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BRIEF REPORT

Detection of human rhinoviruses in the lower respiratory tract of lung transplant recipients

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Abstract The occurrence of human rhinoviruses (HRV) and its relationship to clinical and histopathological findings were investigated in 127 bronchoalveolar lavage specimens from 36 lung transplant recipients by real-time RT-PCR. In addition, 286 samples from 235 other immunocompromised and immunocompetent patients were also studied. HRV was detected in 41.7% of lung transplant recipients vs 14.5% of other patients ($p < 0.0001$), and no differences in viral load were observed. Acute respiratory insufficiency was found in 15 cases, three of which were HRV positive (viral load, $6.3x10^6$ RNA copies/ml in one patient with chronic graft dysfunction). A diagnosis of pneumonia was made in 10 out of 127 cases, two of which were HRV positive (viral load, 10^3-10^4 in cases of co-infection). Acute rejection was diagnosed in 12 cases, three of which were HRV positive (viral load, 10^3 in two cases of co-infection and 10^5 in a single infection). HRV infection may involve the lower respiratory tract, particularly in the presence of an impaired pulmonary background, such as a transplanted lung. Clinical evaluation should take into account the viral load, with a load of $>10^5$ possibly being associated with clinical symptoms, although lower loads can be detected in both symptomatic and asymptomatic patients.

Human rhinoviruses (HRV) belong to the family *Picornaviridae* and account for 30-50% of cases of common cold and related upper respiratory tract complications [1, 2]. A pathogenic role for HRV in the lower respiratory tract has been reported, particularly in immunocompromised patients, although studies are controversial, and very few data are available concerning lung transplant (LT) recipients [3–8]. The success of LT may be limited by many factors, including organ infection/disease and acute rejection (AR), which appears to be a harbinger of chronic graft dysfunction [9]. In a recent study, an HRV load of $>1x10^5$ RNA copies/ml in bronchoalveolar lavage (BAL) fluid was associated with respiratory tract infection, while lower loads were detected in the absence of overt clinical symptoms [6]. An association between AR and persistent HRV infection has been described [10], whereas no association between HRV and new onset of chronic graft dysfunction was found in a recent study [11].

The aim of this study was to investigate the occurrence of HRV in BAL specimens from LT recipients and evaluate its association with acute respiratory illness, pneumonia, and AR.

Over a two-year period (March 2007-2009), all 127 consecutive BAL specimens from 36 patients having received a LT at the University Hospital San Giovanni Battista of Turin were tested. Clinical features are summarized in Table 1. Specimens were collected as routine follow-up at month 1 and subsequently at three-month intervals, and additional samples were collected for investigating clinical signs/symptoms and/or new infiltrates on chest X-ray. A standard therapeutic regimen was given to all

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Table 1 Clinical features of lung transplant recipients

Features	N = 36 (%)
Age (year)	
Mean ± SD	49.2 ± 16.6
Range	19–67
Sex (M/F)	23/13
Underlying disease	
Cystic fibrosis	12 (33.3%)
COPD/emphysema	11 (30.5%)
Pulmonary fibrosis	9 (25%)
Hyaline membrane disease	1 (2.8%)
Alpha-1-antitrypsin deficiency	1 (2.8%)
Sarcoidosis	1 (2.8%)
Bronchiectasis	1 (2.8%)
Type of lung transplant	
Single	15 (41.6%)
Double	19 (52.8%)
Re-transplant	2 (5.6%) both double

SD standard deviation, *COPD* chronic obstructive pulmonary disease

patients: long-term immunosuppression with cyclosporine or tacrolimus (in patients with cystic fibrosis), mycophenolate mofetil and prednisone (to be tapered at low dosage or discontinued); and antiviral prophylaxis with ganciclovir or valganciclovir (450 mg twice daily) from day 21 for 3 weeks, together with HCMV-IG assays (Cytotect Biotest) at days 1, 4, 8, 15, 30 (1.5 ml/kg body weight) and every month until 1 year (1 ml/kg body weight), irrespective of HCMV mismatching. Multiple BAL procedures were performed in 26 patients (mean, 4.5; range, 2-9). Histopathological analysis was done on surveillance transbronchial biopsies using H&E, Periodic acid-Schiff, and Masson's trichrome stains. AR and small-airway inflammation (lymphocytic bronchiolitis) were diagnosed according to the 2007 criteria of the International Society for Heart and Lung Transplantation [12].

Moreover, in the same time period, 286 BAL samples obtained from 235 patients (141M/94F; mean age, 57.3 years; range, 16-83) who were either immunocompromised (159 specimens from 126 patients, including 70 specimens from 52 transplant patients other than LT [18 bone marrow, 15 liver, 10 kidney, 8 heart, 1 heart+kidney] and 89 specimens from 74 otherwise immunocompromised individuals: chemotherapy, HIV-infection or long-term use of corticosteroids) or immunocompetent (127 specimens from 109 patients) were investigated. These specimens were referred to the Virology Unit of the University Hospital San Giovanni Battista of Turin for clinical and/or radiological indications (including fever, cough, dyspnea, newly developed radiological abnormalities in 269 cases) or for follow-up of a previous positivity with no defined sampling frequency. Samples from the same patient collected less than two weeks apart were excluded; each specimen was evaluated independently. In these categories of individuals, in contrast to the defined sampling intervals in LT patients, when there was multiple sampling, the sampling intervals were not determined and were established on the basis of clinical judgement. Informed consent was obtained in all cases.

The following features were recorded: demographics, microbiological/virological results of BAL testing (including a molecular panel detecting 15 respiratory viruses [HCMV, HHV-6 and -7, EBV, HSV-1 and -2, human enteroviruses, human bocavirus, human coronaviruses, influenza A and B viruses, parainfluenza viruses 1-3, RSV, adenoviruses] as described previously [13]), and discharge diagnosis (International Classification of Diseases, codes 480.XX-486.XX for pneumonia, 460.XX-466.XX for acute respiratory insufficiency or other acute respiratory conditions and 33.22 for bronchoscopy). The BAL procedure, specimen processing and quantitative detection of HRV RNA were performed as described previously, with a limit of detection of 220 genome equivalents/ml [14, 15].

Statistical analysis was performed using the χ^2 test and t test. A p value <0.05 was considered statistically significant.

The results are summarized in Table 2. Overall, HRV was detected in 16 of 127 samples (12.6%) from 15 of 36 LT recipients (41.7%), vs 35 of 286 samples (12.2%) from 34 of 235 other patients (14.5%). In particular it was detected in 16 of 127 specimens (12.6%) from 16 of 109 immunocompetent patients (14.7%); 19 of 159 specimens (11.9%) from 18 of 126 immunocompromised patients (14.3%), including 11 of 70 specimens (15.7%) from 10 of 52 recipients of transplants other than lung (19.2%). Among the HRV-positive specimens from LT patients, 10 (62.5%) were in the first year following transplantation, and six were at >1 year. The prevalence of HRV-positivity was significantly higher in LT recipients than in all of the other patients ($p < 0.0001$), both immunocompetent ($p = 0.001$) and immunocompromised ($p < 0.0001$), and in comparison to recipients of transplants other than lung ($p = 0.04$). The viral load did not differ significantly between the different subgroups (mean ± standard deviation: $4.10x10^5 \pm 1.57x10^6$ RNA copies/ml BAL in LT, median $8.8x10^3$; $1.49x10^6 \pm 2.28x10^6$ in immunocompromised patients others than LT, median 25624; $2.03x10^5 \pm 9.60x10^5$ in immunocompetent patients, median $9.36x10^3$).

HRV was detected as a single agent in five LT specimens (3.9%) from as many patients (13.9%): four were collected as a follow-up in the absence of overt clinical symptoms (viral load, 10^3-10^4); one was from a patient

Table 2 Prevalence of HRV positivity in lung transplant (LT) recipients and other patients. Comparison of prevalence of HRV in LT vs control populations. Comparisons and statistical significance are reported for specimens and for patients. n.s., not significant. Prevalence of HRV was not significantly different between LT and control populations when considering specimens. Prevalence of HRV (both overall positivity and positivity as single agent) was significantly higher in LT patients vs other patients. See text for comments

	HRV positive			
	Specimens N (%)	p	Patients N (%)	p
LT	16/127 (12.6%)		15/36 (41.7%)	
	as single agent 5/127 (3.9%)		as single agent 5/36 (13.9%)	
Others	35/286 (12.2%)	n.s.	34/235 (14.5%)	<0.0001
	6/286 (2.1%) as single agent		6/235 (2.6%) as single agent	0.006
Immunocompetent	16/127 (12.6%)		16/109 (14.7%)	0.001
Immunocompromised	19/159 (11.9%)		18/126 (14.3%)	<0.0001
Transplant recipients other than LT	11/70 (15.7%)		10/52 (19.2%)	0.04

with respiratory insufficiency and chronic graft dysfunction during evaluation for re-transplantation (viral load, $6.28x10^6$). In the other patients, HRV was detected as a single agent in six cases, all of which were from immunocompromised patients (three transplant recipients and three haematological patients on chemotherapy; viral load, 10^3-10^4 in five in the presence of clinical signs/symptoms and/or new infiltrates in chest X-rays, and $1.37x10^5$ in one with interstitial infiltrates and a discharge diagnosis of pneumonia). The prevalence of HRV as single agent was significantly higher in LT patients in comparison to all of the other patients ($p = 0.003$) when not specifically considering the group of immunocompromised individuals. In the remaining cases from all of the groups of patients, HRV was mostly detected in co-infection with other viruses (herpesviruses, particularly HCMV, HHV-6 and -7, and influenza virus in three cases from other patients) and/or bacteria (including *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Legionella pneumophila*).

The results are summarized in relation to clinical features in Table 3. Acute respiratory insufficiency or other acute respiratory illness were found in 15 LT cases, three of which were HRV positive (two with viral load 10^3-10^4 and in co-infection, the remaining as described previously). A discharge diagnosis of pneumonia was made in 10 of 127 LT cases (7.9%), two of which were HRV-positive (viral load, 10^3-10^4, co-infected with HCMV in both cases and with bacteria in one). Interstitial pneumonia was diagnosed by histopathology in five LT cases; none were HRV positive. A diagnosis of acute rejection was made for 12 specimens obtained from eight LT patients: three were HRV positive in the corresponding BAL specimen (viral load, 10^3 in two, both with lymphocytic bronchiolitis and co-infection with a β-herpesvirus, and $9.98x10^4$ in one as a single agent with lymphocytic bronchiolitis). HRV-positivity was not consistently detected in consecutive specimens.

In this study, we evaluated the prevalence and clinical role of HRV in the lower respiratory tract in LT recipients. Although HRV is generally temperature-restricted in replication, with optimal growth at 33-35°C, as is found in the upper respiratory tract, the temperatures in the tracheobronchial tree are often lower than core body temperatures, also in relation to external temperature and frequency of ventilation, thus making it permissive for replication, and many serotypes can replicate efficiently at core body temperature [8, 16].

The involvement of the lower respiratory tract has been confirmed by our data, as HRV was detected in approximately 12%-15% of specimens from LT recipients and other patients without significant differences in prevalence, although for individual patients, the rate of detection of HRV in at least one specimen was significantly higher in LT recipients than in other patients, including immunocompetent and immunocompromised patients as well as other transplant recipients [17–19]. Although this difference could be due to the fact that LT recipients are predisposed to viral infections of the lower respiratory tract, given the dysfunctional pulmonary background and altered local immunity, it is likely that the sampling frequency (surveillance at three month-intervals) contributes substantially to the higher rate of detection in LT recipients. This could also explain the lack of differences in prevalence when comparing HRV detection in specimens in contrast to the significant difference when considering patients. Nevertheless, the frequency of detection in LT patients did not imply a difference in viral load between the different subgroups.

In a recent study, a viral load of $>1x10^5$ RNA copies/ml BAL has been associated with respiratory tract infection in

Table 3 Results of HRV detection in relation to clinical features in lung transplant recipients (panel A) and other patients (panel B). N, number of specimens collected in the presence of a clinical feature; N+, number of specimens that were HRV positive. A histopathological diagnosis was available only for LT recipients. HRV was detected as a single agent when not otherwise indicated

	N+ (%)	Notes
(A) BAL from lung transplant recipients		
N = 127		
Acute respiratory insufficiency or other acute respiratory illness	3 (20%)	Load 10^3-10^4 RNA copies/ml BAL and co-infection (with viruses and/or bacteria) in two cases and $6.28x10^6$ in one
N = 15		
Pneumonia (discharge diagnosis)	2 (20%)	Load 10^3-10^4 RNA copies/ml BAL and co-infection (with viruses and/or bacteria) in both
N = 10		
Interstitial pneumonia (histopathological analysis)	0	–
N = 5		
Acute rejection	3 (25%)	Load 10^3 RNA copies/ml BAL in two (both with lymphocytic bronchiolitis), $9.98x10^4$ in one; all in co-infection with viruses
N = 12		
Surveillance bronchoscopy	1 (4.2%)	Load 10^3 RNA copies/ml BAL
N = 24		
Abnormalities at chest X-ray	0	–
N = 9		
(B) AL from other patients		
N = 286		
Acute respiratory insufficiency or other acute respiratory illness	3 (8.3%)	Load 10^3 RNA copies/ml BAL in two (one co-infected with bacteria) and $>10^5$ in one
N = 36		
Pneumonia (discharge diagnosis)	10 (14.5%)	Load 10^3 RNA copies/ml BAL and co-infected (with viruses and/or bacteria) in six cases and $>10^5$ in four (three co-infected with viruses and/or bacteria)
N = 69		
Surveillance bronchoscopy	0	–
N = 17		
Abnormalities at chest X-ray	2(15.4%)	Load 10^3 RNA copies/ml BAL in one case and $>10^5$ and co-infection in one
N = 13		

LT patients, while loads of 10^3-10^4 were invariably detected in the absence of overt clinical symptoms [6]. Similarly, a viral load of $>1x10^5$ was always associated with respiratory symptoms in immunocompetent adults, while lower levels did not appear to correlate with the kinetics of clinical symptoms. In our study, only one LT patient (viral load 10^6, HRV as a single agent) presented respiratory insufficiency, while all of the other HRV-positive LT recipients had a viral load of 10^3-10^4, also in the presence of clinical signs and/or symptoms, and similar loads were also detected in surveillance specimens, thus confirming the presence of HRV in the lower respiratory tract, although it should be considered that real-time PCR methods vary among different studies, and results cannot be extrapolated to all assays. It should be noted that LT recipients with a viral load of 10^3-10^4 copies/ml in the presence of symptoms were all coinfected, thus demonstrating the clinical role played by other respiratory agents. Clinical follow-up and sampling intervals excluded that the low level of virus present in the epithelial lining fluid was due to a previous infectious episode. In the other patients, HRV was detected frequently in co-infection with other viruses, and a viral load of $>10^5$ was invariably associated with overt clinical signs and/or symptoms, as expected.

In contrast to immunocompetent subjects, HRV clearance in immunocompromised patients may be delayed with prolonged shedding, although we did not observe this in patients repeatedly tested, such as LT recipients. In a study on HRV detection in BAL from 68 LT recipients [10], Kaiser et al. described three patients with lower respiratory symptoms and graft dysfunction, two with AR and persistent infection over a 12-month period. In our study, AR was detected in 12 cases, three of which were HRV positive, although only one had a viral load of approximately 10^5 RNA copies/ml, with HRV detected as single agent, while the other two displayed viral loads of 10^3 and co-infections. In another LT recipient, respiratory insufficiency and chronic graft dysfunction were evidenced in association with HRV-positivity as single agent and a viral load $>10^6$. However, no case of persistent HRV detection

was evidenced. These data, like those on LT recipients reported in literature, have to be considered taking into account the small numbers, thus preventing a definitive evaluation and suggesting the need for further studies.

In conclusion, HRV infection may involve the lower respiratory tract; this has been demonstrated in different categories of patients, including those with an impaired pulmonary background, such as a transplanted lung, thus suggesting the opportunity to evaluate HRV in the diagnostic molecular work-up. It could be hypothesized that the clinical evaluation of HRV in the context of LT should take the viral load into account, with levels of $>10^5$ RNA copies/ml BAL potentially being associated with overt clinical signs/symptoms, although lower levels of HRV can also be detected in both symptomatic and asymptomatic patients, and differences in real-time PCR assays should be considered. Considering the small number of positive specimens in the LT cohort and the large number of co-infections [20], no definitive conclusions can be made on this issue, and further studies on larger series are required. The association with AR and chronic graft dysfunction should be studied further, although it could be hypothesized that HRV, similar to other community-acquired respiratory viruses, contributes to the onset of a cascade of events that potentially lead to acute and chronic graft dysfunction.

Conflict of interest The authors have no conflict of interest.

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