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Virological diagnosis in community-acquired pneumonia in immunocompromised patients

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ABSTRACT: Community-acquired pneumonia (CAP) is a serious lower respiratory tract infection associated with significant morbidity and mortality in immunocompromised patients. The present study evaluated the clinical spectrum of CAP in immunocompromised hosts and the role of respiratory viruses, as well as the yield of viral diagnostic methods.

Conventional microbiological tests were routinely performed in immunocompromised patients with CAP. Nasopharyngeal swabs were processed for respiratory viruses by indirect immunofluorescence assay, cell culture and PCR. Four groups were defined according to aetiology of CAP, as follows: group 1 (nonviral), group 2 (mixed, nonviral and viral), group 3 (only viral) and group 4 (unknown aetiology).

Over a 1-yr period, 92 patients were included. An aetiological diagnosis was achieved in 61 (66%) patients: 38 (41%), group 1; 12 (13%), group 2; and 11 (12%), group 3. The most frequent pathogen detected was *Streptococcus pneumoniae* (n=29, 48%), followed by rhinovirus (n=11, 18%). PCR identified 95% of respiratory viruses. Clinical characteristics could not reliably distinguish among the different aetiological groups.

Respiratory viruses represent a substantial part of the aetiologies of community-acquired pneumonia in immunocompromised patients and its routine assessment through PCR in nasopharyngeal swabs should be considered in the clinical care of these patients.

KEYWORDS: Community-acquired pneumonia, immunocompromised patients, respiratory virus, virological diagnosis

Community-acquired pneumonia (CAP) is a severe lower respiratory tract infection associated with significant morbidity and mortality in immunocompromised patients. However, the aetiology of this infection may remain elusive in more than half of all cases [1]. Until now, CAP in these patients has been attributed to fungal or bacterial agents for whom empirical therapy is currently recommended [2]. Viral lower respiratory tract infections in immunocompromised patients have generally been ascribed to herpes virus, particularly cytomegalovirus (CMV) [3]. Community-acquired respiratory viruses have been increasingly recognised to be associated with severe respiratory complications among both immunocompetent and immunocompromised patients of all ages [4, 5]. It has been suggested that infection by respiratory viruses (RVs) in immunocompromised patients may be differentiated from those observed in immunocompetent subjects by the following three major characteristics: 1) persistent viral shedding makes them contagious for prolonged periods; 2) high frequency of nosocomial acquisition; and 3) the high frequency of pneumonia and death [4]. The direct role of RVs in causing pneumonia and death is uncertain in many of these patients, who frequently have multiple infections and concomitant noninfectious illnesses. It is also not currently clear whether RVs themselves cause pneumonia or whether they act as predisposing agents to enable bacteria or fungi, for example, to ultimately cause pneumonia [6].

To date, RVs have not been accurately considered because they are not routinely investigated. Serological tests are generally not performed in immunocompromised hosts due to the length of time taken to obtain results and an inadequate antibody response. Although rapid detection methods using fluorescent-labelled antibodies and cell cultures have been more commonly used, molecular detection methods are increasingly being recognised as superior for the

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detection of many RVs, particularly those that are difficult to isolate in culture [4, 6, 7]. Furthermore, multiplex PCR seems highly sensitive for the detection of viruses and is able to identify more co-infections than conventional methods [8].

Early detection of viral respiratory infections in immunocompromised patients may not only facilitate their optimal clinical management but also prevent their transmission [9].

The present authors designed a prospective observational study specifically addressing the incidence of viral CAP requiring hospital admission, the viruses involved and the potential differences in clinical presentation and outcome between viral and nonviral CAP in immunocompromised patients. In addition, the usefulness of PCR to detect RVs from nasopharyngeal samples in immunocompromised adults with CAP was studied.

MATERIALS AND METHODS

Patients

Immunocompromised patients >14 yrs of age admitted to the Hospital Clínic of Barcelona (Barcelona, Spain) with a diagnosis of CAP were prospectively studied from January 2003 to January 2004. Immunosuppression was considered if the patient had undergone solid organ or bone marrow transplantation, had HIV infection, had received steroids in daily doses of >10 mg prednisolone-equivalents for longer than at least the previous 4 weeks, or was receiving chemotherapy for neoplastic disease [10]. CAP was defined as the presence of a new infiltrate visualised by chest radiography together with clinical symptoms suggestive of lower respiratory tract infection and no alternative diagnosis in a patient not admitted to hospital within the previous month and in whom no alternative diagnosis was established during follow-up [11]. Clinical, laboratory and radiological features at presentation, as well as other epidemiological data previously recorded in a specific questionnaire and entered in a computer database, were also taken into account.

For the purpose of the present study, only patients in whom a nasopharyngeal swab for the study of RVs was available were included. Despite special efforts to obtain nasopharyngeal swabs for every patient admitted with CAP, some patients, particularly on busy days and at night, did not have swabs taken as it was not a routine procedure. As PCR for RVs were not immediately tested, their results had no influence on the choice of antiviral therapy. The Ethics Committee of Hospital Clínic had previously approved the study.

Microbiological methods

Microbiological sampling was performed on admission. It included sputum, blood for bacterial culture, urine for *Streptococcus pneumoniae* (Binax Now *S. pneumoniae* Urinary Antigen Test; Emergo Europe, The Hague, The Netherlands) and *Legionella pneumophila* urinary antigen detection (Binax Now *L. pneumophila* Urinary Antigen Test; Trinity Biotech, Bray, Ireland) and nasopharyngeal swabs. Pleural puncture, tracheobronchial aspiration (BAS) and bronchoalveolar lavage (BAL) clinical samples were obtained according to clinical indication or judgement of the attending physician. Conventional tests were used to evaluate the presence of bacterial, parasitic and fungal agents, and nonrespiratory viruses. Sputum, BAS and BAL specimens were stained using Gram and Ziehl–Neelsen methods for bacterial and mycobacteria detection, respectively. In BAL samples, the following additional stains were used: May–Grünwald Giemsa for fungal detection and cellular differential count and Gomori methenamine silver for *Pneumocystis jirovecii*. In BAL specimens, antigen CMV detection was carried out by means of indirect immunofluorescence (Bio-Rad, Marnes-la-Coquette, France). Sputum and pleural fluid samples were qualitatively cultured for bacterial pathogens, fungi and mycobacteria. BAS and BAL samples were homogenised and processed for quantitative culture by serial dilutions for bacterial pathogens; undiluted cultures for *Legionella* spp., fungi and mycobacteria were also carried out. Processing of samples and diagnostic criteria has been described elsewhere [11].

Nasopharyngeal samples were obtained and placed into a tube with viral transport medium, as previously described [12]. Within 24 h after admission, nasopharyngeal swabs and BAL specimens were processed for antigen detection by immunofluoresence assay (IFA) and for isolation of viruses in cell culture. For IFA, samples were stained with fluorescein-conjugated antibody to influenza virus A, influenza virus B, human parainfluenza virus 1-3, adenovirus and respiratory syncytial virus (Respiratory Panel 1, Viral Screening and Identification Kit; Light Diagnostics, Chemicon, Temecula, CA, USA). The presence of viral antigen in respiratory cells was indicated by the appearance of characteristic intracellular apple-green fluorescence in at least one cell. Simultaneously, specimens were inoculated into Madin Darby canine kidney, human Caucasian lung carcinoma (A-549) and human Caucasian larynx carcinoma squamous cell (Hep-2) cell lines (Vircell, Granada, Spain) for isolation of the viruses mentioned previously and herpes simplex virus. BAL samples were also inoculated into MRC-5 (human fibroblasts) for CMV isolation. Cell cultures showing cytopathic effect were harvested and stained for virus identification with IFA as indicated previously. When Hep-2 cell culture had cytopathic effect and was negative by IFA for RVs, IFA for herpes simplex virus (HSV1/HSV2 Culture Identification/Typing Test; MicroTrak; Trinity Biotech) and a pool of enterovirus (Enterovirus Screening Set Ready to Use; Light Diagnostics, Chemicon) were performed. Upon sample collection, an aliquot of each fresh specimen was stored at -80°C until reverse transcriptase (RT)-PCR testing. For RT-PCR, viral genomic RNA and DNA was extracted from a total volume of 200 μL of specimen using the guanidinium thiocyanate extraction method [13]. The lysis buffer included 500 molecules of the cloned amplified product used as an internal control in each reaction tube and then excluded false-negative results due to nonspecific inhibitors or extraction failure. Two independent multiplex-nested RT-PCR assays able to detect from 1–10 copies of viral genomes were performed as described previously [14, 15]. One RT-PCR assay detected influenza virus types A, B and C, respiratory syncytial virus A and B, and adenovirus. Another RT-PCR assay studied parainfluenza viruses 1, 2, 3 and 4, coronaviruses 229E and OC43, rhinoviruses and enteroviruses. In each assay, negative (viral transport medium containing no nucleic acid) and positive controls (cDNAs obtained from the present study's viral lysates or from reference strains) were treated with the same procedure. All positive results were subsequently confirmed by a second independent assay.

Viral aetiology was considered ''presumptive'' if at least one of the following criteria was met: 1) isolation of RVs in cell culture; 2) detection of RVs by RT-PCR in two different and independent assays; and 3) detection of antigens by IFA plus virus isolation or detection by RT-PCR.

Definition of groups of CAP according to aetiology

Four aetiological groups of CAP were defined according to the agents identified, as follows: group 1 (nonviral), with at least one bacterial, fungal or parasitic agent and no viral agents identified; group 2 (mixed) with both nonviral plus viral agents; group 3 (only viral); and group 4, unknown aetiology.

Statistical analysis

Quantitative characteristics were described for each of the four groups by median and interquartile ranges (IQRs). Comparisons among the three groups (1, 2 and 3) were made using the Kruskal–Wallis test, and the Mann–Whitney U-test was used in each of the three pairwise comparisons. Qualitative characteristics were reported as frequencies and percentages for all four groups and compared between the three groups (1–3) using either Fisher's exact or the Chi-squared test; the same test was used for pairwise comparisons. A p-value $\leqslant 0.05$ was considered statistically significant. All tests were two-tailed and the confidence level was set at 95%. Bonferroni's correction of the significance level was used for three pairwise comparisons.

RESULTS

Patients and specimens

Of the 150 immunocompromised patients with CAP admitted to the present authors' hospital during the study period, 92 with nasopharyngeal swabs were included in the study. Of these, 57 (62%) had HIV infection (all but five were receiving antiretroviral therapy), 19 (21%) had neoplastic disease and were receiving chemotherapy, 10 (11%) were undergoing chronic corticosteroid therapy and six (7%) patients had undergone transplantation (four were solid organ recipients and two had undergone bone marrow transplantation). Of the 92 patients, 60 (65%) were male and 32 (35%) female with a median age of 47 yrs (IQR 39–76). A total of 77 (84%) patients were admitted to conventional wards, nine (10%) to the intensive care unit and six (7%) to the intermediate care unit.

Blood cultures and urine antigen detection for *S. pneumoniae* and *L. pneumophila* were performed in all patients. Sputum was collected in 76 cases, with good quality criteria in 62 (82%) [16]. Other samples for microbiological studies were BAS (one patient), pleural fluid (14 patients) and BAL (10 patients).

Aetiology of CAP

Aetiological diagnosis was achieved in 61 (66%) of the 92 patients studied. According to the predefined groups, 38 (41%) cases of CAP were due to nonviral agents (group 1), 11 (12%) to mixed aetiology (group 2) and 12 (13%) to viruses (group 3; table 1).

The most frequent aetiological agent was *S. pneumoniae* in 39 (42%) cases, followed by RVs (n=20, 22%). Rhinovirus was the most common virus, being observed in 12% (n=11) of cases. Others RVs identified were adenovirus (n=5), influenza virus A (n=3) and influenza virus B (n=1). All RVs were recovered from nasopharyngeal swabs and one rhinovirus was also recovered from one BAL sample. Moreover, herpes simplex virus type 1 (HSV1) was identified in five patients; three of these patients were co-infected with *S. pneumoniae*, one with *P. jirovecii* and one with rhinovirus and *S. pneumoniae*. Two HSV1 came from nasopharyngeal swabs and BAL samples, and three only from nasopharyngeal swabs. All five *P. jirovecii* appeared in BAL samples from HIV-infected patients not receiving antiretroviral therapy.

Virological analysis

A total of 20 RVs in 19 patients were identified, 19 out of 20 (95%) by RT-PCR. IFA was unable to detect any virus. Viral culture allowed the isolation of three (15%) viruses only: one adenovirus, one influenza virus type B and one virus influenza type A. The latter could not be detected by RT-PCR due to nonspecific inhibitors in the sample. All rhinoviruses were exclusively identified by PCR. HSV1 was identified by isolation in cell culture and subsequent IFA.

Characteristics of CAP according to the aetiology

The clinical characteristics of the four aetiological groups are summarised in tables 2 and 3.

No significant differences were found in sex, age, toxic habits, previous antibiotic treatment, previous CAP, pneumonia severity score and comorbidities, although 61 (66%) patients had an underlying disease. Only 31 (34%) out of 92 patients included in the present study had been vaccinated against influenza, none of whom presented with this infection.

There were significant differences among CAP groups regarding the condition for immunosuppression (p=0.043), the concomitance of upper respiratory tract infection (URTI; p=0.023) and the seasonality (p=0.006). HIV-infected patients

TABLE 1 Aetiology of community-acquired pneumonia in immunocompromised adults

	Pathogen	Patients n
Group 1	*Streptococcus pneumoniae*	29
	S. pneumoniae + *Haemophilus influenzae*	1
	S. pneumoniae + *Legionella pneumophila*	1
	Pneumocystis jirovecii	3
	Staphylococcus aureus	2
	Streptococcus viridans	1
	L. pneumophila	1
Group 2	Rhinovirus + *S. pneumoniae*	2
	Rhinovirus + *P. jirovecii*	1
	Rhinovirus + *H. influenzae*	1
	Rhinovirus + HSV1 + *S. pneumoniae*	1
	Adenovirus + *S. pneumoniae*	2
	HSV1 + *S. pneumoniae*	3
	HSV1 + *P. jirovecii*	1
Group 3	Rhinovirus	5
	Influenza A virus	3
	Adenovirus	3
	Influenza B virus + rhinovirus	1

HSV1: herpes simplex virus type 1. n=61.

had a higher incidence of nonviral and mixed CAP, whereas patients with neoplastic disease suffered more commonly from viral CAP. An URTI concomitant or prior to CAP was more common when a virus was involved in the aetiology of CAP (mixed CAP and viral CAP), and it happened more often in autumn and winter (fig. 1).

Four patients required mechanical ventilation and finally died. Oddly enough, two had a rhinovirus as the sole aetiological agent ($p=0.05$). In the remaining two patients, the aetiology was unknown.

DISCUSSION

The main findings of the present study were as follows: 1) RVs represented a common aetiology of CAP in immunocompromised patients; 2) clinical characteristics could not reliably distinguish among the different aetiological groups; and 3) PCR proved to be a sensitive and rapid method for diagnosis of viral CAP.

S. pneumoniae was the most common aetiological agent in both nonviral and mixed CAP, in accordance with other recent studies [17]. RVs, led by rhinovirus, represented the second cause of CAP. Previous studies also suggest that rhinovirus may be frequently involved in immunocompromised patients with CAP [18, 19]. Until recently, rhinoviruses were considered to replicate mainly in the upper respiratory tract in patients infected with the common cold. However, experimental data have shown that they can also replicate in the lower respiratory tract in immunocompromised patients with severe pneumonia [20, 21]. In the present study, more than half of the cases with evidence of rhinovirus infection were associated with another pathogen, mainly *S. pneumoniae*. Whether the rhinovirus is a primary cause of the lower respiratory tract disease or its URTI predisposes to bacterial lower respiratory tract infection remains unclear. Recent studies have shown that rhinovirus infection increases the adherence of *S. pneumoniae* to airways epithelial cells [22]. In the present study, patients with rhinovirus plus *S. pneumoniae* did not have a worse clinical evolution than those with rhinovirus alone did. It is noteworthy that in two out of four patients who required mechanical ventilation and finally died rhinovirus was the only pathogen identified. Adenovirus was the second most frequently detected virus, found almost exclusively in patients with HIV infection (four out of five adenoviruses detected); it

TABLE 2 Baseline characteristics of patients according to the aetiology of community-acquired pneumonia (CAP)

	Nonviral CAP	Mixed CAP	Viral CAP	Unknown aetiology	Total	p-value#
Patients n	38	11	12	31	92	
Median age yrs	44.5 (38–62)	44 (34–54)	50 (36.5–81)	72 (43–80)	47 (38.5–76)	0.708
Sex male/female	23 (61)/15 (39)	6 (55)/5 (45)	7 (58)/5 (42)	24 (77)/7 (23)	60 (65)/32 (35)	1.000
Admission						
Intermedius	3 (8)	1 (9)	0 (0)	1 (3)	5 (5)	0.786
Ward	31 (82)	8 (73)	11 (92)	27 (90)	77 (85)	
ICU	4 (11)	2 (18)	1 (8)	2 (7)	9 (10)	
Immunosupression						
Treatment with corticosteroids	4 (11)	1 (9)	0 (0)	5 (16)	10 (11)	0.043
HIV infection	29 (76)	5 (73)	7 (58)	13 (42)	57 (62)	
Neoplastic disease	3 (8)	0 (0)	5 (42)	11 (36)	19 (21)	
Transplantation	2 (5)	2 (18)	0 (0)	2 (6)	6 (6)	
Counts of CD4 in HIV patients <200	10 (40)	3(43)	2 (33)	4 (33)	19 (38)	1.000
Any comorbidity	25 (66)	4 (36)	8 (67)	24 (77)	61 (66)	0.208
Diabetes mellitus	7 (18)	1 (9)	1 (8)	4 (13)	13 (14)	0.674
Renal	4 (11)	0 (0)	3 (25)	2 (6)	9 (10)	0.174
Hepatic cirrhosis	10 (26)	1 (9)	3 (25)	1 (3)	15 (16)	0.619
CNS	1 (3)	2 (18)	2 (17)	8 (26)	13 (14)	0.082
Heart	0 (0)	1 (9)	1 (8)	6 (19)	8 (9)	0.138
Pulmonary	12 (32)	2 (18)	4 (33)	16 (53)	34 (37)	0.725
Labial herpes simplex	20 (53)	8 (73)	8 (67)	21 (68)	57 (62)	0.848
Alcohol use >80 g·day^{-1}	14 (37)	3 (27)	2 (17)	8 (26)	27 (29)	0.386
Current smoker	25 (66)	6 (55)	7 (58)	7 (23)	45 (49)	0.753
Antibiotics prior to admission	8 (22)	0 (0)	4 (33)	11 (35)	23 (25)	0.115
Previous pneumonia	12 (32)	4 (36)	7 (58)	13 (42)	36 (39)	0.271
Season						
Spring–summer	19 (50)	0 (0)	4 (33)	14 (45)	37 (40)	0.006
Autumn–winter	19 (50)	11 (100)	8 (67)	17 (55)	55 (60)	

Data are presented as n (%) or median (interquartile range), unless otherwise indicated. ICU: intensive care unit; CNS: central nervous system. #: p-values were obtained by comparing nonviral, mixed and viral CAP.

TABLE 3 Clinical presentation and evolution of patients according to the aetiology of community-acquired pneumonia (CAP)

	Nonviral CAP	Mixed CAP	Viral CAP	Unknown aetiology	Total	p-value#
Patients n	38	11	12	31	92	
Clinical presentation						
Days with symptoms	5 (3–7)	5 (3–13)	4 (3–5.5)	5 (3–7)	5 (3–7)	0.630
Leukocytes $\times 1000 \cdot \mu L^{-1}$	10.3 (6.1–17.3)	9.4 (4.1–14.6)	9.5 (6.8–15.2)	10.4 (8.1–13.4)	1.03 (6.7–15.1)	0.703
C-reactive protein $mg \cdot dL^{-1}$	13.6 (6–30.4)	24.6 (18.7–31.7)	9.4 (3.7–19.3)	17.4 (11.1–29.4)	14.8 (6.8–29.4)	0.111
$P_{O_2}/F_{I,O_2}$ mmHg	295.2 (257.1–314.3)	323.8 (295.2–366.6)	271.4 (219–314.3)	316.6 (257.1–333.3)	295.2 (257.1–333.3)	0.180
Temperature at admission °C	37.7 (37.1–38.6)	38 (36.7–38.1)	37.7 (36.7–38.5)	37.7 (36.7–38.5)	37.7 (36.7–38.5)	0.476
Upper respiratory tract illness	14 (37)	9 (82)	7 (58)	13 (42)	43 (47)	0.023
Expectoration	30 (79)	8 (73)	9 (75)	21 (68)	68 (74)	0.912
Pleuritic chest pain	25 (66)	8 (73)	4 (33)	19 (61)	56 (61)	0.119
Mental confusion	4 (11)	1 (9)	2 (17)	7 (23)	14 (15)	0.848
Radiographic patterns						
Alveolar pattern	30 (88)	8 (80)	12 (100)	27 (90)	77 (90)	0.555
Interstitial pattern	3 (9)	2 (20)	0 (0)	0 (0)	5 (6)	
Mixed pattern	1 (3)	0 (0)	0 (0)	3 (10)	4 (5)	
Lobular affection						
1	29 (81)	5 (50)	9 (75)	23 (77)	66 (75)	0.161
⩾2	7 (19)	5 (50)	3 (25)	7 (23)	22 (25)	
PSI class						
I+II+III	20 (56)	8 (80)	5 (45)	10 (32)	43 (49)	0.276
IV+V	16 (44)	2 (20)	6 (55)	21 (68)	45 (41)	
Evolution						
Days of stay n	5 (3–7)	5 (3–13)	6 (4–7)	6 (4–8.5)	6 (4–10)	0.137
Any complication	10 (27)	3 (27)	3 (25)	7 (23)	7 (23)	1.000
Mechanical ventilation	0 (0)	0 (0)	2 (22)	2 (7)	4 (5)	0.607
Renal failure	7 (19)	2 (20)	3 (25)	4 (13)	16 (18)	0.898
Shock	5 (14)	1 (10)	1 (8)	1 (3)	8 (9)	1.000
Death	0 (0)	0 (0)	2 (20)	2 (8)	4 (5)	0.050

Data are presented as n (%) or median (interquartile range), unless otherwise indicated. P_{O_2}: oxygen tension; F_{I,O_2}: inspiratory oxygen fraction; PSI: pneumonia severity index. #: p-values were obtained by comparing nonviral, mixed and viral CAP. Comparisons among the three groups (1,2 and 3) were made using the Kruskal–Wallis test, and the Mann–Whitney U-test was used in each of the three pairwise comparisons. 1 mmHg=0.133 kPa.

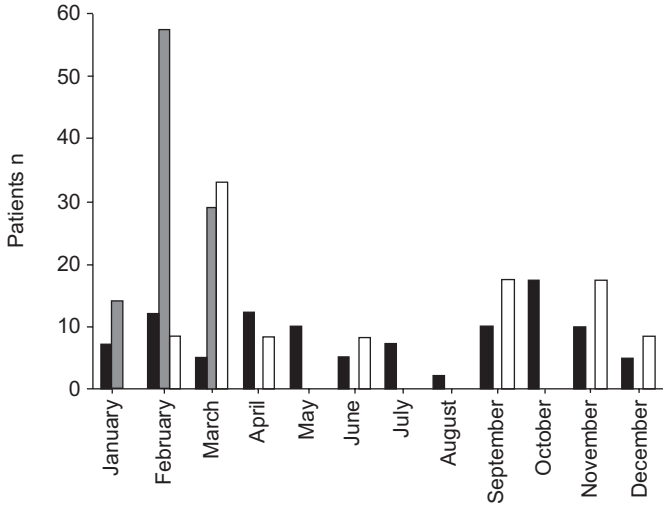

FIGURE 1. Monthly distribution of community-acquired pneumonia cases according to aetiology. ■: nonviral; ■: mixed; □: viral.

was the sole pathogen identified in three out of five patients. The influenza virus has been commonly identified in immunocompromised patients during community outbreaks, with variable incidence and severity of pneumonia [23]. The present authors found only four patients with influenza virus type A and one patient with influenza virus type B; the influenza epidemic during the study period was moderate compared with previous years [24]. None of the patients with CAP associated with influenza virus had been previously vaccinated, and a protective role of influenza vaccination in immunocompromised patients has been suggested [9, 25].

All RVs were detected from nasopharyngeal swabs and, in one case, also from a BAL sample. The current authors believe that these represented true positive results because all the patients included had CAP and so the presence of respiratory viruses in swabs denotes a recent and not latent infection [26], although the present study did not include nonsymptomatic controls. Sputum is not an adequate sample with which to assess the diagnosis of viral pathogens; several sputum contents may easily contaminate cell culture and inhibit the PCR assay. BAL is an invasive procedure that is not routinely performed in

patients with CAP, except for a minority of patients in whom it is clinically indicated.

CMV and HSV1 have been reported as the most common viruses recovered from BAL samples from patients with lower respiratory tract infections in a single institution during a 10-yr period [18]. However, no CMV was detected in the patients of the present study. This discrepancy might be explained by the following three reasons: 1) CMV is a rare agent of CAP in HIV-infected patients; 2) the incidence of CMV infection among transplant recipients has sharply declined since the implementation of routine CMV prophylaxis; and 3) in patients with pneumonia, CMV is almost exclusively found in BAL samples. In the present study, five patients were detected with HSV1, and three out of five only from nasopharyngeal swabs. HSV1 were not considered as RVs, were always associated with another pathogen and, in the present study, were included within the mixed aetiological group. At present, the role of HSV1 as an agent of pneumonia among immunosuppressed patients remains unclear [26]. HSV may reach the lower respiratory tract by aspiration from the upper respiratory tract or by reactivation of the virus at the lungs or trachea, depending on the presence of the virus in the superior cervical and vagal ganglia [27]. BRUYNSEELS *et al.* [28] have shown that the presence of HSV in the throat is highly significant and an independent risk factor for the development of lower respiratory tract infections with HSV [28].

In the present study, an extensive database was used to review the baseline characteristics, clinical presentation and outcome of the patients included. However, few differences were found among the different aetiological groups. There were differences among CAP groups regarding the condition for immunosuppression. In accordance with other reports [29], HIV-infected patients showed a higher incidence of nonviral and mixed CAP, with *S. pneumoniae* and *P. jirovecii* as the predominant causes of CAP in these patients despite the availability of effective prophylaxis. *P. jirovecii* pneumonia was exclusively diagnosed in HIV-infected patients not receiving antiretroviral therapy with $<$200 CD4 per mm^3.

An URTI concomitantly or prior to CAP was more frequent when a respiratory virus was involved (*i.e.* in mixed and viral CAP). In immunocompetent patients, URTI generally gives mild and self-limited symptoms, and the mean duration of the infection is 3–5 days. In contrast, immunocompromised patients often develop worsening symptoms 2–4 days after onset, with progression of the infection from upper to lower respiratory tract [6, 30]. Therefore, some investigators emphasise the need for prevention of respiratory infections and prompt initiation of therapy whenever an URTI is diagnosed [3, 31].

Seasonality is a distinctive feature of viral CAP [6]. The current authors found viral and mixed CAP mainly in the autumn and winter; whereas nonviral CAP was evenly distributed throughout the year.

Similar to other studies, the detection of RVs by RT-PCR was superior to that by cell culture and IFA [8, 12, 32]. Viral culture allows the isolation of the virus, thereby providing direct evidence of infection. This is important for certain purposes that are not yet amenable to PCR (for example, antigenic characterisation and influenza vaccine strain selection), but the yield of cell culture and IFA are closely related to the number and viability of the viruses harboured in clinical samples. In contrast, PCR is able to detect low titres of virus or virus that is replication-incompetent. In adults, the nasopharyngeal viral load might be lower in CAP than in URTI. In a study of URTI performed in the current authors' laboratory, the yield of cell culture was greater than that found in the present study on CAP, and it was similar to that found with RT-PCR assays [33]. Another factor potentially involved in the poor results of cell culture in the present study may be due to the fact that cell culture was not performed for rhinovirus because it is both difficult and time-consuming [34].

In conclusion, the results of the present study demonstrate that respiratory viruses are prevalent aetiologies in immunocompromised patients with community-acquired pneumonia and that clinical characteristics cannot reliably distinguish viral from other aetiologies. Multiplex reverse transcriptase-PCR proved to be a sensitive method providing an early diagnosis, which may be essential for guiding the implementation of antiviral treatment and the preventive measures to avoid nosocomial spread in immunocompromised patients.

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