MAJOR ARTICLE

Upper and Lower Respiratory Tract Viral Infections and Acute Graft Rejection in Lung Transplant Recipients

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Background. Lung transplant recipients are frequently exposed to respiratory viruses and are particularly at risk for severe complications. The aim of this study was to assess the association among the presence of a respiratory virus detected by molecular assays in bronchoalveolar lavage (BAL) fluid, respiratory symptoms, and acute rejection in adult lung transplant recipients.

Methods. Upper (nasopharyngeal swab) and lower (BAL) respiratory tract specimens from 77 lung transplant recipients enrolled in a cohort study and undergoing bronchoscopy with BAL and transbronchial biopsies were screened using 17 different polymerase chain reaction–based assays.

Results. BAL fluid and biopsy specimens from 343 bronchoscopic procedures performed in 77 patients were analyzed. We also compared paired nasopharyngeal and BAL fluid specimens collected in a subgroup of 283 cases. The overall viral positivity rate was 29.3% in the upper respiratory tract specimens and 17.2% in the BAL samples ($P < .001$). We observed a significant association between the presence of respiratory symptoms and positive viral detection in the lower respiratory tract ($P = .012$). Conversely, acute rejection was not associated with the presence of viral infection (odds ratio, 0.41; 95% confidence interval, 0.20–0.88). The recovery of lung function was significantly slower when acute rejection and viral infection were both present.

Conclusions. A temporal relationship exists between acute respiratory symptoms and positive viral nucleic acid detection in BAL fluid from lung transplant recipients. We provide evidence suggesting that respiratory viruses are not associated with acute graft rejection during the acute phase of infection.

Lung transplant recipients are exposed to respiratory viruses circulating year-round in the community [1–6]. In the general population, most of these infections lead to self-limited upper respiratory tract diseases, but protracted respiratory viral infection and lower respiratory tract complications are more likely in lung transplant recipients. This is related to specific risk factors, including direct exposure of the graft to airborne viruses, impaired mucociliary clearance, poor cough reflex, and abnormal lymphatic drainage. Nevertheless, our knowledge of the clinical effect of respiratory viruses is incomplete, particularly when detected by molecular assays applied to lower respiratory tract specimens. This is in part due to the retrospective design of most available studies, the use of different sampling sites (upper vs lower respiratory tract specimens), and the heterogeneity of diagnostic procedures (conventional vs molecular techniques) [1, 2, 4, 6–13].

In addition to the direct consequences of any viral infection, subsequent cellular injury and altered host immunity could also initiate a cascade of immunologic events [14–16], leading to acute and chronic allograft rejection. Although several studies support the association between respiratory viruses and chronic lung rejection [1, 3, 10, 12, 14, 17–19], the link between respiratory viruses and acute rejection has been studied mainly in small case series or by subgroup analysis [1,

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2, 9–11, 20, 21]. On the basis of available evidence, the relationship between viral infection and acute rejection has not been established.

The present investigation was specifically designed to assess the epidemiology of respiratory viruses in bronchoalveolar lavage (BAL) fluid from lung transplant recipients and to analyze the relationship between these viruses and the presence of acute graft rejection. The most sensitive molecular assay methods were used to identify as many as 17 common respiratory viruses in not only the lower but also the upper respiratory tract. Symptoms were carefully and prospectively described, and their association with either respiratory viruses or acute rejection was analyzed.

METHODS

Study population and procedures. During a 27-month study period from November 2003 through March 2006 (covering 3 winter seasons), lung transplant recipients were enrolled in a prospective cohort study [22]. Patients were followed up at the 2 sites of a single transplantation network, including the University Hospitals of Geneva (Geneva, Switzerland) and the University Hospital of Lausanne (Lausanne, Switzerland).

Informed consent was required from each participant, and the study was approved by both institutional ethics committees. Any lung transplant recipient who underwent bronchoscopy with BAL and transbronchial biopsies was eligible, irrespective of the reasons leading to the procedure. On the basis of standardized guidelines, we perform routinely scheduled bronchoscopies at 1, 3, 6, and 12 months after transplantation and yearly thereafter. Other indications for bronchoscopy with transbronchial biopsies include unexplained respiratory symptoms, functional deterioration with a $\geqslant$12% decrease in the forced expiratory volume in 1 s (FEV_1), new chest radiologic infiltrate, and control procedure 1 month after treatment of any rejection of grade A3 or higher. Thus, patients could undergo bronchoscopy for a variety of reasons with additional procedures during the study period. For technical reasons (eg, pathologist availability), transbronchial biopsies are not performed during weekends and holidays at our centers. For safety and ethical reasons, no additional bronchoscopy was performed only for the purpose of the study.

The BAL procedure and the real-time TaqMan reverse-transcription polymerase chain reaction (RT-PCR) assays for the detection of RNA respiratory viruses (influenza viruses A, B, and C; respiratory syncytial viruses A and B; parainfluenza viruses 1, 2, 3, and 4; human rhinovirus; enterovirus; human metapneumovirus; and coronaviruses OC43, 229E, NL63, and HKU1) were performed as described elsewhere [22–24]. The recently identified bocavirus was added. Transbronchial biopsies were performed under fluoroscopic guidance using standard procedures [25] and analyzed according to published guidelines [26] by senior pathologists masked to the viral results. Pooled nasopharyngeal and oropharyngeal swab specimens were obtained from patients who agreed to the procedure. For technical reasons and reasons of cost (>10,500 RT-PCR assays were performed), the procedure (nasopharyngeal and oropharyngeal swabbing) was limited to 80% of the cases selected during the entire study period. Swabs were immediately placed on appropriate transport medium and stored at -80°C until further RT-PCR analysis. Shortly before each bronchoscopic procedure, a specific case report form was completed and symptoms, reasons leading to the BAL procedure, and the presumed diagnosis based on the available clinical data at the time were recorded. Rhinopharyngitis was defined as the presence of acute respiratory symptoms with at least acute rhinorrhea with or without additional signs suggesting acute sinusitis and/or acute pharyngitis (sore throat confirmed by the presence of inflammatory signs on clinical examination). Flu-like illness was defined as the presence of a temperature >37.8°C plus 2 of the following 4 symptoms: cough, myalgia, sore throat, or headache. When available, lung functions measured 2–6 weeks before, at the time of, and 2–6 weeks after the BAL procedure were recorded. FEV_1 and maximal midexpiratory flow of 25%–75% were also recorded. Impaired lung function was defined as a $\geqslant$12% decrease from the previous value.

Statistical analysis. Each episode was classified into 4 categories according to the respective absence (A0 or A1 grade) or presence (A2, A3, or A4 grade) of acute rejection and/or respiratory virus. We considered only acute rejection grade A2 or higher in the analysis, because most centers would not recommend high-dose immunosuppressive treatment for a lower acute rejection grade. The Fisher exact test was used to compare the frequency of respiratory symptoms in each category. Generalized linear latent and mixed models (Stata software, version 10; StataCorp) were used for analysis of lung functions at each episode to take into account the repeated measures in each patient at the 2 study sites. Odds ratios (ORs) for acute rejection associated with respiratory viruses were calculated using the same statistical approach for repeated measures. To investigate a potential time-specific relationship between respiratory viruses and acute rejection, we repeated our analyses of episodes restricted to 4 key periods: 0–3 months, 4–6 months, 7–12 months, and >12 months after lung transplantation.

RESULTS

Population and indications for bronchoscopy. Seventy-seven lung transplant recipients underwent 343 bronchoscopies. Patient characteristics are given in Table 1. All patients received induction immunosuppression with anti–interleukin 2 receptor antibodies (basiliximab) postoperatively, followed by a triple immunosuppression regimen of calcineurin or target of rapa-

Table 1. Patient Characteristics ($n = 77$)

Characteristic	Value
Age at lung transplantation, years	
Median (IQR)	48.6 (27.3)
Range	7–66
Male	43 (55.8)
Type of transplantation	
Single lung transplantation	12 (15.6)
Double lung transplantation	65 (84.4)
Baseline condition	
Chronic obstructive pulmonary disease	37 (48.1)
Cystic fibrosis	22 (28.6)
Idiopathic pulmonary fibrosis	6 (10.4)
Pulmonary hypertension	3 (3.9)
Other	9 (11.7)
Bronchoscopic procedures, median no. per patient (range)	4 (1–23)

NOTE. Data are no. (%) of patients, unless otherwise indicated. IQR, interquartile range.

mycin inhibitors, azathioprine or mycophenolate mofetil, and prednisone.

Of the 343 bronchoscopies, 229 (66.8%) were performed as routinely scheduled procedures, 85 (24.8%) were performed for a new clinical condition, and 29 (8.5%) were performed as a control biopsy after treatment for an acute rejection (grade A3 or higher) episode. Some patients included for a routinely scheduled bronchoscopy presented with a new respiratory symptom with or without functional impairment and/or new radiologic infiltrate. Taken together, 224 (65.3%) of 343 cases had at least 1 new respiratory symptom, and 54 (15.7%) had a new radiologic infiltrate. On the basis of the pretest evaluation, acute lung rejection and infection were suspected by the physician in charge, who was masked to microbiological results, in 176 cases (51.3%) and 99 cases (28.9%), respectively. For the remaining 68 cases (19.8%), the clinician did not suspect any particular diagnosis.

RT-PCR assays. The RT-PCR viral assays performed on the 343 BAL fluid specimens revealed an overall positivity rate of 17.2% ($n = 59$). Eight BAL specimens were discarded because they had thawed during an electricity power outage. Rhinovirus was the most frequently encountered virus, followed by coronaviruses and parainfluenza viruses (Table 2). The viral positivity rate for the 283 pooled nasopharyngeal and oropharyngeal specimens tested (82.5% of the total study population) was 29.3% ($n = 84$). The agreement between upper and lower respiratory tract specimens according to each type of viral genus is shown in Figure 1. Although influenza viruses, human metapneumovirus, and bocavirus were mostly (or even exclusively for bocavirus) found in the upper airway samples, other viruses (rhinoviruses and respiratory syncytial viruses) were found equally in the upper and lower respiratory tracts. No virus was exclusively found in the lower airways.

Association between clinical suspicion and final diagnosis. Because BAL fluid specimens were collected for a variety of clinical conditions, we were able to analyze the association among symptoms, the diagnosis suspected by the physician in charge, and the subsequent presence of a proven upper and/or lower respiratory tract viral infection. We found a significant association between positive viral nucleic acid detection in BAL fluid and the presence of at least 1 new respiratory symptom ($P = .012$), in particular cough ($P = .001$) and sputum pro-

Table 2. Type and Number of Respiratory Viruses Detected in Bronchoalveolar Lavage (BAL) Fluid Specimens

Variable	Value
Total no. of analyzed BAL fluid specimens	343
Virus-negative BAL fluid specimens	284 (82.8)
Virus-positive BAL fluid specimens	59 (17.2)[a]
Total no. of identified viruses	63
Type of identified viruses	
Rhinovirus	22 (37.3)
Coronaviruses[b]	17 (28.8)
Parainfluenza viruses[c]	13 (22.0)
Respiratory syncytial viruses[d]	7 (11.9)
Human metapneumovirus	3 (5.1)
Influenza viruses[e]	1 (1.7)
Enterovirus	0 (0)

NOTE. Data are no. (%), unless otherwise indicated.

[a] Dual infection in 4 cases.
[b] Coronavirus 229E, OC43, NL63, or HKU1.
[c] Parainfluenza virus 1, 2, or 3.
[d] Respiratory syncytial virus A or B.
[e] Influenza virus A, B, or C.

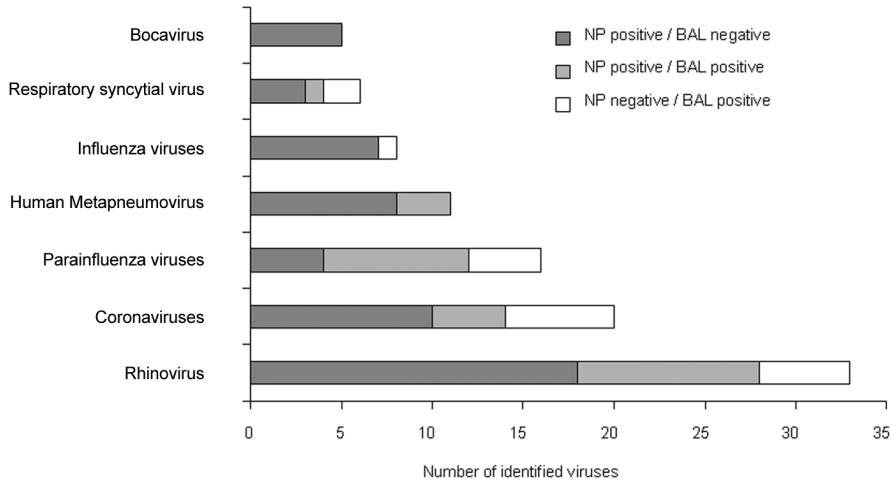

Figure 1. Agreement between upper and lower respiratory tract reverse-transcription polymerase chain reaction results. Of 283 samples simultaneously obtained from the upper (nasopharyngeal and oropharyngeal [NP]) and lower (bronchoalveolar lavage [BAL]) respiratory tract, 184 harbored no virus and 99 were positive for at least 1 respiratory virus at at least 1 site. The total number of identified respiratory viruses was 107. This figure shows their detailed distribution.

duction ($P = .04$). Table 3 gives the clinical findings associated with virus positivity for upper and lower tract specimens. Physicians in charge suspected an infection in 20 (42.6%) of the 47 cases with virus-positive BAL fluid, compared with 57 (24.2%) of the 236 cases that were virus negative. A history of rhinopharyngitis was documented in 20 (23.8%) of 84 cases with a virus-positive nasopharyngeal swab specimen, compared with 22 (11.1%) of 199 of those that were negative.

Acute rejection grade, lung function, and viral infection. Of 343 biopsy specimens, 149 (43.4%) showed no evidence of acute rejection, 78 (22.7%) revealed minimal (A1) rejection, and 116 (33.8%) were graded A2 or higher. Similar to the analysis given in Table 3, we first tested the accuracy of the pretest clinical evaluation performed by the physician in charge. When acute rejection was suspected by the clinician in charge, this was histologically confirmed in 64.1% of cases in the absence of respiratory virus and in 84.6% of cases when a respiratory virus was simultaneously identified in BAL fluid (Table 4). Consistent with the results shown in Table 3, the presence of any respiratory symptoms, cough, or sputum was significantly associated ($P < .004$ for all) with the presence of a viral infection in BAL fluid, irrespective of the presence or absence

Table 3. Clinical Findings in the Upper and Lower Respiratory Tracts According to Viral Reverse-Transcription Polymerase Chain Reaction Results ($n = 283$)

Variable	NP negative and BAL negative	NP positive and BAL negative	NP negative and BAL positive	NP positive and BAL positive	P[a]
No. (%) of cases	185 (65.4)	51 (18.0)	14 (4.9)	33 (11.7)	
Clinical findings					
Fever (temperature, ⩾37.5°C)	11.9	9.8	7.1	18.2	.694
Rhinopharyngitis	11.4	21.6	7.1	27.3	.037
Flulike illness	7.6	17.6	21.4	21.2	.016
Cough	25.9	31.4	35.7	66.6	<.001
Sputum	15.1	27.4	14.3	48.5	<.001
Dyspnea	29.7	15.7	42.9	36.4	.080
FEV_1 decrease of ⩾12%	20.5	21.6	21.4	48.5	.057
New radiologic infiltrate	15.1	11.8	0.0	18.2	.417
⩾1 symptom	60.5	60.8	85.7	84.8	.012
No symptoms	39.5	39.2	14.3	15.2	.012
Suspicion of infection	24.3	23.5	50.0	39.4	.070

NOTE. Data are percentage of cases, unless otherwise indicated. BAL, bronchoalveolar lavage; FEV_1, forced expiratory volume in 1 s; NP, pooled nasopharyngeal and oropharyngeal swabs.

[a] Calculated using the Fisher exact test.

Table 4. Clinical Findings According to the Presence of Acute Rejection (AR) and/or Respiratory Virus in the Lower Respiratory Tract ($n = 343$)

Variable	Virus negative and no AR	Virus negative and AR	Virus positive and no AR	Virus positive and AR	P[a]
No. (%) of cases	181 (52.8)	103 (30.0)	46 (13.4)	13 (3.8)	
Clinical findings					
Fever (temperature, ⩾37.5°C)	14.4	10.7	21.7	7.7	.322
Rhinopharyngitis	12.2	15.5	21.7	23.1	.320
Flulike illness	8.8	9.7	19.6	7.7	.195
Cough	30.9	26.2	56.5	53.8	.001
Sputum	20.4	16.5	39.1	46.2	.004
Dyspnea	30.9	28.2	41.3	38.5	.415
New radiologic infiltrate	16.6	14.6	13.0	23.1	.812
⩾1 symptom	59.1	64.1	84.8	92.3	.001
No symptom	40.9	35.9	15.2	7.7	.001
Suspicion of infection	28.2	22.3	45.6	30.8	.036
Suspicion of AR	40.3	64.1	56.5	84.6	<.001

NOTE. Data are percentage of cases, unless otherwise indicated.

[a] Calculated using the Fisher exact test.

of acute rejection. Impairment of lung function related to acute rejection was also significantly worsened by the presence of a viral infection (Figure 2). In patients with simultaneous acute rejection and lower respiratory tract viral infection, the FEV_1 recovery rate was significantly slower than in patients who had acute rejection without simultaneous viral infection ($P < .05$). We found that the OR for acute rejection was <1 in the presence of a viral infection for each of the 4 periods studied (OR for any period, 0.41; 95% confidence interval [CI], 0.20–0.88). The overall rate of viral infection in grade A0 and A1 cases was 25.4% (46 of 181), compared with 12.6% (13 of 103) in grade A2 or higher. Thus, patients with a lower acute rejection grade were twice as likely to be positive for viral infection than those with a higher rejection grade (OR, 2.0; 95% CI, 1.04–3.9). When repeating these analyses with the presence of virus in the upper respiratory tract as a predictor of acute rejection, we did not find an association between upper respiratory tract viral infection and acute rejection (OR, 1.11; 95% CI, 0.6–2.05). In a supplementary analysis, we verified the occurrence of acute rejection during a 30-day and 90-day period after baseline bronchoscopy. At 30 and 90 days, the probability of acute rejection was associated with the presence of acute rejection at baseline

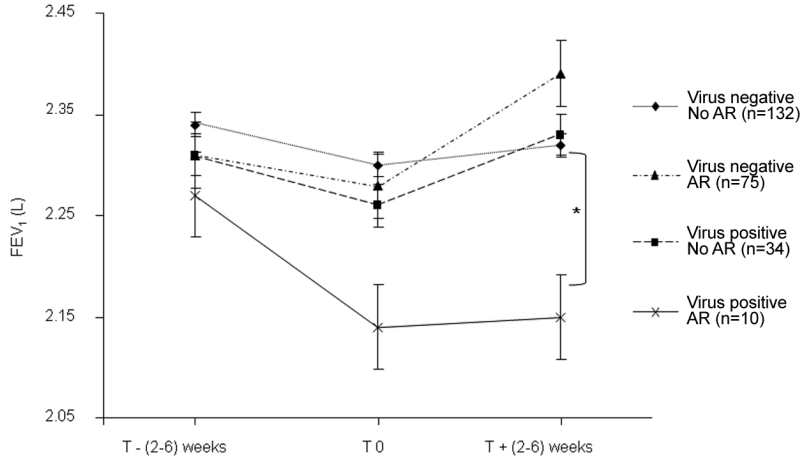

Figure 2. Lung function evolution according to the presence of acute rejection (AR) and/or positive viral nucleic acid detection in bronchoalveolar lavage fluid. Results are expressed at each time point as mean forced expiratory volume in 1 s (FEV_1) values ± standard errors, in liters. The FEV_1 values for the 3 time points were available for 251 (73.2%) of 343 cases. T − (2–6) weeks, FEV_1 baseline value measured 2–6 weeks before the event leading to bronchoscopy; T0, FEV_1 measured immediately before bronchoscopy; T + (2–6) weeks, FEV_1 value measured 2–6 weeks after bronchoscopy. *$P < .05$.

Table 5. Acute Rejection (AR) Rate 30 and 90 Days after Bronchoscopic Procedure According to the Presence of AR and/or Respiratory Virus in the Lower Respiratory Tract (Bronchoalveolar Lavage [BAL] Fluid)

Variable	AR rate, proportion (%)	P[a]
Rate at 30 days (n = 75)		.683
Virus negative		
AR positive	14/24 (58.3)	
AR negative	7/30 (23.3)	
Virus positive		
AR positive	3/6 (50.0)	
AR negative	5/15 (33.3)	
Rate at 90 days (n = 185)		.558
Virus negative		
AR positive	32/78 (41.0)	
AR negative	18/72 (25.0)	
Virus positive		
AR positive	6/12 (50.0)	
AR negative	7/23 (30.4)	

NOTE. The table includes 75 episodes for which a bronchoscopic procedure was performed within a 30-day period and 185 episodes for which a procedure was performed within a 90-day period. Patients with virus-positive BAL samples were as likely to be given diagnoses of AR at 30 and 90 days (odds ratio for 30 days, 1.22; 95% confidence interval, 0.46–3.24; odds ratio for 90 days, 1.30; 95% confidence interval, 0.62–2.69).

[a] Calculated using the Fisher exact test.

but not with the presence of virus (OR for 30 days, 1.22; 95% CI, 0.46–3.24; OR for 90 days, 1.30; 95% CI, 0.62–2.69) (Table 5).

DISCUSSION

Using an extensive panel of molecular assays, we were able to show that 17.2% of prospectively collected BAL fluid specimens from adult lung transplant recipients were positive for at least 1 respiratory virus. On the basis of a pretest clinical evaluation, patients testing positive were significantly more likely to present with respiratory symptoms (86.4%, compared with 60.9% with no identified virus). All analyzed individual symptoms were systematically more frequent in the presence of a respiratory virus, particularly cough and sputum, which were up to 3 times more common. Consistent with these observations, the pretest clinical evaluation performed by the physicians in charge considered that an infection was more likely in those with a final positive viral detection. Furthermore, lung function proved to recover significantly slower in the presence of a respiratory virus. All these findings corroborate that a positive association exists between positive viral nucleic acid detection in BAL fluid and the presence of an acute respiratory illness in lung transplant patients. This is also concordant with other investigations that have linked the detection of respiratory viruses by RT-PCR to symptoms [1, 2, 4, 6, 10, 13, 27]. However, our investigation differs from these studies by its prospective design, the large panel of respiratory viruses screened, the systematic use of BAL specimens, and the integration of pretest clinical evaluations performed by the physicians in charge. Many of the previous studies limited the detection strategy to upper respiratory tract specimens and used an array of molecular tools that was often restricted to a subgroup of viruses (eg, rhinoviruses, coronaviruses, or bocaviruses were not systematically tested). Finally, the link with pretest clinical conditions was not detailed in many of these reports.

Because of the availability of lung biopsy specimens in all cases, we were also able to assess the presence of acute rejection according to the presence of viral infection. A relationship between acute viral infection and subsequent acute rejection has been reported in small case series, but this possible association has not been appropriately confirmed. Viral infection might trigger a chain of immunologically mediated events, leading to subsequent rejection or lung dysfunction. This is important because several studies have identified viral infection as a distinct risk factor for the development of bronchiolitis obliterans syndrome and chronic graft dysfunction [1, 3, 4, 10, 12, 14, 17, 18]. Our investigation had the capacity to address this question because we were able to analyze a high number of lung biopsy specimens together with viral screening at the level of the lower respiratory tract. The biopsy analysis revealed that viral respiratory tract infections were not associated with simultaneous acute rejection. Furthermore, we could show that the probability of acute rejection did not increase 30 and 90 days after a lower airways respiratory viral infection. When present, however, viral infections caused more severe lung dysfunction and significantly hampered the short-term functional recovery in the case of concomitant acute rejection. Because the clinician in charge was masked to any viral result, a positive RT-PCR result did not modify the treatment of simultaneous acute rejection. Thus, a less aggressive treatment of acute rejection in the presence of a virus cannot explain the slower recovery of lung function in these patients. This suggests that respiratory viruses per se do not promote acute rejection during the acute phase but could certainly worsen lung function and impair recovery from acute rejection episodes. Paradoxically, we even observed a negative association between an episode of respiratory viral infection and the subsequent cumulative risk of developing acute rejection. This should not be considered a protective effect of viral infections; more likely, it is a lack of an association or a chance effect, and we refrain from drawing any other conclusions. However, this strongly suggests that when analyzing biopsy specimens according to reference guidelines [26], pathologists should not be misguided by the presence of a viral infection.

Of note, our study design limited follow-up to a few weeks, and we cannot exclude the possibility that the viral infection

triggered a chain of immunologically mediated events, leading to subsequent rejection or lung dysfunction. This is important because several studies have identified viral infection as a distinct risk factor for the development of bronchiolitis obliterans syndrome and chronic graft dysfunction [1, 3, 4, 10, 12, 14, 17, 18].

Our investigation also provided the unique opportunity to compare viral detection in the upper (pooled nasopharyngeal and oropharyngeal swabs) and lower respiratory tract in a large number of paired specimens. The positivity rate in the upper respiratory tract was 29.6%, compared with 16.6% in the lower tract. This difference in recovery rate is similar to that observed in other smaller studies [12], but, to the best of our knowledge, few or none of the previous investigations systematically analyzed paired specimens collected during the same procedure. An important observation is that when recovered only in the upper respiratory tract, respiratory viruses were less likely to be associated with lower respiratory symptoms. Another interesting observation is that only 7.0% of negative nasopharyngeal specimens were associated with a discordant positive BAL viral screening, thus suggesting a high negative predictive value. However, this value should be balanced with the relatively low prevalence of each individual respiratory virus in BAL fluid specimens and the possible technical issues related to nasopharyngeal and pharyngeal swabbing that could negatively affect the recovery rate. Our population was first selected on the basis of the need to perform a BAL procedure; for this reason, we caution that a lower respiratory tract viral infection in lung transplant recipients cannot be definitely ruled out by a nasopharyngeal swab. Yet this could be a reasonable initial screening strategy that needs to be confirmed in further studies and individually for each type of virus.

In conclusion, our study demonstrates that there is a temporal relationship in lung transplant recipients between the emergence of acute respiratory symptoms and positive respiratory viral nucleic acid detection in BAL fluid specimens. When detected only in the upper respiratory tract, viral infections are less likely to be associated with respiratory symptoms and graft dysfunction. We also provide solid evidence suggesting that respiratory viruses per se do not promote acute graft rejection, at least during the acute phase of infection, but that they do worsen graft function recovery when simultaneously present with acute rejection.

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Potential conflicts of interest. All authors: no conflicts.

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