

# Respiratory Viruses in Lung Transplant Recipients: A Critical Review and Pooled Analysis of Clinical Studies

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**Lung transplant recipients present an increased risk for severe complications associated with respiratory infections. We conducted a review of the literature examining the clinical relationship between viral respiratory infection and graft complications. Thirty-four studies describing the clinical impact of influenza, respiratory syncytial virus, parainfluenza, human metapneumovirus, rhinovirus, enterovirus, coronavirus, bocavirus or adenovirus were identified. The detection rate of respiratory viral infection ranged from 1.4% to 60%. Viruses were detected five times more frequently when respiratory symptoms were present [odds ratio (OR) = 4.97; 95% CI = 2.11–11.68]. Based on available observations, we could not observe an association between respiratory viral infection and acute rejection (OR = 1.35; 95% CI = 0.41–4.43). We found a pooled incidence of 18% (9/50) of bronchiolitis obliterans syndrome (BOS) in virus-positive cases compared to 11.6% (37/319) in virus-negative cases; however, limited number of BOS events did not allow to confirm the association. Our review confirms a causal relationship between respiratory viruses and respiratory symptoms, but cannot confirm a link between respiratory viruses and acute lung rejection. This is related in part to the heterogeneity and limitations of available studies. The link with BOS needs also to be reassessed in appropriate prospective studies.**

**Key words:** Acute rejection, bronchiolitis obliterans, influenza, lung transplantation, viral infection

**Abbreviations:** OR, odds ratio; BOS, bronchiolitis obliterans syndrome; RSV, respiratory syncytial virus; PIV, parainfluenza virus; HMpV, human metapneumovirus; LTRs, lung transplant recipients; PCR,

polymerase chain reaction; CI, confidence interval; ICU, intensive care unit; BAL, bronchoalveolar lavage; FEV<sub>1</sub>, forced expiratory volume in 1 sec.; OB, obliterative bronchiolitis.

**Received 12 October 2010, revised 14 January 2011 and accepted for publication 24 January 2011**

## Introduction

Respiratory viruses comprise different viruses, such as influenza, respiratory syncytial virus (RSV), parainfluenza (PIV), human metapneumovirus (HMpV), rhinovirus, enterovirus, bocavirus and adenovirus. Although most respiratory viral infections cause self-limited upper respiratory diseases, lung transplant recipients (LTRs) are particularly prone to develop complications (1–6). This is related to the immunosuppressive therapy that could promote protracted infection, but also to the direct exposure of the graft to the infectious agent together with an impaired mucociliary function and lymphatic drainage, and the absence of cough reflex. Apart from the direct infection-related morbidity, it is commonly accepted that these infections could promote rejection and subsequently lead to bronchiolitis obliterans syndrome (BOS), the main limitation to long-term survival. However, this association is based on reports that have focused mainly on paramyxoviruses or on influenza and adenovirus to a lesser extent (7). These studies are heterogeneous and have several technical limitations in terms of design, case selection and diagnostic procedures (1,8).

Over the years the improvement of molecular tools, including real-time PCR technology, has contributed to increase the sensitivity of our diagnostic procedures and new species (HMpV, coronavirus NL63 and HKU1, bocavirus, rhinovirus C) have also emerged. To assess appropriately the evidence supporting a role of respiratory viruses as a cause of symptoms and graft complications in LTRs, we conducted a systematic review of the published literature (9).

## Methods

We searched the MEDLINE database from 1 January 1985 to 31 March 2010 using the following key words: 'lung transplant recipients or immunocompromised hosts' and 'influenza, parainfluenza, RSV, metapneumovirus, coronavirus, bocavirus, adenovirus and respiratory viruses', respectively. In

addition, reference lists from review articles and selected papers were hand-searched and matched to our database. Only peer-reviewed original articles reporting at least three lung transplant cases with a description of virological methods, design and clinical end-points were included.

Data were collected in standardized report forms with the following information: year of the screening period; design (cohort, case series, retrospective, prospective); age and size of the population screened; number and type of specimens/viruses tested; number of virus-positive episodes analyzed; type of assays used; clinical conditions; association with acute rejection/chronic rejection/BOS and histopathological results; antiviral treatment and survival rate. The potential limitations and any other comments considered as relevant were noted.

We calculated confidence intervals (CI) around proportions for studies on viral frequency using the Agresti and Coull method. Odds ratios (OR) were calculated for each study to determine the association of respiratory viruses with acute rejection or respiratory symptoms. Due to significant heterogeneity between studies, we used random effect models to calculate meta-analytic summaries of the association between respiratory viruses and acute rejection or respiratory symptoms. All analyses were performed with STATA 11 (77845; College Station, TX, USA).

## Results

### **Main study characteristics**

We identified 34 studies; 26 focused on LTRs only and eight analyzed also other immunocompromised populations. Viruses considered in our review cover influenza A, B and C, RSV A and B, PIV 1–4, HMPV, rhinovirus, enterovirus, coronavirus 229E, OC43, NL63 and HKU1, bocavirus and adenovirus, but not herpes viruses. The main characteristics of the 34 studies (1–34) are presented in Table 1 and it can be estimated that more than 4000 specimens from LTRs have been screened for the presence of at least one of the above-mentioned respiratory viruses. In approximately one-third of studies (29%), screening was within the frame of prospective cohort studies investigating the cause and/or the clinical impact of acute viral respiratory tract infections; all others were retrospective or case series.

In 21 (61.7%) studies, patients were recruited from outpatient clinics; in six (17.6%), patients were hospitalized; one recruited both in- and outpatients and the recruitment setting could not be determined precisely for the remaining six (2,11,12,20,24,34). Diagnostic procedures were performed only in symptomatic patients in 12 (35.2%) studies, and in 20 (58.8%) they were also performed as routine posttransplant surveillance or as a control procedure after treatment of an acute rejection. Reasons for the procedure were not identifiable in two retrospective studies (2,27). An 8.8% of studies concerned children only, 17.6% enrolled both children and adults and 73.5% were in adults. Clinical conditions analyzed ranged from uncomplicated upper respiratory tract infection to severe pneumonia requiring intensive care unit (ICU) admission.

### **Viral investigations**

Overall, it was possible to identify that viral investigations were performed in upper respiratory specimens (nasopharyngeal swabs or aspirates) in 8.8% of studies and in bronchoalveolar lavage (BAL) specimens in 38.2%. Both types of specimens were used in the remaining 52.9% studies, but in 61.1% of these it was not possible to clearly establish the respective proportion of upper versus lower respiratory specimens. We identified only one study that compared systematically upper versus lower respiratory tract viral screening performed simultaneously in a given individual (28). Thus, we were unable to compare the respective sensitivity and role of viral screening in the upper versus the lower respiratory tract.

As expected, there was a significant heterogeneity of the different diagnostic procedures used. At least one molecular assay was used in 53% of investigations and only five studies used a large panel to target at least 12 of the above-mentioned 18 viruses. In the older studies, classical methods, such as immunofluorescence or viral culture, were the sole diagnostic tools. The type of technique used (immunofluorescence-based, culture or nucleic acid detection) and the completeness of the screening performed in each of the available studies selected are shown in Table 1. In terms of viral screening, influenza was screened in most studies (91% and 88% for influenza A and B, respectively, but only 3% for influenza C), followed by RSV (85%), PIV 1–3 (82–85%, but only 15% for PIV 4) and adenovirus (71%). Other respiratory viruses that require mainly molecular assays to be detected were less frequently screened; 41% for rhinovirus (rhinovirus C screened in only one study); 35% for HMPV; 11–24% for the different subtypes of coronaviruses; 18% for enterovirus and 12% for bocavirus. Overall, only 15% of studies screened at least 75% of the 18 respiratory viruses listed in Table 1. When assessable, the overall detection rate of respiratory viral infection in the screened population varied from 1.4% to 60%. This wide range can be explained in part by the heterogeneity of the population enrolled (asymptomatic cases versus subjects with limited upper respiratory symptoms versus patients hospitalized with complications). Table S1 depicts the prevalence of virus positivity for each individual study and as a pooled prevalence according to diagnostic method, number of viruses screened and sample size. As expected, the virus positivity rate was higher for studies with small sample size using PCR technique and screening for numerous viruses. For example, studies using PCR techniques had a higher detection rate (12.0%) compared to those not using PCR (1.4%). This can be explained in part by the greater number of viruses searched for by PCR techniques (8.4% for studies identifying nine or more viruses; 3.0% for those identifying eight or less viruses).

The respective contribution of each species in positive cases was available in 82% of studies. When a large panel of molecular tools was used, viruses most frequently detected were rhinovirus and coronavirus. In the three studies

**Table 1:** Main characteristics of 34 studies exploring the role of respiratory viruses in lung transplant recipients

Ref.	Publication year	First Author	Design	Period	Population <sup>1</sup>	Size of the LTRs population studied	Number of respiratory specimens tested in LTRs		Percent of positive episodes analyzed in LTRs* (CI 95%)	Diagnostic method	Influenza				RSV				Parainfluenza				Coronavirus															
							NA	NA			A	B	C	1	2	3	4	A	B	C	1	2	3	4	HMpV	OC43	22E	NL63	HKU1	Rhino	Entero	Boca	Adeno					
10	1995	Apaolachi AM	Retrospective case series	1985-92	C	NA	NA	NA	NA	Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
11	1997	Holt ND	Retrospective case series	1987-94	A	137	1820	1820	1.1 (0.7-1.7)	IF, Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
1	1998	Palmer SM	Retrospective case series	1992-97	A	122	NA	NA	NA	IF, Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
12	1998	Bridges ND	Prospective case series	1994-96	C	16	NA	NA	≥ 22 <sup>§</sup>	IF, Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
13	1998	Kinzman S	Retrospective case series	1993-94	A	NA	NA	NA	4	IF, Ag., Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
8	2001	Vilchez RA	Retrospective case series	1990-00	A	454	NA	NA	24	Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
14	2001	Garantziotis S	Retrospective case series	1997-00	A	3	NA	NA	3	IF, Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
15	2002	Weinberg A	Prospective case series	1999-00	A	93	116	NA	26.7 (19.5-35.4)	Ag., Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
16	2002	Vilchez R	Retrospective case series	1989-99	A	NA	NA	NA	39	Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
17	2002	Billings JL	Retrospective case series	1986-97	A/C	219	NA	NA	40 <sup>3,4</sup>	Ag., Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
18	2002	Vilchez RA	Retrospective case series	1990-00	A	454	NA	NA	19	Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
2	2003	McCurdy LH	Retrospective case series	1992-00	A	15	NA	NA	17 <sup>§</sup>	IF, Ag., Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
19	2003	Hopkins P	Prospective case series	2000	A	18	18	0	50.0 (29.0-70.9)	IF, Cult., Serol.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
7	2004	Khalifah AP	Retrospective case series	1996-02	A	228	NA	NA	21	IF, Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
4	2004	Garbino J.	Retrospective case series	1996-00	A <sup>5</sup>	NA	1001	1001	1.0 (0.5-1.9)	Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
3	2004	Garbino J.	Retrospective cohort study	2001-02	A	57	57	18	32.0 (21.0-44.0)	Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	2005	Sumino KC	Prospective case series	2002-03	A/C	426	270	2	0.7 (0.02-28.8)	IF, PCR, Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
21	2005	Larcher C	Prospective cohort study	2003-04	A/C	26	49	NA	24.4 (14.0-38.0)	IF, Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
22	2005	Kumar D	Prospective cohort study	2001-03	A	100	NA	0	37	IF, Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
23	2005	Glanville AR	Prospective case series	2002-04	A	NA	NA	0	18	IF, Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
5	2006	Milstone AP	Prospective cohort study	1999-00	A	50	49	5	17 <sup>§</sup>	Cult., PCR, Serol.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
24	2006	Gerna G	Prospective cohort study	2001-04	A	75	128	72	22.6 (16.0-30.6)	IF, PCR, Cult.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
25	2007	Dare R	Prospective cohort study	2004-05	A/C	114	234	234	6	Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
6	2008	Hopkins P	Prospective cohort study	2003-06	A	89	199	20	62 <sup>§</sup>	Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
26	2008	Miyakis S	Prospective cohort study	2007	A/C	53	86	86	4	IF, Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
27	2008	Ison MG	Retrospective cohort study	2004-05	A	77	NA	NA	NA	IF	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
28	2009	Soccal P	Prospective cohort study	2003-06	A	77	626	343	98 <sup>§</sup>	PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
29	2009	Gottlieb J	Prospective cohort study	2005-06	A	388	950	180	34 <sup>§</sup>	IF, Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Continued.

Table 1: Continued

Ref.	Publication year	First Author	Design	Period	Population <sup>1</sup>	Size of the LTRs population studied	Number of respiratory specimens tested in LTRs	Number of BAL screened in LTRs	Number of positive episodes analyzed in LTRs	Percent of positive test <sup>2</sup> (CI 95%)	Diagnostic method	Influenza A B C	RSV A B	Parainfluenza 1 2 3 4	HMpV	OC43	22E	NL63	Coronavirus HKU1	Rhino	Entero	Boca	Adeno
30	2009	Gerna G	Prospective cohort study	2006-07	AC	67	108	84	22 <sup>3</sup>	20.3 (13.7-28.9)	IF, PCR	+	+	+	+	+	+	-	-	+	+	+	+
31	2009	Liu M	Retrospective multicenter study	1988-07	C	576	NA	NA	101 <sup>3</sup>	NA	IF, Cult., PCR	+	+	+	-	-	-	-	-	+	+	-	+
32	2009	Costa C	Prospective case series	2007-08	A	18	58	58	7	12.0 (5.6-23.1)	Cult., PCR	+	+	+	+	+	+	+	+	+	+	+	+
9	2009	Pelaez A	Retrospective case series	2005-07	A	NA	NA	NA	5	NA	IF, Cult.	-	-	-	-	-	-	-	-	-	-	-	-
33	2009	Liu V	Retrospective case series	2006-07	A	NA	NA	NA	30 <sup>3</sup>	NA	Cult.	+	+	+	+	+	+	+	+	+	+	+	+
34	2010	Kumar D	Prospective case series	2003-05	A	93	582	582	81 <sup>3</sup>	13.9 (11.0-16.9)	IF, PCR, Cult.	+	+	+	+	+	+	+	+	+	+	+	+

Note: Grey cells indicate viruses that were screened and for which clinical data are provided. Ref., reference; PCR, Detection based on nucleic acid amplification (RNA or DNA); Ag, Viral antigen detection by rapid test or other similar techniques; IF, Viral detection by different direct or indirect immunofluorescence or ELISA or other similar methods; Cult, Viral isolation on cell culture; Rhino, Enterovirus, Adenovirus, bocavirus and adenovirus, respectively. <sup>1</sup>A = adult, C = children (<18 years old). <sup>2</sup>Number of positive episodes/number of specimens tested in lung transplant recipients (LTRs). <sup>3</sup>In these studies, one case is defined as one episode (one given LTR can experience multiple episodes). <sup>4</sup>This report includes 18 cases reported in a first paper by Wendt et al. in 1995. <sup>5</sup>In this study, children are <16 years old. & Positive episode refers to positive for respiratory RNA virus, adenovirus or bocavirus in upper and/or lower respiratory tract specimens. Herpes viruses (cytomegalovirus, herpes simplex virus and varicella zoster virus) are not reported.

that screened at least 14 respiratory viruses (22,28,34), rhinovirus represented 35–55% of all positive cases and coronaviruses 13–27%. When including three supplementary studies screening up to 12 or 13 viruses (3,24,29), the most frequent virus detected was still rhinovirus (8.8–55.5%; Table S1). Of note, for some targets, such as coronaviruses, not all species (OC43, E229, NL63, HKU1) were included.

**Respiratory symptoms and lung function**

Ten of 34 studies compared the rate of viral infections observed in symptomatic cases versus those without respiratory symptoms. We found that smaller studies tended to include more symptomatic patients and that larger studies were associated with a lower virus detection rate (22.3% for studies with less than 150 specimens versus 0.6% for studies with 150 specimens or more; Table S1).

Figure 1A highlights that in all but one study, the association between laboratory-proven respiratory viruses and symptoms was present. We found that viruses were detected five times more frequently when respiratory symptoms were present (OR = 4.97; 95% CI = 2.11–11.68). In terms of objective assessment of the graft function during the acute phase, lung function assessment was available in 53% of studies and showed a forced expiratory volume (FEV<sub>1</sub>) decline that ranged from –5% to –30% for the overall enrolled population. The FEV<sub>1</sub> decline was usually similar or even more important among symptomatic patients, but very few studies provided a specific comparison of FEV<sub>1</sub> variability according to the presence or absence of a viral illness (28), which prevented further analysis.

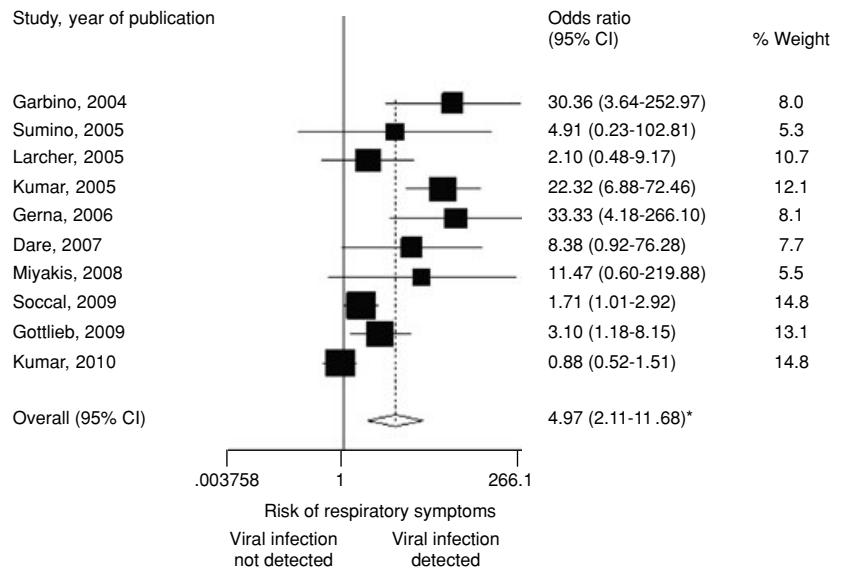
**Outcome and antiviral interventions**

Short-term crude mortality rate was evaluated in 52.9% of studies and ranged from 0% to 25%. Antiviral treatment was used in 53% of studies, mostly ribavirin for RSV infection, but also neuraminidase inhibitors and amantadine for influenza infection. Of these studies, 72% discussed treatment efficacy, but only 28% considered treatment efficacy as an end-point. Based on the clinical outcome of treated subjects, it is reported that early antiviral therapy might be associated with a reduction of complications and mortality. Nevertheless, given the small number of cases, the lack of randomization and appropriate control groups, and the absence of analysis reporting a precise rate of reduction in mortality and/or morbidity, these trends could only be considered as non evidence-based conclusions.

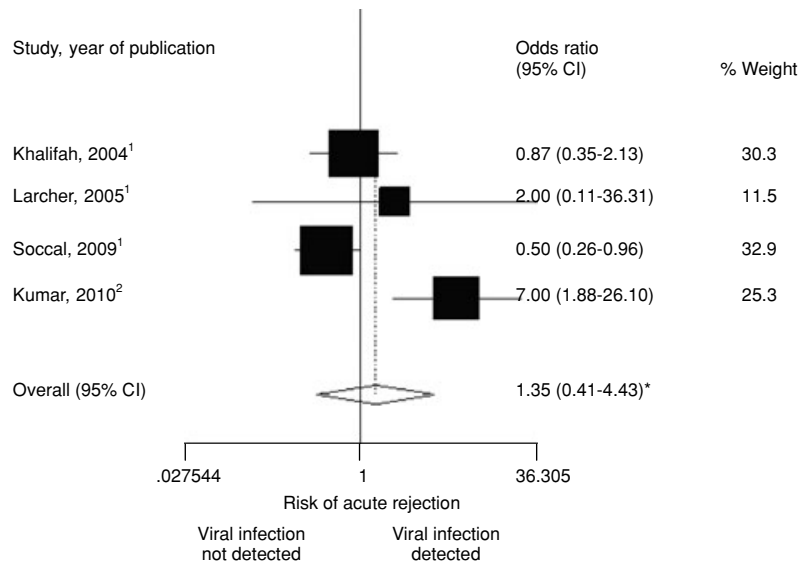
**Graft rejection**

Twenty-five of 34 studies representing more than 2900 LTR specimens reported that transbronchial lung biopsies had been performed and a total of 923 pathological examinations were potentially available. However, the presence of acute rejection or obliterative bronchiolitis (OB) was reported only in 68% and 2.6% of cases, respectively. Among a total of 282 virus-positive and 553 virus-negative cases, 21 (61.8%) studies reported histopathological

**A**



**B**



**Figure 1: Odds ratio of (a) respiratory symptoms and (b) acute graft rejection according to the presence or absence of respiratory viral infections in lung transplant recipients.**

<sup>1</sup>Biopsy-proven.

<sup>2</sup>Biopsy-proven or FEV<sub>1</sub> decline >/= 20%.

\*Random effect.

results and an acute rejection rate. Three studies were not suitable for the present analysis, thus leaving 19 studies reporting a total of 267 acute rejection events graded  $\geq$  A2. In these 19 investigations, the frequency of acute rejections  $\geq$  A2 ranged between 5.9% and 47.6% (Table S4). The association with acute rejection can only be estimated by comparing the rate observed in virus-positive cases with the one observed in virus-negative cases; this was available in only four studies (7,21,28,34). One study suggests a significant positive association (34), which could not be confirmed in the three others (Figure 1B). Overall, we found no statistically significant association between respiratory

viruses and acute rejection (OR = 1.35; 95% CI = 0.41–4.43). OB/BOS incidence following respiratory viral infections was reported for a period of time ranging from a few months to 1 year. In 11 studies (32.5%), all except one (1) used either biopsy-proven chronic rejection (defined by the presence of OB) or a sustained FEV<sub>1</sub> decline of 20% according to the International Society for Heart and Lung Transplantation guidelines (35). BOS incidence (Table 2) following a respiratory viral infection ranged from 5.4% to 62.5% in virus-positive cases and was reported in only three studies for virus-negative cases (5,21,29) with a rate ranging from 9.1% to 52.9%. Pooled incidence rates

**Table 2:** Summary of studies analyzing the potential association between new onset of bronchiolitis obliterans syndrome and/or obliterans bronchiolitis and respiratory viral infections in lung transplant recipients

Reference	Virus-positive cases (n = 201)		Virus-negative cases (n = 757)		Type or number of viruses considered for this analysis	Statistical analysis if available
	Total cases	No. (%) with BOS	Total no. of cases	Number (%) with BOS		
1	10	4 (40.0)	NA	NA	8	NA
12	9	4 (44.4)	NA	NA	Adenovirus only	Cox proportional hazards p <0.0001
8	22	7 (32.0)	NA	NA	PIV only	NA
14	3	3 (100.0)	NA	NA	Influenza only	NA
7	21	13 (62.0)	207	NA	8	p = 0.27, 0.02 and 0.01 for BOS 1, 2 and 3, respectively
21	9	2 (22.2)	17	9 (52.9)	HMpV only	NA
5	15	1 (6.7)	28	3 (10.7)	8	p value = non significant
6	37	2 (5.4)	NA	NA	HMpV and RSV only	NA
29	26	6 (23.0)	274	25 (9.1)	13	Rate of BOS higher among CARV-positive group (Kaplan-Meier curve; p = 0.01)
34	16 <sup>1</sup>	10 (62.5)	45	NA	16	NA
Pooled cases	50	9 (18)	319	37 (11.6)		p = 0.242
17 <sup>2</sup>	33	NA	186	NA	7	Previous CARV infection does not predispose to OB/BOS (relative risk 1.1; 95% CI 0.52–2.3) <sup>3</sup>

CARV, community-acquired respiratory viruses; BOS, bronchiolitis obliterans syndrome; OB, obliterans bronchiolitis.

<sup>1</sup>The analysis focuses on 16 virus-positive cases initially diagnosed with acute rejection at 3 months.

<sup>2</sup>Statistical analysis performed, but number of BOS cases not provided.

<sup>3</sup>In a subset analysis, lower CARV infection predisposes to BOS3 (Cox proportional hazards regression model; RR 2.3, 95% CI 1.1–4.9).

of these three studies revealed a BOS incidence of 18% (9/50) in virus-positive cases compared to 11.6% (37/319) in virus-negative cases. The low number of BOS events analyzed in these three investigations limited our ability to provide any meta-analytic summary that could be considered as relevant. Four of the 11 studies (Table 2) provided a statistical analysis testing the potential association with BOS, but two without providing clearly the BOS rate in virus-negative cases. One (17) failed to show any significant association and three (7,12,29) described a significant higher rate of BOS in subjects experiencing a respiratory viral infection.

## Discussion

During seasonal peaks, LTRs living in the community are exposed to RNA and DNA respiratory viruses. Given the concomitant presence of a significant immunosuppression and impaired protective mechanisms of the grafted lung, these viral infections will promote complications and graft rejection (7,21,22,34). In the present review of 34 studies, our goal was to assess the strength and the characteristics of this association in available clinical reports and whether this translates into an observable association in real-life conditions. Incomplete microbiological investigations or insensitive diagnostic tools limited the completeness of viral investigations; only the most recent reports have used a

large panel of molecular tests and can provide a less biased image of the respective role of each viral agent. In the early 1990s, studies used mainly viral culture or direct immunofluorescence and, if available, PCR was limited to influenza, RSV or parainfluenza viruses. The recent emergence of new viruses such as HMpV, coronavirus NL63, coronavirus, HKU1, bocavirus and human rhinovirus C need to be included in any modern molecular panel; these new agents have been systematically studied in two studies only. Interestingly, when tested, the so-called 'common cold' viruses like rhinoviruses and coronavirus revealed to be the most frequent compared to others such as influenza or paramyxoviruses, an observation consistent with other hospital-based studies. Depending on the type and number of technique used, the size of the study or enrolment criteria, the observed frequency of viral infections can dramatically change—ranging for each individual virus from less than 1% to more than 20% in our pooled analysis (Table S1).

The clinical significance of a positive viral nucleic acid detection result is a critical point that needs to be confronted with the presence or absence of respiratory symptoms. This type of analysis has been done in at least 11 studies (Table S3 and Figure 1A) in which LTRs submitted to a routine respiratory screening for graft follow-up were used as controls and compared to symptomatic cases. It was consistently shown that in the presence of a viral

infection, the likelihood of respiratory symptoms was five times higher. This observation could guide clinicians in their interpretation of microbiological results in an era where increasingly sensitive molecular diagnostic panels are available. Even if a background positivity rate is expected, for example, following unnoticed or asymptomatic infection, or when seasonal outbreaks are ongoing in the community, these viruses likely contributed to symptom production in most cases and cannot be regarded as innocent bystanders (3,22,28,29).

Although mainly expected in the upper respiratory tract, viral infections are also present in lower respiratory specimens. This raises several issues such as the respective ability of each respiratory virus to infect the lower respiratory tract and whether all of them should be considered as equally able to cause graft complications in LTRs. Despite being expected, our pooled analysis was unable to confirm a positive association between acute rejection and a previous viral infection. However, this conclusion needs to be considered carefully since the three largest studies (7,28,34) representing 96% of all cases brought discordant results; two of these failed to observe a positive association (7,28), whereas a third (34) reported a 33.3% rejection rate in 48 virus-positive cases compared to 6.7% in virus-negative cases ( $p$  value = 0.001). Of note, this latter study considered not only biopsy-proven cases as rejection criteria, but also a FEV<sub>1</sub> decline of 20% or more. Another potential limitation of our pooled analysis is related to the heterogeneity of the design of each study: some reported an acute rejection rate during the acute phase of the viral infection and others during a follow-up period of 3 months. Although the present report focuses on respiratory viruses, it must also be kept in mind that these agents could be associated, or promote other bacterial or fungal infections that subsequently could lead to graft complications.

With regard to chronic rejection, a relationship between a previous respiratory viral infection and the subsequent development of BOS was reported as statistically significant in three studies (14,24,41). In at least seven other studies in which BOS incidence was evaluated or discussed, the risk could not be linked to respiratory viruses or was not evaluable. The median number of virus-positive cases in the 10 studies in which BOS was analyzed was five (range 1–13). Four of these studies compared the rate in virus-positive versus negative cases for a total of only nine BOS events in those virus-positive cases (Table 2). The low number of events, incompleteness, heterogeneity and the retrospective design of published reports, did not allow us to conduct any appropriate statistical analysis. Of note, some studies have suggested that selected viruses, such as RSV, PIV, influenza and possibly (7,21,22,34) HMPV (6,21), are particularly prone to trigger graft rejection. In most of these studies, control groups were incomplete and thus again the clinical relationship between one specific respiratory viral family and graft rejection needs to be reconsidered carefully.

In conclusion, our review confirms a causal relationship between respiratory viral infections and respiratory symptoms, even when these infections are documented by molecular assays. However, the respective role of each respiratory virus, especially with respect to picorna- and coronavirus, needs to be reconsidered. Although it is certain that lower respiratory viral infection will promote graft complication, we highlight that the clinical link between respiratory viruses and acute lung rejection or BOS needs to be characterized in prospective and appropriately designed cohort studies.

## Acknowledgments

This work was supported by a grant of the Swiss National Science Foundation attributed to L. Kaiser (3200B-101670).

## Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Table S1:** Frequency of viral infections observed in lung transplant recipients according to individual study characteristics

**Table S2:** Range of virus detection per family in the 6 studies where at least 12 viruses were screened

**Table S3:** Relative proportion of virus-positive cases according to the presence of respiratory symptoms in lung transplants recipients screened for respiratory viruses

**Table S4:** Summary of studies analyzing the potential association between acute rejection and respiratory viral infections in lung transplant recipients

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