Incidence and outcomes of respiratory viral infections in lung transplant recipients: a prospective study

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INTRODUCTION

Influenza, respiratory syncytial virus (RSV), parainfluenza (PIV), human metapneumovirus (HMPV), rhinovirus, enterovirus, coronavirus, human bocavirus and adenovirus are the most frequent causes of respiratory infections in humans. These viruses circulate in the community with different seasonal patterns and can lead to serious complications, mainly in individuals with predisposing risk factors such as immunosuppression or pre-existing lung disease.5–7 Lung transplant recipients (LTR) are particularly prone to developing lower respiratory tract infections. This is related to their immunosuppression and also to the exposure of the graft to viral agents, impaired mucociliary function, altered lymphatic drainage and the absence of a cough reflex. These infections may have the potential to promote secondary bacterial infections, acute rejection (AR) and subsequently lead to chronic graft dysfunction.

The availability of highly sensitive diagnostic panels targeting the nucleic acids of multiple respiratory viruses now allows for the exploration of the complex relationship between specific respiratory viruses and clinical outcomes in LTR. Previous investigations and a systematic review have shown that viral infections detected by molecular assays are associated with respiratory symptoms, but their association with AR remains controversial.6–7 Nonetheless, both the retrospective nature of most studies and the difficulties in conducting a complete panel of molecular assays in a standardised manner allow for only weak inferences regarding the role of viruses in this patient population. The aims of our study were to investigate the...
Experiments were validated only if the resulting cycling 96-well plates and inhibition was controlled by spiking each speci- men with a quantified standard using canine distemper virus. Experiments were validated only if the resulting cycling threshold (CT) value was within expected ranges. PCR detection was considered positive if duplicated by a second complete experiment on an original aliquoted specimen and with a CT value of ≤39.

Statistical methods
The incidence of viral infection was estimated with Stata V11 (Statacorp, College Station, Texas, USA) using the Poisson routine. Incident rate ratios were estimated as categorical functions of the post-transplant period and bronchiolitis obliterans syndrome (BOS).

To compare predictors of viral infections, we applied general- ised linear or logistic latent models using the xtreg or xtolotg commands of Stata. We chose this method to take into account the repeated measures in each LTR. ORs with CIs for AR (stage 2 or 3) and respiratory symptoms associated with respiratory viruses were obtained using the same approach, controlling for study site. The tacrolimus trough levels, which were abnormally distributed, were compared between virus-positive and virus-negative visits after log transformation.

Because the role of picornavirus is controversial, we carried out sensitivity analyses excluding infection with picornavirus.

RESULTS
Patients and baseline characteristics
On average, patients received lung transplants a mean (SD) of 4.0 (4.1) years before study entry; nearly one-third (n=31; 27.7%) were transplanted within the previous 3 months. Conditions leading to lung transplantation were mainly chronic obstructive lung disease (42.8%) and cystic fibrosis (27.7%); median age at transplantation was 49.5 years. Most patients were on triple immunosuppressive therapy and 16% had BOS stage 1 or more at study entry (table 1). Patients had a median of five study visits and an average follow-up of 827 days (range 52–1037). A total of 903 visits were analysed: 570 (63.1%) during screening periods defined by the study protocol; 124 (13.7%) routine post-transplantation visits; and 209 (23.2%) due to new or worsening respiratory symptoms (see online supplementary appendix table 1).

BAL and transbronchial biopsy
BAL fluid was obtained in 276 cases. In 153 specimens (55.4%), no pathogen was detected. A virus was present in 34 (12%), at least one bacterium in 73 (26.4%) and at least one fungus in 23 (8.3%). Thirty-two specimens (11.6%) were polymicrobial. Multiple infection was found in 14/34 virus-positive BAL speci- mens: Pseudomonas aeruginosa (n=6), Aspergillus spp (n=3), Candida spp (n=3), Escherichia coli (n=2), Streptococcus pneumoniae (n=1) and non-tuberculous mycobacteria (n=1).

Two hundred and thirty-five TBB specimens were classified according to the pathological AR grade (see online supplementary appendix tables 1 and 2). LTR with moderate or severe AR were frequently treated for rejection (A2 83.3%; A3 6.7%; ≤A39). A total of 903 visits were analysed: 570 (63.1%) during screening periods defined by the study protocol; 124 (13.7%) routine post-transplantation visits; and 209 (23.2%) due to new or worsening respiratory symptoms (see online supplementary appendix table 1).

Incidence of viral infections
The viral infection rate was 19.3% (174/903) overall and 12.3% (34/276) in the BAL subgroup. Sixty-eight of 112 LTR (61%) had one or more viral infections (see online supplementary appendix figure 1). Three had 10 or 11 viral infections; all were BOS stage 3 and at least 5 years post-transplant. The incidence of viral infection affecting upper and/or lower airways was 0.83 per patient-year (95% CI 0.45 to 1.52). Patients transplanted within...
the previous year did not have a higher incidence compared with those more than 1 year post-transplant (incidence rate ratio (IRR) 1.57 (95% CI 0.63 to 3.93); p=0.329). Incidence was not associated with baseline BOS stage, comparing BOS 0 with later stages (IRR 1.76 (95% CI 0.72 to 4.30); p=0.215).

Distribution of viral infections
Picornavirus was the most frequently detected virus for all visit types (105/174, 60.3%) and among positive BAL specimens (20/34, 59%) (figure 1). Sequence analysis and specific assays revealed that almost all picornaviruses were in fact rhinoviruses (data not shown). All viruses had a relatively similar distribution among upper and lower respiratory specimens (figure 1A). During the study period, 10 LTRs repeatedly screened positive for picornavirus. In three cases picornavirus infection lasted from 120 to 180 days, while it was shorter in others. However, we could not distinguish between re-infection and protracted infection as genome-sequencing techniques were not employed in this study.

Viruses other than picornavirus were four times more likely to be identified at emergency visits (40/72, 65.6%) than at routine or screening phase visits (29/102, 28.4%; OR 3.7 (95% CI 1.7 to 8.1); p=0.010) (figure 1B). Adenovirus, PIV, RSV and HMpV were also more frequently detected at emergency visits. At the screening visits, 48% of patients reported new or worsening symptoms (figure 2). The proportion of positive viral tests was 10.0% (range 2.3–20.0%) for those without new symptoms and 19.6% (range 6.4–29.7%) for those with new symptoms. Strong seasonal patterns were observed—for example, viral infections were less frequent during the summer and in influenza cases were observed mainly in February to March (figure 2).

Factors associated with viral infections
Higher tacrolimus serum trough levels were observed in patients with concurrent viral infections (p=0.015; table 2). Time since transplantation and BOS stage were not associated with viral

Table 1  Main characteristics of lung transplant recipients at study entry (n=112)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>58/54</td>
</tr>
<tr>
<td>Age at lung transplantation (years)</td>
<td>49.5 (11–66)</td>
</tr>
<tr>
<td>Time since lung transplantation</td>
<td></td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>31 (27.7)</td>
</tr>
<tr>
<td>3 months to 1 year</td>
<td>10 (8.9)</td>
</tr>
<tr>
<td>1–3 years</td>
<td>18 (16.1)</td>
</tr>
<tr>
<td>3–5 years</td>
<td>15 (13.4)</td>
</tr>
<tr>
<td>5–10 years</td>
<td>27 (24.1)</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>11 (9.8)</td>
</tr>
<tr>
<td>Diagnosis leading to lung transplantation</td>
<td></td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>48 (42.0)</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>31 (27.7)</td>
</tr>
<tr>
<td>Idiopathic pulmonary fibrosis</td>
<td>6 (5.4)</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>9 (8.0)</td>
</tr>
<tr>
<td>Other</td>
<td>18 (16.1)</td>
</tr>
<tr>
<td>Immunosuppressive therapy</td>
<td></td>
</tr>
<tr>
<td>Calcineurin inhibitors</td>
<td>107 (96)</td>
</tr>
<tr>
<td>Mycophenolate mofetil or azathioprine</td>
<td>112 (100)</td>
</tr>
<tr>
<td>mTOR inhibitors</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Systemic corticosteroids</td>
<td>110 (98)</td>
</tr>
<tr>
<td>Bronchiolitis obliterans syndrome (BOS) stage</td>
<td></td>
</tr>
<tr>
<td>BOS 0</td>
<td>71 (63.4)</td>
</tr>
<tr>
<td>BOS 0p</td>
<td>20 (17.9)</td>
</tr>
<tr>
<td>BOS 1</td>
<td>9 (8.0)</td>
</tr>
<tr>
<td>BOS 2</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td>BOS 3</td>
<td>6 (5.4)</td>
</tr>
<tr>
<td>BOS undetermined</td>
<td>3 (2.7)</td>
</tr>
</tbody>
</table>

mTOR, mammalian target of rapamycin.

Figure 1  Distribution of viruses in lung transplant recipients. (A) Viruses recovered in upper and lower respiratory specimens. (B) Viruses recovered according to visit type.
respiratory tract infection. Patients ≥1 year post-transplant, however, were five times more likely to have lower respiratory infections detected via BAL specimens than those transplanted within the previous year.

Viral infections were more likely to be detected during an emergency visit (34.0% vs 14.6%, p=0.001) compared with a screening period and when new symptoms were reported (OR 2.9 (95% CI 1.9 to 4.6), figure 3). Fever and sore throat were

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**Table 2** Factors associated with respiratory tract viral infection identified by reverse-transcription PCR in lung transplant recipients

<table>
<thead>
<tr>
<th>Time since transplantation</th>
<th>Visits with upper (NPS) or lower (BAL) respiratory tract viral searches (n=903)</th>
<th>Visits with lower (BAL) respiratory tract viral searches (n=276)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Positive (19.3%)</td>
<td>Negative (12.3%)</td>
</tr>
<tr>
<td>N=729</td>
<td>N=174</td>
<td>N=242</td>
</tr>
<tr>
<td>93 months, n (%)</td>
<td>120 (16.7)</td>
<td>82 (6.8)</td>
</tr>
<tr>
<td>3 months to 1 year, n (%)</td>
<td>126 (23.2)</td>
<td>48 (18.6)</td>
</tr>
<tr>
<td>1–3 years, n (%)</td>
<td>411 (20.1)</td>
<td>51 (23.9)</td>
</tr>
<tr>
<td>&gt;3 years, n (%)</td>
<td>p=0.0296†</td>
<td>p=0.0011†</td>
</tr>
<tr>
<td>Tacrolimus level, μg/L, median (IQR)</td>
<td>n=397, 8.7 (3.2)</td>
<td>n=139, 8.9 (3.8)</td>
</tr>
<tr>
<td>Log-transformed tacrolimus level</td>
<td>2.18 (0.36)</td>
<td>2.21 (0.36)</td>
</tr>
<tr>
<td>Treatment for acute rejection during the preceding 3 months, n (%)</td>
<td>65 (17.7)</td>
<td>64 (9.2)</td>
</tr>
<tr>
<td>Study phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening period visits, n (%)</td>
<td>487 (14.6)</td>
<td>49 (7.6)</td>
</tr>
<tr>
<td>Routine visits, n (%)</td>
<td>105 (19.3)</td>
<td>113 (7.5)</td>
</tr>
<tr>
<td>Emergency visits, n (%)</td>
<td>137 (34.5)</td>
<td>80 (23.3)</td>
</tr>
<tr>
<td>p=0.001†</td>
<td></td>
<td>p=0.001</td>
</tr>
</tbody>
</table>

*p=0.296† p=0.001† p=0.015† p=0.092† p=0.0011† p=0.001

†Visits with BAL (n=276) are a subset of the total visits (n=903).

Figure 2: Overall percentage, number and type of positive viral test by screening period and presence of symptoms: y right axis: number of positive tests; y left axis: percentage of positive tests during the screening period. S±, presence or absence of new symptoms.

Lung transplantation

strongly associated with viral infections (43.1% and 49.3%, respectively); rhinorrhea and cough were also very frequent (figure 3). Among all visits where BAL was performed, an emergency visit (OR 6.2 (95% CI 1.5 to 26.2)) and sputum production (OR 3.9 (95% CI 1.4 to 10.6)) were significantly associated with viral infection. Serum C-reactive protein and procalcitonin levels did not appear to be associated with viral infection (see online supplementary appendix table 3).

Symptoms and short-term outcomes
Table 3 demonstrates the symptomatology, hospitalisation rate and lung function change associated with each viral agent. Of note, one-third of cases with picornavirus infection did not report any new or worsening symptoms. The most prevalent symptom for picornavirus was rhinorrhea (43.0%), while fever (14.3%) and myalgia (7.6%) were uncommon. At the other end of the spectrum, influenza, RSV and PIV were almost systematically associated with new symptoms. Fever was present in 61.1% of patients with influenza. The sum of new symptoms, ranging from 0 to 9, varied according to specific viruses. Lower scores were found for picornavirus (mean (SD) 2.2 (2.3) new symptoms) and human bocavirus (mean (SD) 2.5 (3.5), and higher scores for influenza (4.4 (3.0), PIV (4.3 (1.6) and RSV (3.9 (2.4)). Overall, new infiltrates on chest x-ray were rare, except for patients with influenza (22.2%). The hospitalisation rate varied markedly among viruses. Hospitalisation rates during viral respiratory infections were 50.0% (95% CI 30.0% to 70.1%) for transplant recipients with influenza or PIV, 18.9% (95% CI 12.5% to 26.8%) for those with picornavirus, coronavirus or human bocavirus, and 12.9% (95% CI 9.8% to 15.1%) for patients without viral or bacterial infection. LTR with influenza or paramyxoviruses (RSV PIV) were three times more likely to be hospitalised (OR 3.1 (95% CI 1.8 to 5.1); p<0.001) than those without viral or bacterial infection. Patients with picornavirus or coronavirus infection tended to be more frequently hospitalised (OR 1.7 (95% CI 0.9 to 3.3); p=0.080) than those without viral or bacterial infections.

Among the 12 patients who died during the study, picornavirus (2 patients) and adenovirus (1 patient) infections were diagnosed shortly before death.

Lung function changes
Many LTR were too ill to perform acceptable quality lung function tests at an emergency visit, but function was poorer in those with viral infection. LTR with viral infection had a mean loss of 106 mL (95% CI −157 mL to −56 mL) on forced expiratory volume in 1 s (FEV1) compared with pre-infection lung function. At recovery, function returned to pre-infection values. Table 3 and online supplementary appendix figure 2 show lung function changes compared with pre-infection values. When excluding picornavirus from the analyses, lung function change was within a similar range (−106 mL (95% CI −189 mL to −23 mL).

Acute rejection (AR)
Biopsy-proven AR was not associated with concurrent viral infection (OR 0.4 (0.1 to 1.3)), nor was initiation of AR therapy within the next 3 months (OR 0.8 (0.4 to 2.0)) (see online supplementary appendix table 4). Table 2 in the online appendix details the frequency of virus associated with acute vascular or bronchiolar rejection grades. There was still no association when picornavirus was excluded from the analysis. The individual clinical course of an initial viral infection identified via BAL is shown in online supplementary appendix figure 3. Following the incident lower respiratory tract infection, only three cases with picornavirus, influenza or coronavirus experienced AR at 90, 120 and 150 days, respectively (table 3). One additional patient had an upper respiratory picornavirus infection 90 days before AR.

DISCUSSION
We show that respiratory viral infections are very common in LTR. Picornavirus, in particular rhinovirus, was the most frequent causative agent recovered in both upper and lower respiratory specimens, followed by coronavirus and influenza. However, the clinical course of each of these viral infections was variable. Of note, influenza and paramyxoviruses were associated with a higher symptom load, contributing to 50% of emergency visits and hospitalisations. Symptoms most often encountered with picornavirus or coronavirus are cough and rhinorrhea, but one-third of our study population infected with these agents were asymptomatic. Conversely, influenza, paramyxovirus and adenovirus, which are less common, were
Table 3

<table>
<thead>
<tr>
<th>Symptomatology and hospitalisation rate associated with viral infection in lung transplant recipients (n=174)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picornavirus (n=105)</td>
</tr>
<tr>
<td>New respiratory symptoms</td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Rhinorrhoea</td>
</tr>
<tr>
<td>Dyspnoea</td>
</tr>
<tr>
<td>Thoracic pain</td>
</tr>
<tr>
<td>Sputum</td>
</tr>
<tr>
<td>Myalgia</td>
</tr>
<tr>
<td>Hospitalisation</td>
</tr>
</tbody>
</table>

All values expressed as percentages with 95% CI or mean with SD.

HMpV, human metapneumovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Interestingly, picornavirus circulates year-round with an alternating seasonal pattern. This contrasts with other viruses like influenza which are found during seasonal epidemics only. We further observed that the epidemiology and impact of viruses differed in the upper and lower respiratory tracts. Picornavirus and coronavirus were largely predominant in the upper respiratory tract and two-thirds were found during routine or screening visits. Again, this suggests a limited clinical impact of picornavirus and coronavirus when recovered in the upper respiratory tract, as these were not patient-initiated visits.

Picornavirus and coronavirus were also predominant in the lower respiratory tract. Of 34 viral infections detected in 276 BAL samples, 20 (59%) were caused by picornviruses, mainly rhinoviruses. Compared with influenza or paramyxoviruses which are known to infect the lower respiratory tract, this proportion is higher than previously reported. This may be due in part to the sensitivity of the assays used, the population selected or the study design: all cases were systematically screened, even in the absence of overt clinical symptoms.13

In LTR, rhinovirus has been shown to adapt to the lower respiratory tract and cause protracted and chronic infections.16 19 We further describe distinct symptom patterns triggered by specific respiratory viruses. Our study supports previous observations that systemic signs and symptoms such as fever, fatigue and myalgia are often reported for influenza or RSV, but rarely for picornavirus: 60% of influenza cases presented with fever compared with fewer than 15% of picornavirus cases. Nevertheless, symptom overlap in our study population limited our ability to accurately predict the virus type. Our study confirms that immunosuppressed patients manifest fever inconsistently.20

This contrasts with community-based studies of influenza where subjects are immunocompetent and not exposed to steroids and report a prevalence of fever of 68%–86%.24 Lower FEV1 or forced vital capacity (FVC) compared with pre-infection values were observed in patients with viral infection. Mallia et al22 recently reported that rhinovirus infection in patients with chronic obstructive pulmonary disease resulted in decreased peak flow without significant loss in FEV1 or FVC. A more significant loss of function was observed in cystic fibrosis patients with viral infection, mostly due to rhinovirus.23 Although the inability to obtain spirometry measurements in acutely ill patients is an unavoidable limitation, our study supports previous reports showing that viral infection acutely affects function in LTR.6 24

It has been theorised that viral infections promote acute graft rejection.8 While some case series suggest an association between the two,25 26 other studies—some prospective—have found no association.6 27 A meta-analysis could not confirm this link.7 Similarly, the present prospective study shows no temporal relation between respiratory viruses and biopsy-proven AR. However, this finding does not preclude an association between viral infection and chronic graft rejection.

We identified elevated tacrolimus levels as a possible risk factor for viral infection. To our knowledge, no prospective study has yet employed both a quantitative evaluation of immunosuppression and concomitant viral screening in LTR. As calcineurin inhibitors weaken the lymphocyte-mediated immune response, it is plausible that viruses are not contained, leading to prolonged shedding and protracted infection.

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103 (259) − 22 (279) − 71 (415) 251 (220) − 181 (115) − 180 (56)
The study included prespecified periods during which transplant recipients were screened for viral infection, independent of symptomatology. Asymptomatic carriage of viral RNA or DNA in the upper respiratory tract was found at 10% of screening visits, most belonging to picornavirus. While viral RNA, mostly from rhinovirus, was detected in up to 30% of asymptomatic immunocompetent children, Johnston et al found only 4% of asymptomatic adults to be carrying rhinoviruses. However, the true prevalence of asymptomatic viral carriage in immunocompetent adults remains uncertain and thus cannot be compared with the rate observed in our study population during screening periods.

The strengths of our study are its prospective design with a full record of all visits over more than 30 months, as well as the use of an extensive panel of RT-PCR viral tests. It is unlikely that clinically significant viral infections were missed in our cohort. The seven screening periods covering all four seasons offered a unique opportunity to evaluate the incidence of asymptomatic viral infection. The fact that lung function could not be evaluated in many patients may lead to an underestimation of lung function loss due to acute viral infection, but does not alter our conclusions.

This large prospective study of LTR found that respiratory viral infections could be diagnosed in one-third of emergency visits. Viral infections transiently diminish lung function and lead to more hospitalisations, often including invasive procedures such as bronchoscopy, but are not associated with AR. Asymptomatic viral infection is rare and occurs most often with picornavirus. Specific antiviral therapy in LTR is limited and its efficacy as yet unproven.

The incidence and clinical impact of viral infections in LTR demonstrated by this study suggest a need for trials investigating both preventive and protective interventions in this population.

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Contributors P-OB designed the study, analysed the data and drafted the manuscript. J-DA designed the study, supervised patient enrolment and drafted the manuscript. PMS designed the study and supervised patient enrolment. JM-S and CB enrolled patients. TR and LN participated in study organisation. LT and SVB performed the laboratory analyses. PM participated in specimen collection. GW collected patient data, supervised the database and analysed the data. LK designed, planned and supervised the study. All authors contributed to the manuscript and approved the final version.

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Competing interests None.

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