Herpes simplex virus (HSV) pneumonia in the non-ventilated immunocompromised host: Burden and predictors

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SUMMARY

Objectives: To evaluate burden and predictors of HSV pneumonia among immunocompromised patients not undergoing invasive mechanical ventilation according to a tailored diagnostic algorithm.
Methods: This prospective, observational study included immunocompromised adults with pneumonia non-responding to empirical antibiotic therapy. Bronchoalveolar lavage (BAL) specimens were cultured for bacteria, mycobacteria and fungi. Real-time PCR for Herpesviruses and other microorganisms were performed on BAL and other specimens. Cytological examination of BAL samples was carried out for identification of intranuclear inclusion bodies and immunohistochemical staining for HSV.
Results: We enrolled 45 patients (mean age 64.6 years) from January 2015 to June 2016. Nineteen (42.2%) cases tested positive for HSV-1 PCR on BAL. According to our definitions, 11 (24.4%) patients had HSV-1 pneumonia with viral loads ranging between 10^3 copies/mL and 10^7 copies/mL. HSV-1 positive throat swab (OR 85.2, 95% CI 5.83–1245.1, $P < 0.001$) and solid organ transplant (SOT) (OR 53.3, 95% CI 1.37–2072.8, $P < 0.03$) as underlying condition were found to be independently associated with HSV pneumonia by multivariable analysis.
Conclusions: HSV pneumonia turned out to be relatively common and should be investigated especially in individuals with HSV positive throat swab and SOT. Interventional studies are needed to assess the real clinical impact of HSV pneumonia in immunocompromised patients.

Introduction

Herpes simplex virus (HSV) is known to be an infrequent cause of pneumonia affecting mostly severely immunocompromised hosts.[1] However, HSV pneumonia has rarely been described in such patient population probably considered not clinically relevant or unrecognized.[2–5] On the other hand, HSV ventilator-associated pneumonia (VAP) results to be not uncommon among immunocompetent patients and has been frequently reported in literature.[6–10] Currently, the diagnosis of HSV lower respiratory tract (LRT) infections including pneumonia and tracheobronchitis remains very demanding. In fact, since shedding of HSV in the oropharyngeal secretions occurs in 2-3% of individuals in the general population,[6,11] the significance of HSV presence in the LRT secretions is challenging to interpret. Similarly, the use of acyclovir in pneumonia due to HSV remains controversial. HSV pneumonia should be diagnosed by histological evidence of pneumonia combined with viral isolation from the lung tissue.[1] Alternatively, the diagnosis may be achieved demonstrating the cytopathic effects of HSV with evidence of intranuclear inclusion bodies, positive HSV immunohistochemical staining (INH) and high viral load in bronchoalveolar lavage (BAL) specimens obtained by fiberoptic broncoscopy (FOB).[9,12,13] We therefore performed this prospective study to evaluate the burden and predictors of HSV pneumonia in immunocompromised patients not undergoing invasive mechanical ventilation according to a tailored diagnostic algorithm.

Patients and methods

This is a prospective, observational study enrolling consecutive patients with pneumonia admitted to the University Hospital of

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Trieste, Italy. This institution is a 780-bed tertiary care hospital located in Northeastern Italy.

Characteristics of patients

During the 24-month study period (January 2015–June 2016) we enrolled immunocompromised adults (aged ≥ 18 years) affected by community-acquired pneumonia (CAP) or hospital-acquired pneumonia (HAP) non-responding to 3–5 days of empirical antibiotic therapy by current standards of clinical practice. They had immunocompromising conditions including ongoing solid tumors or haematologic disorders or receiving chemotherapy ≤ 6 months, connective tissue disorders, HIV infection, chronic obstructive pulmonary disease (COPD, Gold stage 3-4), pulmonary fibrosis, bronchiectasis, chronic dialysis, bone marrow and solid organ transplants. In addition, other inclusion criteria were immunosuppressive regimens such as steroids (prednisone ≥ 0.5 mg/kg daily for at least 4 weeks), anti-TNF drugs, chemotherapy or radiotherapy (≤ 1 month before enrollment), and other immunosuppressive regimens (cyclophosphamide, tacrolimus, everolimus, azathioprine, cyclosporine, mycophenolate mofetil, methotrexate, sirolimus). The study excluded patients with ventilator-acquired pneumonia (VAP), those who had received agents active against HSV in the previous month, pregnant women and those with contraindications for FOB.

Diagnostic algorithm

Following clinical failure of empirical antibiotic therapy, chest CT scan and FOB with BAL were proposed to all patients. The FOB macroscopic aspect was considered abnormal in case of erythematous, edematous, and friable mucosa. The sampling area was selected on the basis of pulmonary infiltrate location. An oropharyngeal swab was collected for real-time PCR testing for HSV. BAL specimens were cultured for bacteria and fungi. Cultures for mycobacteria were done when requested by clinicians. BAL fluid and serum galactomannan concentrations were determined by the Platelia *Aspergillus* enzyme immunoassay (Bio-Rad Laboratories, Marnes-la-Coquette, France) using optical density index cutoffs of 0.5 and 1.0 to evaluate the diagnostic performance of the test. Real-time PCR for Herpesviruses, namely HSV, Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) (Elitech Molecular Diagnostic, Torino, Italy), Parainfluenza, Metapneumovirus, Respiratory syncytial virus(RSV) (Argene Biomerieux, Firenze, Italy), *Mycoplasma pneumoniae, Chlamydia pneumoniae, Aspergillus* spp (Elitech Molecular Diagnostic, Torino, Italy) were performed in BAL specimens. In the same specimens, real-time PCR for Influenza viruses, Adenovirus and *Pneumocystis jirovecii* were obtained by in-house methods following previously described methods.[14–16] HSV and CMV were tested also in blood specimens. HSV serology (IgG and IgM) was tested by chemiluminescence immunoassay (DiaSorin, Vercelli, Italy) in all cases. Cytological examination of BAL specimens was carried out splitting the sample from each patient into two parts. The first one was prepared for identification of intranuclear inclusion bodies indicative of intracellular viral infection. The second part of the sample was fixed in formalin and included in paraffin for setting up a cell-block for ICH and testing with a rabbit polyclonal antibody against HSV (DAKO Autostainer, CA, US).[12,13] Characteristics of patients including demographics, underlying diseases and Charlson comorbidity index, immunosuppressive regimens, type of pneumonia (CAP and HAP), severity of pneumonia (Pa O_2/Fi O_2 ratio, CURB index for CAP), chest CT scan patterns of pulmonary abnormalities, routine laboratory data including peripheral leucocyte count and serum C-reactive protein (CR-P, normal value < 5 mg/dL), microbiological findings, BAL cytological and INH data, and duration of hospitalization were stored in a database.

Diagnostic criteria of HSV pneumonia

Patients were classified as having proven HSV pneumonia if HSV was the only pathogen identified by PCR in the BAL specimen in concurrence of cytopathologic effect and/or HSV positive IHC in cells obtained from the same BAL specimen. Probable HSV pneumonia was defined by the presence of HSV and other pathogens in combination with cytopathologic effect and/or HSV positive IHC in cells obtained from the same BAL specimen. Finally, patients were classified as having possible HSV pneumonia if HSV was identified by PCR (with viral load ≥ 10^5 copies/mL) in the BAL specimen with or without other pathogens despite no cytological or IHC evidence of HSV infection.

Potential predictors of HSV pneumonia and risk factors for mortality

Potential predictors of HSV pneumonia including demographics, duration of hospitalization, underlying diseases and immunosuppressive regimens, type of pneumonia, patterns of chest CT scan, throat HSV testing and FOB macroscopic abnormalities were evaluated. We limited the outcome evaluation, namely crude mortality of pneumonia, to 15 days to increase the likelihood that mortality could be attributable to pneumonia. Analysis of risk factors for 15-day mortality included demographics, type of pneumonia, peripheral leucocyte count and serum C-reactive protein (CRP), CURB index, Pa O_2/Fi O_2 ratio, smoking, previous ICU stay, corticosteroids, chemotherapy and immunosuppressive regimens, duration of hospitalization, diagnosis of HSV pneumonia and other pneumonia.

Statistical methods

Continuous variables with normal distribution were expressed as mean ± standard deviation (SD) and those without normal distribution as median and the range between the first and the third quartile. Discrete variables were expressed as frequencies and percentages. Statistical comparison of patient characteristics was performed using the Chi-square or the Fisher's exact test for categorical variables, and the Student's *t*-test or the Mann-Whitney *U*-test for continuous variables. Independent predictors of HSV pneumonia and 15-day mortality were investigated by a backward stepwise logistic regression model of multivariable analysis where every variable with a *P*-value < 0.1 at univariable analysis had been included. The area under the receiver-operating characteristic (ROC) curve, with 95% confidence interval (CI), and the Hosmer-Lemeshow test were calculated to assess the discriminatory power and the calibration of the models, respectively. The accuracy of prediction was defined as low (AUC = 0.5-0.7), moderate (AUC = 0.7-0.9), and high (AUC = 0.9–1). All tests were two-sided with the alpha level set at 0.05 for statistical significance. Data analyses were performed using the SPSS software package for Windows, version 13.0 (SPSS, Inc., Chicago, Illinois, US).

Results

Forty-five patients (mean age 64.6 ± 14 years, male 65.4%) fulfilled the enrollment criteria. The most common underlying conditions were solid tumors, haematologic disorders, COPD, connective tissue disorders and SOT. More than half of patients were receiving steroids and around a quarter of them no immunosuppressive regimen. Twenty-seven (60%) cases had CAP and the remaining cases HAP. Chest CT scan findings included areas of ground-glass attenuation (42.2%), airspace consolidation (62.2%) and excavation (15.6%) according to prevalent bilateral and multifocal distributions. Baseline characteristics of patients including CURB index for CAP and Pa O_2/Fi O_2 ratio are shown in Table 1. All patients tested positive

Table 1
Baseline characteristics of study population.

Variable	mean or *n*	range or % or SD
Age	64.6 years	29-83 years
Sex (male)	29	65.4%
CAP	27	60%
HAP	18	40
Underlying diseases		
Cancer/haematologic disorders	17	37.8%
Connective tissue disorders	6	13.3
Solid organ transplants	5	11.1
Others§	10	22.2
Immunosuppressive regimens		
Steroids	26	57.8%
Chemotherapy	8	17.8
Others	12	26.7
No immunosoppressive regimen	12	26.7
Charlson comorbidity score	4.8	± 2.38
CURB 65 (CAP)	312	1.71
PaO2/FiO2 ratio	207.5	63
Chest CT scan findings		
ground-glass attenuation	19	42.2%
consolidation	28	62.2
excavation	7	15.6
unilateral distribution	17	37.8
bilateral distribution	28	62.2
unifocal distribution	15	33.3
multifocal distribution	30	66.6

Note: n: number of cases; SD: standard deviation; CAP: community-acquired pneumonia; HAP: hospital-acquired pneumonia; COPD: chronic obstructive pulmonary disease; § pulmonary fibrosis ($n=2$), bronchiectasis ($n=3$), chronic dialysis ($n=4$), HIV ($n=1$).

for HSV serology (IgG) with the exception of one patient positive for both HSV IgG and IgM. The patients underwent chest CT scan and FOB which visualized macroscopic abnormalities in 25 (55.6%) cases. HSV-1 was detected in BAL specimens of 19 (42.2%) cases with viral loads ranging between 10^3 copies/mL and 10^7 copies/mL. In addition, HSV-1 was found in the throat swabs of 11 (24.4%) cases and blood specimens of 4 (8.8%) cases. CMV was detected in BAL specimens of 12 (26.6%) cases and blood specimens in 5 (11.1%) cases. EBV, RSV, and Influenza viruses were found in BAL specimens of 13 (28.9%), 4 (8.8%) and 2 (4.4%) cases, respectively. Lastly, *P. jirovecii, Aspergillus* spp, bacteria, and *M. pneumoniae* were detected in BAL specimens of 4 (8.8%), 6 (13.3%), 20 (44.4%), and 1 cases, respectively. Galactomannan testing resulted to be positive in 9 (20%) and 2 (4.4%) cases, respectively in BAL and blood specimens. Isolated bacteria from BAL specimens included *Pseudomonas aeruginosa* (7 isolates), *Staphylococcus aureus* (6), *Klebsiella pneumonia* (3), *Acinetobacter baumannii* (2), *Enterobacter cloacae* (1), *Stenotrophomonas maltophilia* (1), and *Proteus mirabilis* (1). As regards BAL specimens collected for cytological examination and IHC for HSV, 5 specimens were insufficient and 8 were inadequate for the presence of purulent respiratory secretions. Therefore, only 32 (71%) BAL specimens were found to be adequate and showed intranuclear viral inclusion bodies and HSV-positive cells by IHC in 6 cases, respectively. Table 2 summarizes laboratory data, hospitalization duration and previous antimicrobial regimens at time of FOB. According to our diagnostic algorithm, 11 (24.4%) patients had HSV pneumonia. Of them, 4 patients had proven, 3 probable and 4 possible HSV pneumonia. Among the remaining cases, 12 (26.7%) patients had bacterial pneumonia, 4 CMV, 4 RSV, 3 *P. jirovecii*, 1 EBV, 1 influenza pneumonia, and 1 pulmonary aspergillosis. No causative agent could be found in 8 (17.8%) cases. Fig. 1 shows the diagnostic flow-chart of pneumonia.

Univariable analysis showed that potential predictors of HSV pneumonia turned out to be CAP ($P=0.086$), SOT ($P=0.085$), and HSV positive throat swab ($P<0.0001$) (Table 3). By multivariable analysis, HSV positive throat swab and SOT as underlying condition were found to be independently associated with HSV pneumonia (Table 4). ROC curve analysis showed that the value of AUC was 0.945 (95% CI 0.834, 0.991).

The overall 15-day mortality was 22.2% and higher (27.3%) in patients with HSV pneumonia than those with other pneumonia (20.6%) ($P=0.69$). The univariable analysis of risk factors for 15-day mortality showed that Charlson comorbidity index and duration of hospitalization were associated to poor outcome (Table 5). No risk factors turned out to be independently associated with 15-day mortality by multivariable analysis. Seven (63.6%) of 11 patients with HSV were treated with acyclovir. The 15-day mortality rates were 28.6% (2 out of 7 patients) among acyclovir treated patients and 25% (1 out of 4 patients) among those untreated.

Discussion

HSV pneumonia is a not well defined clinical entity occurring in both immunocompromised patients and immunocompetent hosts, the latter generally during mechanical ventilation.

Diagnostic criteria of HSV pneumonia: review of literature

At present, there are no defined criteria for the diagnosis of HSV pneumonia in the absence of histopathological findings consistent with HSV infection. For instance, according to a study on patients with solid tumors proven HSV pneumonia was defined by the presence of HSV in the LRT secretions in combination with viral cytopathic effect from the same specimens, probable HSV pneumonia if HSV as the sole pathogen identified in the latter specimens, possible HSV pneumonia if HSV in combination with copathogens in the same respiratory specimens.[4] In another study on patients undergoing mechanical ventilation, the definition of HSV pneumonia strictly required HSV detection by PCR or culture and cytological or histopathological evidence of intranuclear inclusion bodies from same BAL specimens or biopsy.[8] In such series, patients with HSV pneumonia had higher BAL viral loads than those without suggesting that HSV viral load could be a strong predictor of HSV pneumonia. However, although cytological or histopathological criteria show high specificity they lack

Table 2
Laboratory findings, hospitalization durations and previous antimicrobial regimens at time of FOB.

Variables	HSV pneumonia $N=11$ n or mean ± SD	bacterial pneumonia $N=12$ n or mean ± SD	other defined pneumonia $N=17$ n or mean ± SD	undefined pneumonia $N=5$ n or mean ± SD
HSV-PCR				
HSV-1 (BAL)	11	4	2	2
HSV viral load $\geq 10^5$cp/mL	8	0	0	0
HSV viral load $< 10^5$cp/mL	3	4	2	2
HSV-1 (blood)	4	0	0	0
HSV-1 (throat swab)	8	3	1	0
HSV serology				
IgM positive	1	0	0	0
IgG positive	11	9	11	2
Cytology (BAL)				
nuclear inclusions	6	0	4	0
IHC	6	0	3	0
nuclear inclusions+IHC	5	0	1	0
Culture (BAL)				
Bacteria	5	12	0	0
Aspergillus spp	2	1	0	0
PCR (BAL)				
M. pneumoniae	0	0	1	0
P. jirovecii	1	0	3	0
Aspergillus spp	2	0	4	0
CMV	3	4	5	0
Influenza	1	0	1	0
RSV	0	0	4	0
EBV	5	4	4	0
other investigations				
CMV-PCR (blood)	3	1	1	0
Galactomannan (BAL)	5	2	2	0
Galactomannan (blood)	1	0	1	0
hospitalization days	20.3±15.8	26.6±15.5	14.5±15.3	17±16.8
previous antimicrobial regimen				
Amoxicillin Clavulanate	0	0	1	2
Piperacillin Tazobactam	4	5	9	1
Ceftriaxone	0	1	1	1
Ceftazidime	0	1	0	0
Meropenem or Imipenem	5	2	5	0
Clarithromycin	0	1	3	1
Levofloxacin	0	2	3	3
Vancomycin	8	8	5	1
Gentamicin or Amikacin	1	1	5	0
Others*	3	4	2	0

Note: FOB: fiberoptic bronchoscopy; *N*: total available sample size; n: number of cases; SD: standard deviation; BAL: bronchoalveolar lavage; cp:copies; IHC: immunoistochemical staining; Others*: linezolid ($n=4$), metronidazole ($n=3$),oseltamivir ($n=2$).

sufficient sensitivity as demonstrated in a study showing negative open lung biopsy findings compared to autopsy-proven HSV pneumonia.[1] For the purpose of defining the clinical role of HSV DNA viral load in respiratory specimens, a retrospective study on critically ill patients with suspected VAP demonstrated that PCR HSV-1 load $> 10^5$ genome equivalents/mL in BAL specimens was associated with an increased mortality and even sometimes with histologically proven HSV pneumonia.[9] Similarly, quantitative detection of HSV DNA in BAL fluid was found to be a potential diagnostic tool for the detection of viral infection of the LRT in a largely immunocompromised patient population. In fact, in this series a threshold of HSV DNA levels ≥ 5.0 log in BAL specimens was associated with mortality suggesting that such quantitative threshold would increase the specificity of HSV presence in the LRT fluid.[13]

Burden of HSV pneumonia

Over 42% of our patients tested positive for HSV-1 on BAL specimens with viral loads ranging between between 10^3 copies/mL and 10^7 copies/mL, similarly to what reported in previous studies including immunocompromised patients.[13,17] Based on our diagnostic definitions, 24.4% of immunocompromised patients with pneumonia not responding to empirical antibiotic therapy had HSV-1 pneumonia. Since all patients except one of them had IgG positive HSV serology, the vast majority of our patients with HSV pneumonia showed reactivation of HSV infection. According to previous studies with different or unspecified enrollment criteria, 5 (9.8%) of 51 recipients of heart-lung transplantation had HSV pneumonia confirmed by histopathological findings.[18] In another series, 6 (2.45%) episodes of HSV-1 pneumonia have been reported among 245 patients following orthotopic liver transplantation.[19] In a more recent study evaluating diagnosis of pneumonia in 63 patients with autoimmune diseases under maintenance regimen of immunosuppression, 6 (10%) cases had positive HSV-1 BAL-PCR but only 2 (3.2%) cases of HSV-1 pneumonia were confirmed by cytological abnormalities from BAL specimens.[5] Similarly, in our series, detection of HSV DNA in BAL specimens was relatively common but 8 of 19 cases had HSV shedding with low viral load $< 10^5$/mL in BAL specimens and no cytological or IHC findings being therefore diagnosed as bacterial or undefined pneumonia. Unfortunately, only almost three-quarters of BAL specimens were adequate for cytological and IHC investigations. However, we believe that the latter tests should be pursued for confirming the diagnosis of HSV pneumonia, especially in the case of low HSV viral load in LTR specimens.[12,20]

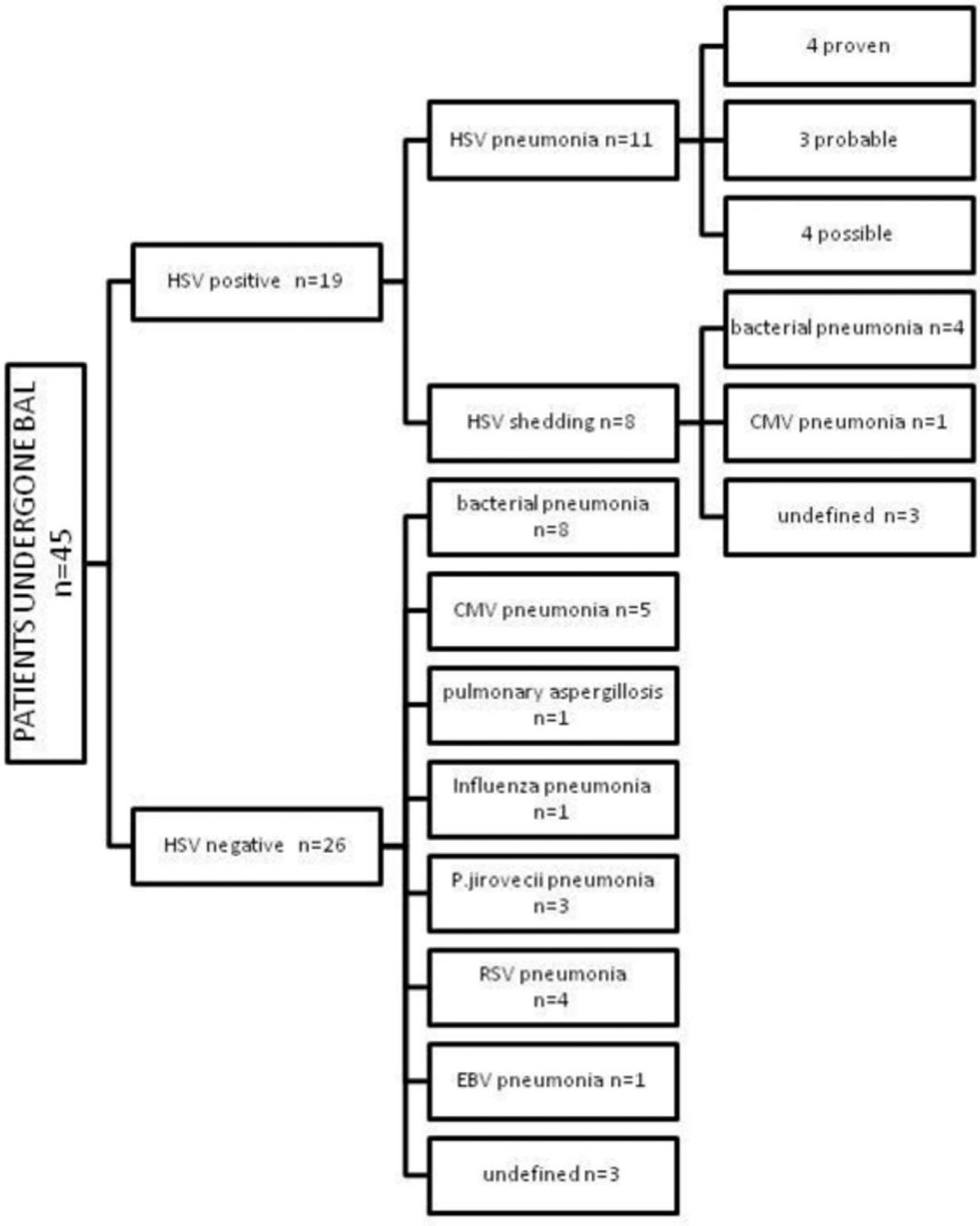

Fig. 1. Diagnostic flow-chart of pneumonia. Note: BAL: bronchoalveolar lavage; *n*: number of cases.

Predictors of HSV pneumonia

To-date, predictors of HSV pneumonia among immunocompromised patients remain poorly investigated. In a retrospective study on pneumonia in patients affected by autoimmune diseases under immunosuppressive therapy, detection of HSV in respiratory secretions was associated with stronger immunosuppressive regimens and, especially, vasculitic diseases.[5] We have demonstrated that positive HSV throat swab testing and SOT as underlying disease were found to be highly significant and independent predictor factors for the development of HSV pneumonia. Similarly, positivity of HSV in throat swab turned out to be predictor of HSV pneumonia in two previous studies which evaluated critically ill and non-immunocompromised patients undergoing prolonged mechanical ventilation, respectively.[6,8] In the latter study, other risk factors associated with HSV bronchopneumonitis were HSV oral-labial lesions and macroscopic bronchial lesions visualized during bronchoscopy.[8] A subsequent study on these series demonstrated that HSV pulmonary infection resulted from the reactivation of genetically related HSV isolates in the oropharynx progressively infecting the LTR.[21] Alternatively, HSV might reach the LRT by haematogenous seeding and this is thought to lead to diffuse bilateral interstitial pneumonia, as supported by a previous study on immunocompromised patients.[1] In our series, data support mainly the former pathogenetic hypothesis since only 4 of 11 patients with HSV pneumonia had HSV positivity in both BAL and blood specimens. Most of our cases with HSV pneumonia showed radiographic areas of consolidation with bilateral and multifocal

Table 3
Univariable analysis of potential predictors of HSV pneumonia.

Variables	Total pneumonia $N=45$ n(%) or mean ± SD	HSV pneumonia $N=11$ n(%) or mean ± SD	Other pneumonia $N=34$ n(%) or mean ± SD	OR	95% CI
Male gender	29 (64.4)	8 (72.7)	21 (61.7)		
Age (years)	64.6 ± 14	62 ± 16.3	65.4 ± 13.4		
Hospitalization duration (days)	19.04 ± 15.8	20.3 ± 15.2	18.6 ± 16.2		
Previous ICU stay duration (days)	10 (22.2)	3 (27.3)	7 (20.6)		
CAP	27 (60)	7 (63.6)	11 (32.4)	3.66	0.88, 15.2
HAP	18 (40)	4 (36.4)	23 (67.6)		
Chest CT scan					
ground-glass attenuation	19 (42.2)	4 (36.4)	15 (44.1)		
consolidation	28 (62.2)	7 (63.6)	21 (61.7)		
Excavation	7 (15.6)	1 (9.1)	6 (17.6)		
unilateral distribution	17 (37.8)	4 (36.4)	13 (38.2)		
bilateral distribution	28 (62.2)	7 (63.6)	21 (61.7)		
unifocal distribution	15 (33.3)	3 (27.3)	12 (35.3)		
multifocal distribution	30 (66.6)	8 (72.7)	22 (64.7)		
Comorbidities					
haematological and solid neoplasia	17 (37.8)	3 (27.3)	14 (41.2)		
COPD	7 (15.6)	1 (9.1)	6 (17.6)		
connective tissue disorders	6 (13.3)	2 (18.2)	4 (11.8)		
SOT	5 (11.1)	3 (27.3)	2 (5.9)	6	0.85, 42.2
Others	10 (22.2)	2 (18.2)	8 (23.5)		
Immunosuppressive therapy					
Corticosteroids	26 (57.8)	7 (63.6)	19 (55.9)		
chemotherapy and/or others	20 (44.4)	6 (54.5)	14 (41.2)		
None	12 (26.7)	3 (27.3)	9 (26.4)		
HSV positive throat swab	12 (26.7)	9 (81.8)	3 (8.8)	46.5	6.7,322.6
Abnormal aspect (FOB)	25 (55.6)	7 (63.6)	18 (52.9)		

Note: N: total available sample size; *n*: numer of cases; OR: odds ratio; CI: confidence interval; ICU: intensive care unit; CAP: community-acquired pneumonia; HAP: hospital-acquired pneumonia; COPD: chronic obstructive pulmonary disease; SOT: solid organ transplant; FOB: fiberoptic bronscoscopy

Table 4
Multivariable analysis of potential predictors of HSV pneumonia.

Variable	Standard error	OR	95% CI	*P*-value
CAP	1.33	7.96	0.58, 109.5	0.12
SOT	1.86	53.3	1.37, 2072.8	0.0012
HSV positive throat swab	1.36	85.2	5.83, 1245.1	0.033

Note: CAP: community-acquired pneumonia; SOT: solid organ transplant; OR: odds ratio; CI: confidence interval.

distribution on chest CT scan. However, no specific differences in CT patterns were found between HSV pneumonia and other pneumonias. This is in accordance with what has been previoulsly observed in both immunocompromised and immunocompetent patients with HSV pneumonia.[22–24]

Outcome of HSV pneumonia

Regarding the outcome of our patients, the overall 15-day mortality was 22.2% and resulted to be mildly higher in patients with HSV pneumonia than in those with other pneumonia, as previously reported by others.[6,8,9] No predictor was found to be independently associated to mortality. However, our study protocol did not provide pre-established management and therapeutic regimens, and the choice of antimicrobial therapy was at clinical discretion of the attending physician. Indeed, only 7 of 11 patients

Table 5
Univariable analyses of risk factors for 15-day mortality.

Variables	Total cases $N=45$ mean ± SD or n (%)	Alive $N=35$ mean ± SD or *n* (%)	Dead $N=10$ mean ± SD or *n* (%)	*P*-value
Age (years)	64.6 ± 14	65.6 ± 13.3	69.2 ± 15.1	0.24
Age ≥ 50 years	38 (84.4)	29 (82.9)	9 (90)	1
CAP	27 (60)	22 (62.8)	5 (50)	0.72
HAP	18 (40)	13 (37.1)	5 (50)	0.72
leucocyte count (/mm^3)	10077 ± 7667	9079 ± 4494	13568 ± 13916	0.052
CR-P (mg/dL)	123.3 ± 115,9	112.8 ± 115	160.1 ± 117.6	0.13
Charlson Comorbidity Index	4.8 ± 2.38	4 ± 2.2	6 ± 2.4	0.014
CURB (CAP)	3.12 ± 1.71	3 ± 1.7	3 ± 1.7	0.8
PaO2/FiO2	207.5 ± 63	212 ± 61.6	191 ± 68.6	0.36
Smoking	14 (31.1)	9 (25.7)	5 (50)	0.24
Previous ICU stay	10 (22.2)	8 (22.8)	2 (20)	1
Corticosteroids	26 (57.8)	22 (62.8)	4 (40)	0.28
Chemotherapy and/or others	20 (44.4)	16 (45.7)	4 (40)	1
No immunosoppressive regimen	12 (26.7)	9 (25.7)	3 (30)	1
Hospitalization duration (days)	19.04 ± 15.8	15.9 ± 13.7	30 ± 18.4	0.0056
HSV pneumonia	11 (24.4)	8 (22.8)	3 (30)	0.69
Other pneumonia	27 (77.1)	27 (77.1)	7 (70)	0.69

Note: N: total available sample size; *n*: number of cases; SD: standard deviation; CAP: community-acquired pneumonia; HAP: hospital-acquired pneumonia; CR-P: serum C-reactive protein; ICU: intensive care unit.

with HSV pneumonia were treated with acyclovir in our series. According to a study on patients with solid cancer, mortality was not significantly lower in patients treated with acyclovir or other antivirals active against HSV compared with that of untreated.[4] On the contrary, in another retrospective study on critically ill patients with positive HSV culture in LRT secretions, acyclovir treatment was positively linked to ICU-mortality reduction but only in the subgroup with HSV detected in the BAL specimens.[25] However, this study included a patient population with unspecified HSV detection in the respiratory tract secretions. In summary, further data are needed to define the effect of acyclovir or other antivirals on the outcome of HSV pneumonia.

Study limitations and strenghts

There are several limitations of our study that should be acknowledged. Firstly, this was a single centre study and the sample size was relatively small which limited its generalizable conclusions. However, the study design was sound and the enrollment criteria were strict. Secondly, the range of immunocompromising conditions was relatively large. Thirdly, over a quarter of BAL specimens were inadequate for cytological and IHC examinations, limiting partially our diagnostic algorithm. Finally, the evaluation of the clinical impact of different etiologies was questionable due to the fact that the therapeutic decisions on management of pneumonia were left to the discretion of the attending physician. Notwithstanding these limitations, to our knowledge the present is the first prospective study evaluating HSV pneumonia in immunocompromised patients through a diagnostic algorithm including HSV PCR with threshold of viral load testing, cytological and IHC examinations in BAL specimens. In our series, we achieved to determine that HSV pneumonia affects almost a quarter of immunocompromised patients with pneumonia non-responding to empirical antibiotic therapy. However, our data should be confirmed in further studies. We believe that the present diagnostic algorithm can support the diagnosis of HSV pneumonia and represent the premise for a therapeutic trial in immunocompromised patients.

Conclusions

Our data suggest that HSV-1 pneumonia is relatively common among immunocompromised patients not undergoing mechanical ventilation and should be investigated especially in individuals with HSV positive throat swab and SOT. The role of acyclovir and other antiviral agents active against HSV remains undefined and only interventional studies will be able to assess the real clinical impact of HSV pneumonia in this patient population.

Author contributions

Conception and design of the study: LR, CM, DP, MM; subject recruitment, sample processing and acquisition of data: DP, BA, MC, MF, RC, SL; data interpretation, statistical analyses and writing/revising the manuscipt: LR, SL, GG, MM, CM. Authors had access to all data in the study and had final responsibility for the decision of submit for publication.

Declarations of interests and funding

All the authors declare no conflict of interest related to this work.

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Ethical approval and patient consent

This study was approved by the local Ethics Committee (No. 50/2013) and informed consent was obtained from all patients.

References

1. Ramsey PG, Fife KH, Hackman RC, Meyers JD, Corey L. Herpes simplex virus pneumonia. *Ann Intern Med* 1982;**97**:813–20.
2. Cunha BA, Eisenstein LE, Dillard T, Krol V. Herpes simplex virus (HSV) pneumonia in a heart transplant : diagnosis and therapy. *Heart Lung* 2007;**36**:72–8.
3. Ferrari A, Luppi M, Potenza L, Riva G, Morselli M, Imovilli A, et al. Herpes simplex virus pneumonia during standard induction chemotherapy for acute leukemia: case report and review of literature. *Leukemia* 2005;**19**:2019–21.
4. Aisenberg G, Torres H, Tarrand J, Safdar A, Bodey G, Chemaly RF. Herpes simplex virus lower respiratory tract infection in patients with solid tumors. *Cancer* 2009;**115**:199–206.
5. Witt MN, Braun GS, Ihrler S, Schmid H. Occurence of HSV-I-induced pneumonitis in patients under standard immunosoppressive therapy for rheumatic, vasculitic, and connective tissue disease. *BMC Pulmon Med* 2009;**9**:22.
6. Bruynseels P, Jorens PG, Demey HE, Goossens H, Pattyn SR, Elseviers MM, et al. Herpes simplex virus in the respiratory tract of critical care patients: a prospective study. *Lancet* 2003;**362**:1536–41.
7. Simoons-Smit AM, Kraan EM, Beishuizen A, Strack van Schijndel RJ, Vandenbroucke-Grauls CM. Herpes simplex virus type 1 and respiratory disease in critically-ill patients: real pathogen or innocent bystander ? *Clin Microb Infect* 2006;**12**:1050–9.
8. Luyt CH, Combes A, Deback C, Aubriot-Lorton MH, Nieszkowska A, Trouillet JL, et al. Herpes simplex virus lung infection in patients undergoing prolonged mechanical ventilation. *Am J Respir Crit Care Med* 2007;**175**:935–42.
9. Linssen CFM, Jacobs JA, Stelma FF, van Mook WNKA, Terporten P, Vink C, et al. Herpes simplex virus load in bronchoalveolar lavage fluid is related to poor outcome in critically ill patients. *Intens Care Med* 2008;**34**:2202–9.
10. De Vos N, Van Hoovels L, Vankeerberghen A, Van Vaerenbergh K, Boel A, Demeyer I, et al. Monitoring of herpes simplex virus in the lower respiratory tract of critically ill patients using real-time PCR: a prospective study. *Clin Microb Infect* 2009;**15**:358–63.
11. McMillan JA, Weiner LB, Higgins AM, Lamparella VJ. Pharingitis associated with herpes simplex virus in college students. *Pediatr Infect Dis J* 1993;**12**:280–4.
12. Cunha BA *Pneumonia essentials*. 3rd ed. Sudbury ,MA, USA: Jones & Bartlett; 2010. p. 139–58.
13. Gooskens J, Templeton KE, Claas EC, van Bussel MJ, Smit VT, Kroes AC. Quantitative detection of Herpes simplex virus DNA in the lower respiratory tract. *J Med Virol* 2007;**79**:597–604.
14. Ritzwoller DP, Kleinman K, Palen T, Abrams A, Kaferly J, Yih W, et al. Comparison of syndromic surveillance and a sentinel provider system in detecting an influenza outbreak—Denver. *Colorado, 2003. MMWR* 2005;**54**:151–6.
15. Damen M, Minnaar R, Glasius P, van der Ham A, Koen G, Wertheim P, et al. Real-time PCR with an internal control for detection of all known human adenovirus serotypes. *J Clin Microbiol* 2008;**46**:3997–4003.
16. Fujisawa T, Suda T, Matsuda H, Inui N, Nakamura Y, Sato J, et al. Real-time PCR is more specific than conventional PCR for induced sputum diagnosis of Pneumocystis pneumonia in immunocompromised patients without HIV infection. *Respirology* 2009;**14**:203–9.
17. Tang JW, Lin M, Chiu L, Koay ESC. Viral loads of Herpes Simplex virus in clinical samples–A 5-year retrospective analysis. *J Med Virol* 2010;**82**:1911–16.
18. Smyth RL, Higenbottam TW, Scott JP, Wreghitt TG, Stewart S, Clelland CA, et al. Herpes simplex virus infection in heart-lung transplant recipients. *Transplantation* 1990;**49**:735–9.
19. Liebau P, Kuse E, Winkler M, Schlitt HJ, Oldhafer K, Verhagen W, et al. Management of Herpes simplex virus type 1 pneumonia following liver transplantation. *Infection* 1996;**24**:130–5.
20. Oda Y, Okada Y, Katsuda S, Nakanishi I. Immunohistochemical study on the infection of Herpes simplex virus, Human cytomegalovirus, and Epstein-Barr virus in secondary diffuse interstitial pneumonia. *Hum Pathol* 1994;**25**:1057–62.
21. Deback C, Luyt CE, Lespinats S, Depienne C, Boutolleau D, Chastre J, et al. Microsatellite analysis of HSV-1 isolates : from oropharynx reactivation toward lung infection in patients undergoing mechanical ventilation. *J Clin Virol* 2010;**47**:313–20.
22. Aquino SL, Dunagan DP, Chiles C, Haponik EF. Herpes simplex virus 1 pneumonia: patterns on CT scans and conventional chest radiographs. *J Comput Assist Tomogr* 1998;**22**:795–800.
23. Chong S, Kim TS, Cho EY. Herpes simplex virus pneumonia: high-resolution CT findings. *Brit J Radiol* 2010;**83**:585–9.
24. Brodoefel H, Vogel M, Spira D, Faul C, Beck R, Claussen CD, et al. Herpes-simplex virus 1 pneumonia in the immunocompromised host: high-resolution CT patterns in correlation to outcome and follow-up. *Europ J Radiol* 2012;**81**:e415–20.
25. Traen S, Bochanen N, Ieven M, Schepens T, Bruynseels P, Verbrugghe W, et al. Is acyclovir effective among critically ill patients with herpes simplex in the respiratory tract ? *J Clin Virol* 2014;**60**:215–21.