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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 community-acquired pneumonia in immunocompromised hosts, 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. We defined 4 groups according to aetiology of CAP: group 1 (non viral), group 2 (mixed, non-viral and viral), group 3 (only viral) and group 4 (unknown aetiology).

Over a one year period, 92 patients were included. An aetiological diagnosis was achieved in 61 (66%) patients: 38 (41%), group 1; 12 (13%), group 2; and 11 (13%), 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 aetiologic groups.

Respiratory viruses represent a substantial part of the aetiologies of CAP 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

INTRODUCTION

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 the 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 herpesvirus, particularly cytomegalovirus (3). Community-acquired respiratory viruses have been increasingly recognized 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 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 non-infectious illnesses. Neither is it currently clear whether RVs themselves cause pneumonia or whether they act as predisposing agents such as bacteria or fungi to ultimately cause pneumonia (6).

Up to now, RVs have not been accurately considered because they are not routinely investigated. Serological tests are generally not performed in immunocompromised hosts because long time to get results and inadequate antibody response. Although rapid detection methods using fluorescent-labeled antibody and cell culture have been more commonly used, molecular detection methods are becoming increasingly recognized as superior for detection of many RVs, particularly for those with difficult isolation in

culture (4, 6, 7). Furthermore, multiplex PCR seems highly sensitive for the detection of viruses and able to identify more coinfections 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).

We 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 non-viral CAP in immunocompromised patients. In addition, we studied the usefulness of PCR to detect RVs from nasopharyngeal samples in immunocompromised adults with CAP.

MATERIALS AND METHODS

Patients

Immunocompromised patients older than 14 years admitted to Hospital Clínic of Barcelona with a diagnosis of CAP were prospectively studied up from January 2003 to January 2004. Immunosuppression was considered if the patient had undergone solid organ or bone marrow transplantation, had human immunodeficiency virus (HIV) infection, had received steroids in daily doses > 10 mg prednisolone-equivalent for more 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 visualized on 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 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. Because PCR for RVs were not immediately tested, their results had no influence on the choice of antiviral therapy.

The Ethics Committee of Hospital Clinic 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) and *Legionella pneumophila* urinary antigen detection (Binax Now *Legionella pneumophila* Urinary Antigen Test) 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, non-respiratory viruses and fungal agents. Sputum, BAS and BAL specimens were stained using Gram and Ziehl-Neelsen methods for bacterial and mycobacteria detection respectivately. In BAL samples, additional stains used were May-Grünwald Giemsa for fungal detection and cellular differential count and Gomori methenamine silver for *P. carinii*. In BAL specimens, antigen cytomegalovirus detection was done by means of indirect immunofluorescence (Bio-Rad, 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; also undiluted cultured for *Legionella spp* , fungi and mycobacteria were done.

Processing of samples and diagnostic criteria has been described elsewhere (11).

Nasopharyngeal samples were obtained as described previously and placed into a tube with viral transport medium as 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). The presence of viral antigen in respiratory cells was indicated by the appearance of characteristic intracellular apple-green fluorescence in ≥ 1 cell. Simultaneously, specimens were inoculated into MDCK (Madin Darby Canine Kidney), A-549 (Human Caucasian lung carcinoma) and Hep-2 (Human Caucasian larynx carcinoma squamous cell) cell lines (Vircell, Granada, Spain) for isolation of the viruses mentioned above and herpes simplex virus. BAL samples were also inoculated into MRC-5 (human fibroblats) for cytomegalovirus (CMV) isolation. Cell cultures showing cytopathic effect were harvested and stained for virus identification with IFA referenced above. When Hep-2 cell culture had cytopathic effect and was negative by IFA for RVs, IFA for herpes simplex virus (HSV 1/HSV 2 Culture Identification/Typing Test, MicroTrak) and a pool of enterovirus (Enterovirus Screening Set Ready to Use, Light Diagnostics, CHEMICON, Temecula, CA) were performed. Upon sample collection, an aliquot of each fresh specimen was stored at –80ºC until 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 internal control in each reaction tube and then excluded false-negative results due to non-specific inhibitors or extraction

failure. Two independent multiplex nested RT-PCR assays able to detect from 1 to 10 copies of viral genomes were performed as described previously (14, 15). One RT-PCR assay detected influenza viruses 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 our 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: RVs isolation in cell culture; detection of RVs by RT-PCR in two different and independent assays; detection of antigens by IFA plus virus isolation or detection by RT-PCR.

Definition of groups of CAP according to aetiology

Four aetiologic groups of CAP were defined according to the agents identified: group 1 (non-viral), with at least one bacterial, fungal or parasitic agent and no viral agents identified; group 2 (mixed) with both non-viral 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 and the Mann-Whitney 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 the Fisher's exact or χ^2 test; and the same test was used for

pairwise comparisons. A p value of 0.05 or less 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 our hospital during the study period, 92 with nasopharyngeal swabs were included in the study. Of these, 57 (62%) had HIV-infection (all but 5 receiving antiretroviral therapy), 19 (21%) had neoplastic disease and were receiving chemotherapy, 10 (11%) were undergoing chronic corticosteroid therapy and 6 (7%) patients had undergone transplantation (4 solid organ recipients and 2 bone marrow transplantation). Of the 92 patients, 60 (65%) were men and 32 (35%) women with a median age of 47 years (IQR 39-76). Seventy-seven (85%) patients, were admitted to conventional wards, 9 (10%) to the intensive care unit (ICU) and 6 (5%) to intermediate care unit.

Blood cultures and urine antigen detection for *S.pneumoniae* and *Legionella pneumophila* were perfomed in all patients. Sputum was collected in 76 cases, with good quality criteria in 62 (82%) (16). Other samples for microbiological studies were BAS (1 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 non-viral 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 (n=39, 42%) cases followed by RVs (n=20, 21%). Rhinovirus was the most common virus, being observed (n=11,

12 %). 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 1 rhinovirus was also recovered from 1 BAL sample. Moreover, in 5 patients we identified herpes simplex virus type 1 (HSV1), 3 of whom were coinfected with *S.pneumoniae*, 1 with *P.jirovecii* and 1 with rhinovirus and *S.pneumoniae*. Two HSV 1 came from nasopharyngeal swabs and BAL samples and 3 only from nasopharyngeal swabs. All 5 *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 not able to detect any virus. Viral culture allowed the isolation of 3 (15%) viruses only: 1 adenovirus, 1 influenza virus type B and 1 virus influenza type A. The latter could not be detected by RT-PCR due to non-specific 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 summarized in Tables 2 and 3.

No significant differences were found in gender, 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 had a higher incidence of non-viral and mixed CAP whereas patients with neoplasic disease suffered more commonly viral CAP. An URTI concomitant or prior to the 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.

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 that: 1) respiratory viruses represented a common aetiology of CAP in immunocompromised patients; 2) clinical characteristics could not reliably distinguish among the different aetiologic 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 non-viral 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 with 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 our study, patients with rhinovirus plus *S.pneumoniae* did not have a worse clinical evolution than those with rhinovirus alone did. Of note, 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, being found almost exclusively in patients with HIV infection (4 out of 5 adenoviruses detected); it was the sole pathogen identified in 3 out of 5 patients. Influenza virus has been commonly identified in immunocompromised patients during community-outbreaks, with variable incidence and severity of pneumonia (23). We only found 4 patients with influenza virus type A and 1 patient with influenza virus type B; the influenza epidemic during the study period was moderate compared with previous years (24). None of 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 from a BAL sample too. We 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 our study did not include non-symptomatic controls. Sputum is not an adequate sample 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 excepting for a minority of patients in whom it is clinically indicated.

Cytomegalovirus and HSV 1, have been reported as the most common viruses recovered from BAL samples of patients with lower respiratory tract infections in a single institution during a 10 year period (18). However, no CMV was detected in our patients. This discrepancy might be explained by 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 report, we detected 5 patients with HSV1, 3 out of 5 only from nasopharyngeal swabs. HSV1 were not considered as RVs, were always associated with another pathogen and we included them 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). Bruuynseels et al, have shown that the presence of HSV in the throat is highly significant and 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 non-viral and mixed CAP, being *S.pneumoniae* and *P.jirovecii* 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 less than 200 CD4 per mm^3.

An URTI concomitantly or prior to the CAP was ore frequent when a respiratory virus was involved (mixed CAP and viral CAP). In immunocompetent patients, URTI generally gives mild and self-limited symptoms, and the mean duration of the infection

is 3 to 5 days. In contrast, immunocompromised patients often develop worsening symtoms 2 to 4 days after onset, with progression of the infection from upper to lower respiratory tract (6, 30). Therefore, some investigators emphasize 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). We found viral and mixed CAP, mainly in the auntumn and winter; whereas non viral CAP was evenly distributed throuhgout 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 therefore providing direct evidence of infection. This is important for certain purposes that are not yet amenable to PCR (for example, antigenic characterization and influenza vaccine strain selection), but the yield of cell culture and IFA are closed related to the amount and viability of the viruses harbored in clinical samples. In contrast, PCR is able to detect low titres or not replication-competent virus. In adults, the nasopharyngeal viral load might be lower in CAP than in URTI. In a study on URTI performed in our 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 we did not perform cell culture for rhinovirus because this is difficult and time-consuming (34).

In summary, the results of this study demonstrate that RVs are prevalent aetiologies in immunocompromised patients with CAP and that clinical characteristics cannot reliably distinguish viral from other aetiologies. Multiplex RT-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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REFERENCES

1. File TM. Community-acquired pneumonia. Lancet 2003; 362:1991-2001

2. Bodey GP. Community respiratory viral infections in the immunocompromised host: past, present, and future directions. Am J Med 1997; 17; 102(3A):77-80.

3. Barton TD, Blumberg EA. Viral pneumonias other than cytomegalovirus in transplant recipients. Clin Chest Med 2005; 26(4):707-20.

4. Englund JA. Diagnosis and Epidemiology of Community-Acquired Respiratory Virus Infections in the Immunocompromised Host. Biol Blood Marrow Transplant 2001; 7:2S-4S.

5. de Roux A, Marcos MA, Garcia E, Mensa J, Ewig S, Lode H, Torres A. Viral community-acquired pneumonia in nonimmunocompromised adults. Chest. 2004; 125(4):1343-51.

6. Couch RB, Englund JA, Whimbey E. Respiratory viral infections in immunocompetent and immunocompromised persons. Am J Med. 1997; 102(3A):2-9;

7. Roghmann M, Ball K, Erdman D, Lovchik J, Anderson LJ, Edelman R. Active surveillance for respiratory virus infections in adults who have undergone bone marrow and peripheral blood stem cell transplantation. Bone Marrow Transplant 2003; 32(11):1085-8.

8. Freymuth F, Vabret A, Cuvillon-Nimal D, Simon S, Dina J, Legrand L, Gouarin S, Petitjean J, Eckart P, Brouard J. Comparison of multiplex PCR assays and conventional techniques for the diagnostic of respiratory virus infections in children admitted to hospital with an acute respiratoryillness. J Med Virol 2006; 78: 1498-1504.

9. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM Jr, Musher DM, Niederman MS, Torres A, Whitney CG. Infectious diseases society of america/american thoracic society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007;44 Suppl 2:S27-72.

10. Ewig S, Ruiz M, Mensa J, Marcos MA, Martinez JA, Arancibia F, Niederman MS, Torres A. Severe community-acquired pneumonia. Assessment of severity criteria. Am J Respir Crit Care Med 1998; 158(4):1102-8.

11. Danés C, González-Martínez, J, Pumarola T, Rañó A, Benito N, Torres A, Moreno A, Rovira M, Puig de la Bellacasa J. Pulmonary infiltrates in immunosupressed patients: analysis of a diagnostic protocol. J Clin Microbiol 2002; p.2134-2140.

12. Marcos MA, Camps M, Pumarola T, Martinez JA, Martinez E, Mensa J, Garcia E, Penarroja G, Dambrava P, Casas I, Jimenez de Anta MT, Torres A. The role of viruses in the aetiology of community-acquired pneumonia in adults. Antivir Ther 2006; 11:351-359.

13. Casas I, Powell L, Klapper PE, Cleator GM. New method for the extraction of viral RNA and DNA from cerebrospinal fluid for use in the polymerase chain reaction assay. J Virol Methods 1995; 53(1):25-36.

14. Coiras MT, Perez-Brena P, Garcia ML, Casas I. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. J Med Virol 2003; 69(1):132-44.

15. Coiras MT, Aguilar JC, Garcia ML, Casas I, Perez-Brena P. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. J Med Virol 2004; 72(3):484-95.

16. Murray PR, Washington JA. Microscopic and bacteriologic analysis of expectorated sputum. Mayo Clin Procced 1975; 50:339-344.

17. de Roux A, Ewig S, Garcia E, Marcos MA, Mensa J, Lode H, Torres A. Mixed community-acquired pneumonia in hospitalised patients. Eur Respir J 2006; 27(4):795-800.

18. Malcolm E, Arruda E, Hayden FG, Kaiser L. Clinical features of patients with acute respiratory illness and rhinovirus in their bronchoalveolar lavages. J Clin Virol 2001; 21(1):9-16.

19. Ison MG, Hayden FG, Kaiser L, Corey L, Boeckh M. Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia. Clin Infect Dis 2003; 36:1139-43.

20. Imakita M, Shiraki K, Yutani C, Ishibashi-Ueda H. Pneumonia caused by rhinovirus. Clin Infect Dis 2000; 30:611–2.

21. Mosser AG, Brockman-Schneider R, Amineva S, Burchell L, Sedgwick JB, Busse WW, Gern JE. Similar frequency of rhinovirus-infectible cells in upper and lower airway epithelium. J Infect Dis 2002; 185:734–43.

22. Ishizuka S, Yamaya M, Suzuki T, Takahashi H, Ida S, Sasaki T, Inoue D, Sekizawa K, Nishimura H, Sasaki H. Effects of rhinovirus infection on the adherence of *Streptococcus pneumoniae* to cultured human airway epithelial cells. J Infect Dis 2003; 15;188(12):1928-39.

23. Ljungman P, Andersson J, Aschan J, Barkholt L, Ehrnst A, Johansson M, Weiland O. Influenza A in immunocompromised patients. Clin Infect Dis 1993; 17:244-7.

24. CNE. Centro Nacional de Epidemiología. Vigilancia Epidemiológica de la gripe en España. http://cne.isciii.es/htdocs/ve/ve.htm.

25. Ballout A, Goffin E, Yombi JC, Vandercam B. Vaccinations for adult solid organ transplant recipient: current recommendations. Transplant Proc 2005; 37(6):2826-7.

26. Ferrari A, Luppi M, Potenza L, Riva G, Morselli M, Imovilli A, Volzone F, Rossi G, Codeluppi M, Guaraldi G, Torelli G. Herpes simplex virus pneumonia during standard induction chemotherapy for acute leukemia: case report and review of literature. Leukemia 2005; 19(11):2019-21.

27. Warren K, Brown SM, Wroblewska Z, Gilden D, Koprowski H, Subak-Sharpe J. Isolation of Herpes simplex virus from the superior cervical and vagus ganglions of human beings. N Engl J Med 1978; 298: 1068-1069.

28. Bruynseels P, Jorens PG, Demey HE, Goossens H, Pattyn SR, Elseviers MM, Weyler J, Bossaert LL, Mentens Y, Ieven M. Herpes simplex virus in the respiratory tract of critical care patients: a prospective study. Lancet 2003; 362:1536-1541.

29. Rimland D, Navin TR, Lennox JL, Jernigan JA, Kaplan J, Erdman D, Morrison CJ, Wahlquist SP; Pulmonary Opportunistic Infections Study Group. Prospective study of etiologic agents of community-acquired pneumonia in patients with HIV infection. AIDS 2002; 16(1):85-95.

30. Garbino J, Gerbase MW, Wunderli W, Deggernez C, Thomas Y, Rochat T, Ninet B, Schrenzel J, Yerly S, Perrin L, Soccal PM, Nicod L, Kaiser L. Lower

respiratory viral illnesses: improved diagnosis by molecular methods and clinical impact. Am J Respir Crit Care Med 2004; 1;170(11):1197-203.

31. Martino R, Porras RP, Rabella N, Williams JV, Ramila E, Margall N, Labeaga R, Crowe JE Jr, Coll P, Sierra J. Prospective Study of the Incidence, Clinical Features, and Outcome of Symptomatic Upper and Lower Respiratory Tract Infections by Respiratory Viruses in Adult Recipients of Hematopoietic Stem Cell Transplants for Hematologic Malignancies. Biol Blood Marrow Transplant 2005; 11;10:781-796.

32. van Elden, L. J., M. G. van Kraaij, M. Nijhuis, Hendriksen KA, Dekker AW, Rozenberg-Arska M, van Loon AM. Polymerase chain reaction is more sensitive than viral culture and antigen testing for the detection of respiratory viruses in adults with hematological cancer and pneumonia. Clin Infect Dis 2002; 34:177–183.

33. Pumarola T, Dominguez A, Marcos MA, Martínez A, Muñoz C, Cañas A, Ruiz M, Jiménez MT, Salleras L. Vigilancia virólogica de la gripe (2002-2003). Vacunas 2003; 4:119-126

34. Loens K, Goossens H, de Laat C, Foolen H, Oudshoorn P, Pattyn S, Sillekens P, Ieven M. Detection of rhinoviruses by tissue culture and two independent amplification techniques, nucleic acid sequence-based amplification and reverse transcription-PCR, in children with acute respiratory infections during a winter season. J Clin Microbiol 2006; 44(1):166-71.

Table 1. Aetiology of community-acquired pneumonia in the immunocompromised adults

	Pathogen	Patients (n=61)
GROUP 1	*S. pneumoniae*	29
	S.pneumoniae + *H. influenzae*	1
	S. pneumoniae + *Legionella pneumophila*	1
	P. jirovecii	3
	S. aureus	2
	S. viridans	1
	Legionella 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

Table 2. Baseline characteristics of the patients according to the aetiology of CAP.

	Non viral CAP n: 38 (%)	Mixed CAP n: 11 (%)	Viral CAP n: 12 (%)	unknown aetiology n: 31 (%)	Total n:92 (%)	P-value
Median age,years (IQR)	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)	
Neoplasic 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 >80g/d	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)	

Note. Data are number (percentage) unless otherwise specified. *P-value* has been obtained comparing non viral, mixed and viral community-acquired pneumonia (CAP). ICU, intensive care unit; CNS, central nervous system; PSI, pneumonia severity index score; IQR, interquartile range.

Table 3. Clinical presentation and evolution of the patients according to the aetiology of CAP

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	Non viral CAP n: 38(%)	Mixed CAP n: 11 (%)	Viral CAP n: 12 (%)	unknown aetiology n: 31 (%)	Total n:92 (%)	P-value
Clinical presentation						
Days with symptoms *	5 (3-7)	5 (3-13)	4 (3-5.5)	5 (3-7)	5 (3-7)	0.630
Leukocytes x 1000/µl *	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/dl	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
PO_2/FiO_2, mm Hg	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 (ºC)	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	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

Note. * Quantitative characteristics are described with median and interquartile range (IQR), and the rest as number (percentage). *P-value* has been obtained comparing non viral, mixed and viral community-acquired pneumonia (CAP).Comparisons among the three groups (1,2,3) were made using the Kruskal-Wallis test and the Mann-Whitney test was used in each of the three pairwise comparisons.

Figure 1. Monthly distribution of CAP cases according to the etiology