronchoalveolar lavage fluid (BALF) was collected in sterile polypropylene tubes.

2.3 Microbiological Procedure

Samples of BALF were sent for microbiological examination including direct microscopy, cultures, and fungal antigen detection, when appropriate. One milliliter samples of BALF were frozen at −20 °C. Total DNA was extracted from 200 µl of BALF, using EXTRAcell[®] isolation kit. Commercial qualitative amplification assay (PNEUMOTRIS oligomix Alert Kit[®]), based on nested PCR method, was used to detect specific DNA sequences of *L. pneumophila*, *C. pneumoniae*, and *M. pneumoniae* in defrozen BALF samples. Also BETA-GLOBIN oligomix Alert Kit[®], which uses the human β-globin gene as a standard, was used as an external control of DNA extraction and amplification. All reagents described above were supplied by Nanogen Advanced Diagnostics S.r.L. (Turin, Italy), and all investigations were performed in accordance to the manufacturer's instructions. A presumed limit of detection (LOD) of the PCR assay used was established as a few dozen copies/ml.

2.4 Data Collection and Analysis

Data on clinical and radiological signs and symptoms, and the results of microbiological examination of BALF were retrospectively collected and loaded in an electronic database. Additionally, results of other microbiological studies, including blood samples, throat swabs, sputum, urine, or stool were also analyzed.

Consistently with the aim of the study, results were assessed in patients with different types of immunosuppression.

Quantitative variables were presented as median, interquartile range (IQR) and/or ranges, while qualitative variables were presented as number and percentage. A non-parametric Mann-Whitney *U* test or Chi-squared test was used to assess the difference between variables in different groups. A p-value below 0.05 was considered statistically significant. Statistical analysis was performed using a statistical software package (STATISTICA, ver. 9.0, StatSoft Inc., Tulsa, OK).

3 Results

Demographics and data on the underlying diseases are presented in Table 1. Patients were unevenly distributed, with 61 (84.7 %) in the HDM group, 8 (11.1 %) in the MISC group, and 3 (4.2 %) patients in the SORT group. The most common underlying disease was acute myeloid leukemia (AML) which was responsible for almost one third of all causes of immunosuppression. AML was followed by chronic lymphocytic leukemia (n = 10; 13.9 % of causes) and non-Hodgkin lymphoma (n = 9; 12.5 % of causes).

Clinical signs and symptoms as well as radiographic data are demonstrated in Table 2. The major clinical symptoms were fever found in 54 (75.0 %) patients and cough reported by 30 (41.6 %) patients. There were no typical signs and symptoms of lower airway infection in 9 (12.5 %) patients, and pulmonary disease in these patients was diagnosed based on the new radiological findings. Chest radiographs and thorax CT scans were available in 71 (98.6 %) and 66 (91.7 %) of patients, respectively. The most common radiographic manifestation was lung consolidation, found in 50 (69.4 %) patients. There was a predominance of bilateral radiographic lung involvement, which was

Table 1 Underlying diseases in relation to demographic data in 72 immunocompromised patients

Causes of immunosuppression	All patients (n)	Male (n)	Female (n)	Age ^a
Hematologic diseases and malignancies	61	44	17	56 (45–66)
Lymphoproliferative disorders	29	23	6	56 (47–63)
Chronic lymphocytic leukemia	10	8	2	63 (57–73)
Non-Hodgkin lymphoma	9	8	1	55 (52–58)
Hodgkin lymphoma	5	3	2	38 (34–50)
Multiple myeloma	4	3	1	55 (49–60)
Waldenstrom macroglobulinemia	1	1	0	61
Acute leukemias	24	15	9	52 (43–61)
Acute myeloid leukemia	23	14	9	53 (45–61)
Acute lymphoblastic leukemia	1	1	0	22
Myeloproliferative disorders	4	2	2	68 (66–70)
Chronic myeloid leukemia	1	1	0	67
Essential thrombocythemia	1	0	1	61
Idiopathic myelofibrosis	2	1	1	69, 72
Other diseases	4	4	0	74 (60–78)
Bone marrow hypoplasia	2	2	0	78, 79
Bone marrow aplasia	1	1	0	29
Thrombocytopenia treated with steroids	1	1	0	70
Solid organ transplant recipients	3	3	0	46, 61, 70
Kidney	1	1	0	70
Liver	1	1	0	61
Kidney and pancreas	1	1	0	46
Various diseases that affected immune status	8	4	4	63 (55–68)
Rheumatoid arthritis	3	0	3	46, 67, 74
Granulomatosis with polyangiitis	1	1	0	58
Idiopathic pulmonary fibrosis	1	1	0	69
Liver cirrhosis	1	1	0	63
Diabetes mellitus	1	1	0	63
Porphyria	1	0	1	28

Data on patients age are presented as median and interquartile range (IQR)

^aAge of individual patients was presented when fewer than four patients with respective diagnosis were evaluated

Table 2 Clinical and radiological characteristics of patients with pulmonary infections in relation to different underlying conditions

Variable	All patients (n = 72)	HDM group (n = 61)	SOTR group (n = 3)	MISC group (n = 8)	p
Signs and symptoms					
Fever, n (%)	54 (75.0)	47 (77.0)	2 (66.6)	5 (62.5)	0.600
Cough, n (%)	30 (41.6)	25 (41.0)	0	5 (62.5)	0.700
Dyspnea, n (%)	13 (18.0)	10 (16.4)	0	3 (37.5)	0.300
Hemoptysis, n (%)	6 (8.3)	3 (4.9)	0	3 (37.5)	0.016
No symptoms, n (%)	9 (12.5)	8 (13.1)	0	1 (12.5)	0.600
Radiological pattern					
Nodular pattern, n (%)	20 (27.8)	19 (31.1)	0	1 (12.5)	0.300
Consolidations, n (%)	50 (69.4)	40 (65.6)	3 (100.0)	7 (87.5)	0.200
Ground glass, n (%)	18 (25.0)	17 (27.9)	0	1 (12.5)	0.400
Other abnormalities (atelectasis, pleural effusion), n (%)	13 (18.0)	10 (16.4)	1 (33.3)	2 (25.0)	0.600

(continued)

Table 2 (continued)

Variable	All patients (n = 72)	HDM group (n = 61)	SOTR group (n = 3)	MISC group (n = 8)	p
Lung involvement in chest radiograph					
Bilateral, n (%)	37 (52.1)	30 (50.0)	2 (66.7)	5 (62.5)	0.700
Right lung only, n (%)	23 (32.4)	21 (35.0)	0	2 (25.0)	0.400
Left lung only, n (%)	8 (11.3)	6 (10.0)	1 (33.3)	1 (12.5)	0.400
No abnormalities, n (%)	3 (4.2)	3 (5.0)	0	0	0.800
No chest radiograph, n (%)	1 (1.4)	1 (1.6)	0	0	0.900
Lung involvement in CT scan					
Bilateral, n (%)	46 (69.7)	38 (69.1)	2 (66.7)	6 (75.0)	0.800
Right lung only, n (%)	13 (19.7)	11 (20.0)	0	2 (25.0)	0.600
Left lung only, n (%)	7 (10.6)	6 (10.9)	1 (33.3)	0	0.300
No CT scan, n (%)	6 (8.3)	6 (9.8)	0	0	0.600
Various data					
Duration from hospital admission to FOB, days	14 (8–28)	14 (8–30)	14, 23, 38 ^a	15 (11–21)	0.500
Antibiotic treatment prior to FOB; n/n of pts with DA (%)	51/65 (78.5)	45/55 (82.0)	1/2 (50.0)	5/8 (62.0)	0.300
Treatment with antibiotic active against APs; n/n of pts with DA (%)	28/65 (43.1)	24/55 (44.0)	0/2 (0)	4/8 (50.0)	0.400
Neutropenia; n/n of pts with DA (%)	39/49 (79.6)	38/46 (82.6)	0/0 (0)	1/3 (33.3)	0.200
GCS therapy; n/n of pts with DA (%)	31 (43.0)	24/61 (39.3)	3/3 (100.0)	4/8 (50.0)	0.100
Outcome					
Cured/improved, n (%)	40 (55.5)	36 (59.0)	1 (33.3)	3 (37.5)	0.400
Failure, not fatal, n (%)	5 (6.9)	4 (6.5)	NA	1(12.5)	0.900
Fatal, n (%)	11 (15.2)	9 (14.7)	1 (33.3)	1 (1.25)	0.700
Data not available, n (%)	16 (22.2)	12 (13.1)	1 (33.3)	3 (37.5)	0.500

Data are presented as median and interquartile range (IQR) or number (%)

APs atypical pathogens, CT computed tomography, pts patients, DA data available, FOB fiberoptic bronchoscopy, GCS glucocorticosteroid, HDM hematologic disease/malignancy, MISC miscellaneous chronic diseases, SOTR solid organ transplant recipients, NA non-applicable

^aData of individual patients were presented instead of median and IQR when fewer than four patients were evaluated

demonstrated in about half of patients, i.e., in 37/71 (52.1 %) and 46/66 (69.7 %) patients as based on chest radiograph and thorax CT scan, respectively. Isolated right lung involvement was found in 23 chest radiographs and 13 thorax CT scans.

Table 3 presents the clinical, radiological and microbiological characteristics of 9 patients in whom DNA of atypical pathogens was identified in BALF. In none of 72 samples specific *L. pneumophila* DNA sequences were found. *M. pneumoniae* specific DNA was identified in samples collected from 7 (9.7 %) patients. Two

samples (2.8 %) tested positively for *C. pneumoniae* DNA. In all patients with identified atypical pathogens, fever was the most commonly reported symptom. In 6 out of the 9 patients bilateral lung involvement was demonstrated. In 8 patients, co-infections with different microorganisms were detected based on BALF or blood microbiological studies. Despite broad spectrum antibiotic and antifungal therapy, 3 patients died. All those patients had positive results of blood culture, with *A. baumannii* and *P. aeruginosa* found in two and one patients, respectively.

Table 3 Characteristics of patients with atypical bacteria DNA in bronchoalveolar lavage fluid (BALF)

Patient No	Age (yr)	Gender	Group	Fever	Cough	Dyspnea	Chest X-ray	CT scan	Blood samples (culture/tesis)	BALF culture	BALF fungal antigens or PCR	Immunosuppression factor	Outcome
Patients with <i>Mycoplasma pneumoniae</i> infection													
1	64	M	HDM	+	-	-	NA	BL	Negative	Negative	Negative	Neutropenia	Improvement
2	46	M	HDM	+	-	+	RL	RL	<i>S. aureus</i>	Negative	Negative	Neutropenia	Improvement
3	39	M	HDM	-	+	-	BL	BL	Negative	<i>S. pneumoniae</i>	Negative	CHTH	Improvement
4	75	M	HDM	+	-	-	RL	RL	<i>A. fumigatus</i> PCR (+)	<i>S. viridians</i> , <i>C. glabrata</i>	Negative	Underlying disease (CLL)	Improvement
5	51	M	HDM	+	+	+	BL	BL	<i>P. aeruginosa</i>	<i>E. faecium</i>	<i>Candida</i> antigen	Neutropenia, SC	Fatal (ARF due to pneumonia)
6	46	M	SOTR	+	-	-	BL	BL	Negative	<i>E. faecium</i>	Negative	SC	Improvement
7	58	M	MISC	+	+	+	BL	RL	<i>A. baumani</i> ^a , <i>A. fumigatus</i> ^a <i>A. fumigatus</i> antigen	<i>A. fumigatus</i>	<i>A. fumigatus</i> antigen <i>Candida</i> antigen	Neutropenia CHTH, SC	Fatal (ARF due to IPA)
Patients with <i>Chlamydomytila pneumoniae</i> infection													
8	63	M	HDM	+	-	-	RL	RL	Negative	Negative	<i>Candida</i> PCR (+)	Neutropenia	Improvement
9	67	M	HDM	+	-	-	BL	BL	<i>A. baumani</i>	<i>S. viridians</i> , MRSA, <i>E. coli</i>	Negative	SC	Fatal (ARF due to pneumonia)
Patients with <i>Legionella pneumophila</i> infection													
-	-	-	-	-	-	-	-	-	-	-	-	-	-

ARF acute respiratory failure, BALF bronchoalveolar lavage fluid, BL bilateral lung involvement, CHTH chemotherapy, CLL chronic lymphocytic leukemia, HDM hematologic disease/malignancy, IPA invasive pulmonary aspergillosis, M male, MRSA methicillin resistant *Staphylococcus aureus*, MISC miscellaneous chronic diseases, NA not available, PCR polymerase chain reaction, RL right lung involvement, SOTR solid organ transplant recipients, + symptom present, - symptom absent, SC systemic corticosteroids

^aAlso in tissue samples

4 Discussion

The present study demonstrates a low prevalence of atypical pulmonary infections in non-HIV immunocompromised patients. *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila* were found in 9.7 %, 2.8 %, and 0 % of patients, respectively. Thus, the prevalence of these infections in this study was somewhat lower than that usually reported in immunocompetent patients with CAP (Capelastegui et al. 2012; Masiá et al. 2007). On the other hand, the percentage of patients in whom atypical pathogens (except *L. pneumophila*) were identified was slightly higher as compared with other studies in immunocompromised hosts (Cervera et al. 2006; Hohenthal et al. 2005; Jain et al. 2004; Danés et al. 2002). This difference can be easily explained by multiple factors that can influence the results of various studies. These include: environmental factors (community or hospital acquired infection), seasonal and local epidemiological situation, type, severity and duration of immunosuppression, methods applied for pathogen detection and identification, reporting method (per entire study group or per subgroup with specific cause of immunoincompetence), and treatment applied prior to microbiological sampling. Despite all these conditions, most authors agree that atypical pulmonary infections in immunocompromised hosts are rather uncommon. Depending on the source of data, typical bacteria, fungi, and viruses have been responsible for 18–51 %, 8–38 %, and 9–23 % of pulmonary infections in non-HIV immunocompromised patients, respectively (Camps Serra et al. 2008; Jain et al. 2004; Danés et al. 2002; Rañó et al. 2001). In addition, polymicrobial infections caused by the pathogens outlined above have been diagnosed in 7–13 % of patients. Atypical pathogens have been found in single cases only.

In this study, diagnosis of pulmonary infection caused by atypical bacteria was based on a sole microbiological test, i.e., identification of specific DNA sequences in lavage fluid collected directly from the site of infection. The role of

fiberoptic bronchoscopy and bronchoalveolar lavage as a diagnostic tool in immunocompromised patients with pulmonary infiltrates is well established. It has been shown that an early bronchoscopy (<5 days) has a significantly higher diagnostic yield for pulmonary infections than the late bronchoscopy (78 vs. 23 %; $p = 0.02$) (Lucena et al. 2014). The role of diagnostic methods other than culture in the work-up of immunocompromised patients with pulmonary infections has also been positively verified, albeit ELISA tests for the detection of *C. pneumoniae* and/or *M. pneumoniae* antibodies have some limitations, due to well-known cross reactions with other *Chlamydia* and *Mycoplasma* species. Hohenthal et al. (2005) have shown that the use of PCR and antigen detection to identify infectious agents in BALF from patients with hematological malignancies significantly improves the diagnostic yield. Unfortunately, although *M. pneumoniae* and *C. pneumoniae* PCR tests were performed in 37 and 29 BALF samples, respectively, the authors have neither presented nor discussed these results. Similar to the present study, none of the BALF samples evaluated by Hohenthal et al. (2005) tested positively for *Legionella spp.* in PCR tests. There are, however, two points which should be mentioned when comparing the results of these two studies. Firstly, the number of BALF samples evaluated by Hohenthal et al. (2005) has been almost two-fold higher than that in the present study. Secondly, in the Finnish study both PCR method and cultures have been applied and there was one patient with a positive culture but negative *Legionella spp.* PCR test. Thus, we cannot exclude that some patients with legionellosis could have been found in the present study, had other than PCR diagnostic methods been used. Nevertheless, the results of both studies point to a very low prevalence of *L. pneumophila* pulmonary infection in immunocompromised patients. That seems inconsistent with the results of some earlier studies which showed that hematological malignancies are a significant risk factor (rate ratio 22.4) for *L. pneumophila* pneumonia (Marston et al. 1994). Furthermore, as the course

of pulmonary infections in immunocompromised patients is often severe and *L. pneumophila* is a well-known pathogen responsible for severe pneumonias, a higher prevalence of this infection could be expected in immunocompromised patients. Therefore, some methodological issues that could have negatively influenced the prevalence of *L. pneumophila* infections found in the present study should be considered. The hypothesis that extremely low prevalence of *L. pneumophila* infection was related to false negative PCR results is highly unlikely. Contrary to the above mentioned data (positive *L. pneumophila* culture and false negative PCR test) numerous other studies demonstrate that *Legionella* PCR has a sensitivity equal to, or greater than, culture. A PCR test can give false negative results when polymerase inhibitors are present in the biological sample (Hammerschlag 2000). It has been shown that in *M. pneumoniae* infections, throat swabs are preferred over nasopharyngeal samples due to a lower rate of PCR inhibitors (Murdoch 2003). As PCR inhibitors are usually nonspecific, their presence would have caused false negative results not only in terms of *L. pneumophila* infection but also other pathogens, i.e., *M. pneumoniae* and *C. pneumoniae*. This was not the case in our study, as an external control of DNA extraction and amplification was used simultaneously and no inhibition was observed during this study. Early and adequate antibiotic therapy before sample collection can be another cause of false negative results of microbiological studies. In fact, a significant proportion of our patients (65.3 %), including 7/9 patients with atypical bacterial infection, had been treated with macrolides or fluoroquinolones before or at the time of diagnostic bronchoscopy. Prior studies in patients with pneumonia have shown that bronchoalveolar lavage performed within 3 days of antibiotic therapy onset has a diagnostic yield of 63.4 %, while the diagnostic value decreases to 57.6 % and 34.4 %, when lavage is done later on, before and after 14 days of treatment initiation, respectively (Kottmann et al. 2011). The argument against the confounding role of prior treatment for the

results obtained in the present study is that PCR tests allow detecting genetic material of causative pathogen even a few weeks after initiation of antibiotic therapy (Welti et al. 2003).

Interestingly, atypical pathogens were identified in the present study exclusively in males. This may be partially explained by a higher proportion of males (71 %). Nevertheless, we believe this is not a sufficient explanation for this finding. Some gender-related differences in the incidence of atypical bacterial infections have also been reported in previous studies. Gutiérrez et al. (2006) have found the incidence of CAP caused by *C. pneumoniae* and *L. pneumophila* in the general population two-fold and ten-fold higher in males than in females, respectively. Age-related differences in the prevalence of atypical pathogen infections should also be mentioned. In the present study, median age of patients with *M. pneumoniae* infection was 51 years. This is somewhat inconsistent with Gutiérrez et al.'s (2006) findings who have reported the highest incidence of *M. pneumoniae* CAP in young and very elderly people, and the lowest between 45 and 64 years of age. To our knowledge, no specific data have been published on the gender-related or age-related differences in the prevalence of atypical pathogen infection in immunocompromised patients. Therefore, we could not confront our observation with any other. We realize that the number of patients with atypical pathogen infections is too small to draw unequivocal conclusions on the relationship between age or gender and the prevalence of *M. pneumoniae* and *C. pneumoniae* infections.

The mortality rate in our nine patients with *M. pneumoniae* or *C. pneumoniae* infection was relatively high (33.3 %), but we believe that neither was the course of disease nor mortality rate related exclusively to atypical bacterial infection. In this context, it should be underlined that in eight of these patients co-infection with other microorganisms was found (positive BALF and/or blood cultures). Systemic bacterial co-infection was proved in all three patients who died (*A. baumannii* and *P. aeruginosa* cultured from blood samples). This finding is

consistent with the results of three earlier studies that have reported co-infection with at least one another pathogen in 33–64 %, 48–74 %, and 54–63 % patients with *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* infections, respectively (Welti et al 2003; Gleason 2002; Hammerschlag 2000). Perhaps, destruction of the airway epithelial layer and ciliostatic effect of these pathogens, facilitate other bacterial infections.

We are aware of several limitations of this study. Due to a small sample size, 95 % confidence interval could be calculated as 2.9–17.0 % and 0.0–6.8 % for a proportion of *M. pneumoniae* and *C. pneumoniae* infections, respectively. These values may question the confidence of a low prevalence of atypical pathogen infection in the study group. There is a marked disproportion between the number of patients with different causes of immunosuppression. In fact, our study group included mainly patients with hematological malignancies; hence the results refer mostly to this group of immunocompromised patients. That is also why we could not analyze the relationship between underlying diseases and the prevalence or clinical course of atypical infections.

A significant limitation of our study is associated with the use of PCR only to identify atypical bacteria infection. In consequence, we were unable to assess and discuss potential false positive and false negative results. Previous studies, including that by Pignanelli et al. (2009), have shown that a concomitant use of two or more different tests provides a higher diagnostic accuracy. Thus, the question on the true etiology of lower respiratory tract infection in some of our patients is still pending. In cases in which we did not find any putative etiological agent, it could have been any of the common respiratory viruses (metapneumovirus, coronavirus, or bocavirus) that are not routinely detected. Therefore, use of wide-range diagnostic tool, e.g., FilmArray[®] Respiratory Panel based on multiplex nested PCR assay, could be helpful to improve outcome in immunocompromised patients (Dzieciatkowski et al. 2013).

In conclusion, we found that atypical lower airway infections are uncommon in immunocompromised patients. This particularly refers to *L. pneumophila* pneumonia. The majority of atypical pulmonary infections are co-infections rather than single pathogen infections.

Acknowledgment The authors gratefully acknowledge Warsaw Medical University and the Foundation for Patients with Hematological Diseases in Warsaw, Poland for the financial support that enabled the realization of the project.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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