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MAJOR ARTICLE

Community-acquired Respiratory Viruses Are a Risk Factor for Chronic Lung Allograft Dysfunction

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Background. The relationship between community-acquired respiratory viruses (CARVs) and chronic lung allograft dysfunction (CLAD) in lung transplant recipients is still controversial.

Methods. We performed a prospective cohort study (2009–2014) in all consecutive adult patients (≥18 years) undergoing lung transplantation in the Hospital Universitari Vall d'Hebron (Barcelona, Spain). We systematically collected nasopharyngeal swabs from asymptomatic patients during seasonal changes, from patients with upper respiratory tract infectious disease, lower respiratory tract infectious disease (LRTID), or acute rejection. Nasopharyngeal swabs were analyzed by multiplex polymerase chain reaction. Primary outcome was to evaluate the potential association of CARVs and development of CLAD. Time-dependent Cox regression models were performed to identify the independent risk factors for CLAD.

Results. Overall, 98 patients (67 bilateral lung transplant recipients; 63.3% male; mean age, 49.9 years) were included. Mean postoperative follow-up was 3.4 years (interquartile range [IQR], 2.5–4.0 years). Thirty-eight lung transplant recipients (38.8%) developed CLAD, in a median time of 20.4 months (IQR, 12–30.4 months). In time-controlled multivariate analysis, CARV-LRTID (hazard ratio [HR], 3.00 [95% confidence interval {CI}, 1.52–5.91]; $P = .002$), acute rejection (HR, 2.97 [95% CI, 1.51–5.83]; $P = .002$), and cytomegalovirus pneumonitis (HR, 3.76 [95% CI, 1.23–11.49]; $P = .02$) were independent risk factors associated with developing CLAD.

Conclusions. Lung transplant recipients with CARVs in the lower respiratory tract are at increased risk to develop CLAD.

Keywords. bronchiolitis obliterans; chronic rejection; lung transplantation; respiratory virus; viral infection.

In the past 2 decades, lung transplantation has witnessed substantially improved short-term graft survival. In contrast, survival beyond the first year remains severely affected by chronic lung allograft dysfunction (CLAD), with limited treatment options [1]. CLAD developed in 50% of primary adult lung transplant recipients within 5 years of transplantation and 76% by 10 years posttransplant [2]. It is the leading cause of mortality after the first year, accounting for 20%–30% of deaths [3]. The pathophysiology of CLAD is thought to involve a complex interplay between the lung allograft, antidonor immunity, and environmental stimuli including infections that elicit direct nonimmunological and indirect immune effects [4–7]. The most consistent data on the long-term effect of infectious diseases on allograft dysfunction come from viral infections, especially those involving herpesviruses [5]. However, the role of community-acquired respiratory viruses (CARVs) on lung transplant outcomes is still controversial, which is partly due to the fact that CARV infections have not been comprehensively assessed due to several technical limitations in terms of design, case definition, and diagnostic procedures [8–10]. As a potentially modifiable risk factor, a better understanding of the relationship between CARVs and CLAD is extremely important and might improve outcomes for lung transplant recipients (LuTRs), as exposure can be minimized and vaccines and antivirals covering CARVs other than influenza will become available in the near future [5, 11]. The aim of this study was to characterize the potential association between CARVs and CLAD using comprehensive state-of-the-art multiplex nucleic acid testing (NAT) for 16 commonly circulating respiratory viruses (CARVs) for asymptomatic seasonal surveillance as well as for clinically symptomatic events in the long-term follow-up of a LuTR prospective cohort.

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METHODS

Study Setting and Patient Population

The details of this prospective LuTR cohort have been provided previously [12]. In short, the cohort included all consecutive adult patients (≥18 years) undergoing lung transplantation in the Hospital Universitari Vall d'Hebron (Barcelona, Spain) from September 2009 to September 2011. The patients were followed from the first day of hospital discharge posttransplantation until September 2014 or death, whichever occurred first.

Data were collected in a database with the following information: demographic characteristics, baseline disease, pretransplantation data (1 month before transplantation), type of transplant, intraoperative and postoperative variables, and episodes of infections. Data concerning the clinical courses of the patients were prospectively collected during the patients' clinical follow-ups. All data were prospectively entered from patients' medical records in the hospital, microbiology, and histopathology databases using a standardized protocol including bacterial and fungal infections, cytomegalovirus (CMV) testing, and lung allograft biopsy results. The surveillance strategy for performing bronchial biopsies to detect acute rejection (AR), respiratory cultures (for *Pseudomonas aeruginosa* and *Aspergillus* species), and blood CMV loads were based on routine screening or clinician's judgment for diagnosis of clinical events (Supplementary Materials). Data concerning the clinical courses of the patients were prospectively collected during the patients' clinical follow-ups. Details on immunosuppression and prophylaxis protocols are located in the Supplementary Materials.

This study adhered to the principles of the Declaration of Helsinki, formulated by the World Medical Association, and the ethical statement of the International Society for Heart and Lung Transplantation. Informed consent was obtained from all patients prior to their inclusion in the study.

Respiratory Virus Monitoring

Systematic collection of nasopharyngeal swabs (NPSs) was performed in all LuTRs in different settings according to a previously established protocol. These included asymptomatic patients during seasonal changes (around calendar-based seasonal changes at spring, summer, autumn, and winter) in order to study different CARVs according to the season and patients with upper respiratory tract infectious disease (URTID), lower respiratory tract infectious disease (LRTID) and biopsy-proven AR. Furthermore, NPS collection was also performed 1 month and 3 months after URTID. The systematic collection of NPSs was performed during the entire follow-up period of the study.

Moreover, patients were closely followed up by phone calls every week to check any possible respiratory tract infection episode. In cases of new-onset symptoms (fever, sore throat, rhinorrhea, dyspnea, cough, sputum, myalgia, fatigue, thoracic pain), patients were instructed to contact the research team to schedule a prompt visit (<24 hours) at the outpatient clinic or to go to the emergency room.

Respiratory Tract Infectious Disease Definitions

URTID was defined as an illness caused by an acute infection with the onset of sore throat, rhinorrhea, or hoarseness. LRTID included tracheobronchitis and pneumonia. LRTID was defined as a new onset of shortness of breath, cough, sputum, rales, hypoxemia, and/or wheezing. Pneumonia was distinguished from tracheobronchitis if LTRID was associated with a new pulmonary infiltrate on chest radiograph or chest computed tomography [13, 14].

Follow-up and Outcome

Spirometry was performed at discharge after lung transplantation, at routine follow-up visits for monitoring lung allograft function (monthly during first year after lung transplant, followed by 4 annual spirometries), and at any time if clinically indicated. All of the pulmonary function tests were performed in our center following the European Respiratory Society and American Thoracic Society guidelines [15]. Changes in allograft function were reported as the difference in forced expiratory volume in 1 second (FEV_1). Values were expressed as absolute values and the percentage change according to the baseline value. Baseline was defined as the average of the 2 best posttransplant values for FEV_1 and forced vital capacity obtained at least 3 weeks apart [16].

CLAD includes bronchiolitis obliterans syndrome (BOS), restrictive allograft syndrome (RAS), and an overlap syndrome (BOS and RAS). BOS was defined as a persistent (≥3 weeks) FEV_1 drop of 10% or more compared to baseline, with or without histological findings consistent with bronchiolitis obliterans, and other causes of pulmonary dysfunction were excluded. RAS was defined as a persistent decline in forced vital capacity and total lung capacity that is accompanied by a decline in FEV_1 of >20% [16]. The date of CLAD diagnosis was reviewed by 3 study investigators who were part of a clinical lung transplant pulmonologist panel, and results were based on the full consensus of the investigators. Data from each study's patient were independently reviewed by 3 of these investigators. The reviewers were asked to assign the date of diagnosis and type of CLAD based on previously described criteria [16]. Results were based on full consensus among the experts.

Statistical Analysis

To evaluate the potential association of CARV infection and development of CLAD, time-controlled univariate and multivariate models were constructed for events that occurred at different times after transplantation to avoid assignment of risk to subjects before their occurrence. Relevant variables reported as related to CLAD were included for the univariate analysis: AR [17], primary graft dysfunction [18], gastroesophageal reflux disease [19], colonization/infection by *Aspergillus* species

[20], colonization/infection by *P. aeruginosa* [21, 22], and CMV pneumonitis [23]. CARV infection was analyzed as time-dependent risk factor for a 3-month risk period. A 3-month primary endpoint was chosen because it was hypothesized that adverse clinical events occurring soon after viral infection (ie, within 3 months) were more likely to be associated with that infection than events occurring later after [24]. Other variables were analyzed as a time-dependent risk factors for the entire duration of follow-up. Statistically significant variables ($P < .05$) in the univariate analysis were introduced in a multivariate model by use of forward stepwise time-dependent Cox regression model to identify the independent risk factors for CLAD. A P value < .05 was considered significant. Data analyses were performed with Stata 11.2 (StataCorp, College Station, Texas).

RESULTS

Patients' Baseline Characteristics and Epidemiology of CARV Infections

Ninety-eight patients (67 bilateral, 31 single LuTRs) were included, with a mean postoperative follow-up of 3.4 years (interquartile range [IQR], 2.5–4.0 years). The mean age of patients was approximately 50 years, and 63.3% (n = 62) were male. The main baseline diseases leading to lung transplantation were chronic obstructive lung disease (34.7%) and idiopathic pulmonary fibrosis (30.6). The main epidemiological and clinical data of included patients are shown in Table 1.

The epidemiology of CARV infections in this cohort has been previously described [12]. A total of 1094 NPSs were collected, and NPSs tested positive for CARVs in 64.7% (97/150) of patients with URTID, 51.8% (56/108) with tracheobronchitis, 26.4% (9/34) with pneumonia, and 11.5% (68/591) with asymptomatic status. Positivity rates of systematically collected NPS results are shown in Table 2.

Table 1. Demographic Data and Patient Characteristics

Variable	Result
Patients, n	98
Age, y, mean ± SD	49.9 ± 12.6
Male sex	62 (63.3)
Pretransplant diagnosis	
COPD	34 (34.7)
Idiopathic pulmonary fibrosis	30 (30.6)
Cystic fibrosis	12 (12.2)
Primary pulmonary hypertension bronchiectasis	7 (7.1)
Bronchiectasis	4 (4)
Other	11 (11.2)
Transplant type	
Bilateral	67 (68.4)
Single	31 (31.3)
Induction regimen	
Basiliximab	3 (3.1)
Steroids	98 (100)

Data are presented as no (%) unless otherwise indicated.

Abbreviations: COPD, chronic obstructive pulmonary disease; SD, standard deviation.

Table 2. Positivity Rates of Systematically Collected Nasopharyngeal Swabs in Different Clinical Settings

Event	No. (%) of Positive Samples	Patients, N
Asymptomatic	68/591 (11.5)	93
URTID	97/150 (64.7)	69
1 mo after URTID	16/103 (15.5)	60
3 mo after URTID	8/78 (10.2)	50
LRTID, tracheobronchitis	56/108 (51.8)	61
LRTID, pneumonia	9/34 (26.4)	24
Acute rejection	4/30 (13.3)	25
Total	258/1094 (23.6)	98

Abbreviations: LRTID, lower respiratory tract infectious disease; URTID, upper respiratory tract infectious disease.

Overall, picornaviruses (108/234 [46.2%]) were the most frequently encountered CARVs, followed by coronaviruses (46/234 [19.7%]), influenza virus (28/234 [12.0%]), parainfluenza virus (20/234 [8.5%]), and human metapneumovirus (18/234 [7.7%]). Tracheobronchitis was mainly caused by picornavirus (22/56 [39.3%]), followed by influenza virus (9/56 [16.1%]), coronavirus (8/56 [14.3%]), and human metapneumovirus (8/56 [14.3%]); pneumonia was associated mainly with respiratory syncytial virus (3/9 [33.3%]), parainfluenza virus (2/9 [22.2%]), and influenza virus (2/9 [22.2%]).

Association Between CARVs and CLAD

During the study period, a total of 38 LuTRs (38.8%) developed CLAD, consisting of 33 diagnoses of BOS (86.8%), 2 cases of RAS (5.3%), and 3 mixed forms (7.9%). The median time until CLAD diagnosis was 20.4 months (IQR, 12–30.4 months). During the study period, overall mortality was 27.6% (27/98) and was CLAD related in about half of the cases (13/27 [48.1%]).

Univariate analysis of predictors for CLAD revealed that AR (hazard ratio [HR], 3.21 [95% confidence interval {CI}, 1.67–6.17]; $P < .001$), primary graft dysfunction (HR, 2.31 [95% CI, 1.00–5.31]; $P = .04$), positivity for CARVs in general (HR, 2.37 [95% CI, .98–5.75]; $P = .05$), CARV-LRTID (HR, 2.96 [95% CI, 1.51–5.78]; $P = .001$), and CMV pneumonitis (HR, 7.34 [95% CI, 1.66–32.43]; $P = .008$) were significant risk factors (Table 3). In the time-dependent Cox regression multivariate analysis, CARV-LRTID (HR, 3.00 [95% CI, 1.52–5.91]; $P = .002$), AR (HR, 2.97 [95% CI, 1.51–5.83]; $P = .002$), and CMV pneumonitis (HR, 3.76 [95% CI, 1.23–11.49]; $P = .02$) remained as independent risk factors associated with development of CLAD (Table 3). Different CARVs did not exert different effects on CLAD development, or were associated with different CLAD phenotypes (BOS, RAS, mixed BOS/RAS).

DISCUSSION

The results of this prospective study indicate that CARV infections play a significant role as an independent risk factor for CLAD next to acute rejection and CMV pneumonitis. These

Table 3. Risk Factors for Chronic Lung Allograft Dysfunction in Lung Transplant Recipients: Results From Univariate and Multivariate Time-dependent Cox Regression Models

	Univariate Analysis		Multivariate Analysis	
Variable	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Acute rejection	3.21 (1.67–6.17)	.001	2.97 (1.51–5.83)	.002
Primary graft dysfunction	2.31 (1.00–5.31)	.049	…	…
Gastroesophageal reflux	1.50 (.78–2.89)	.224	…	…
Aspergillus spp colonization-infection	1.34 (.61–2.96)	.466	…	…
P. aeruginosa colonization-infection	1.74 (.90–3.34)	.098	…	…
P. aeruginosa de novo colonization-infection	1.22 (.63–2.36)	.558	…	…
CMV pneumonitis	7.34 (1.66–32.43)	.008	3.76 (1.23–11.49)	.020
Any respiratory virus (+)	2.37 (.98–5.75)	.056	…	…
Asymptomatic respiratory virus (+)	0.89 (.42–1.89)	.758	…	…
LRTID respiratory virus (+)	2.96 (1.51–5.78)	.001	3.00 (1.52–5.91)	.002

C statistic = 0.62.

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; HR, hazard ratio; LRTID, lower respiratory tract infectious disease; *P. aeruginosa*, *Pseudomonas aeruginosa*.

results are important and have been intuitively suspected in the past [8], but were clearly revealed through a combination of state-of-the-art multiplex NAT together with a very tight longer-term clinical follow-up of >3 years in our study. Indeed, the association between CLAD and CARV has been discussed in the literature, but an earlier meta-analysis remained inconclusive in this regard [8]. Nevertheless, a retrospective study from Fisher et al performed in a large LuTR population (250 patients) using modern molecular diagnostic techniques reported that symptomatic CARV infections defined as LRTID in our study were independently associated with CLAD within a short time period [9]. Similarly, Allyn et al retrospectively reviewed CARV infections in 563 patients who underwent lung transplantation between 2000 and 2013 and found that only viral pneumonia increased the risk of both CLAD and graft loss after lung transplantation [10]. In these 2 studies, the CARV tests were requested according to the treating physician and there was not a study protocol to compare the impact of CARVs in asymptomatic patients. A prospective cohort of LuTRs who underwent a protocolized testing of CARVs during the first year of transplantation has been recently published [25]. CARV detection during the first year was independently associated with CLAD in this study. However, there was no differentiation between symptomatic and asymptomatic detection of CARVs in the multivariate model. The strengths of our report reside in the prospective design of the study, the extensive assessment of both symptomatic and asymptomatic LuTRs throughout all seasons during a long-term follow-up, and the complete evaluation of other infectious and noninfectious factors that have been linked to CLAD [8–10], enabling us to independently associate CARV-LRTID, as well as CMV pneumonitis, and AR with CLAD in lung transplantation.

It is known that some CARVs have a greater propensity to produce LRTID such as respiratory syncytial virus, parainfluenza, influenza, and human metapneumovirus, which appear particularly potent to trigger CLAD [8]. In our study, no such distinction was observed, emphasizing that the pathologic changes associated with symptomatic LRTID are possibly more important, and also includes presumably less pathogenic CARVs such as picornavirus and coronaviruses [26, 27]. Attachment of CARVs to the bronchial epithelium and subsequent direct cytopathic effect on the respiratory epithelium followed by the release of inflammatory factors/cytokines may enhance the alloimmune response toward the airway epithelium and/or microvasculature leading to chronic lung allograft rejection [28, 29]. Thus, the clinical presentation of LRTID (tracheobronchitis or pneumonia) should alert physicians to initiate a broad diagnosis for AR and CMV as well as multiplex NAT for CARV infections.

The efficacy of intravenous immunoglobulin, monoclonal antibodies, and augmented corticosteroid therapy alone or in combination is not established [11, 30]. Thus, the current study also provides a rationale to develop novel therapeutic options including antivirals to avoid subsequent development of CLAD in the future [31–33]. Such therapies might not only modify the acute URTID episode, but also prevent LRTID and reduce the rate of CLAD. In a phase 2b randomized clinical trial, respiratory syncytial virus (RSV)–infected LuTRs were randomized to aerosolized ALN-RSV01 vs placebo and there was a trend toward a decrease in BOS (13.6% vs 30.3%; $P = .058$) at 180 days [34]. Similarly, our results suggest that maximizing prevention measures against viral infections in LuTRs is mandatory. This includes lifelong seasonal influenza vaccination for LuTRs and the development of further CARV vaccines [35, 36].

Multiplex NAT is a highly sensitive technique and has greatly improved the detection of CARVs [37]. However, the sensitivity of multiplex NAT also complicates clinical interpretation, as the presence of small amounts of viral targets may not necessarily have clinical relevance [38]. In a previous prospective study, an association was identified between

a positive test for CARV and CLAD, regardless of the presence of symptoms [39]. Instead, our results are in line with a recent retrospective study [9], demonstrating in a prospective setting that symptomatic rather than asymptomatic CARV infections were of relevance for CLAD development. Most likely, the underlying pathophysiology reflects the severity of damage caused by the extent of cytopathic virus replication and innate immune responses initiating heterologous immunity [40].

Our study has some limitations. First, identifying the exact time of CLAD diagnosis is difficult in clinical practice. To address this concern, a consensus investigation panel of experts was part of our prospective study design as previously described [12]. Second, we could not compare differences in the risk of CLAD associated with specific CARVs (eg, RSV vs coronaviruses) due to the small number of events in the respective subgroup analysis, despite a relatively large number of LuTRs and prolonged follow-up. Third, we only analyzed NPS data, which permitted us to also include asymptomatic LuTRs outside of scheduled bronchoalveolar lavage visits. Of note, CARV testing in NPS in patients with clinical evidence of LRTID has a high negative predictive value [41, 42]. Fourth, we included in the statistical analysis CARVs infections as a 3-month risk period, and this could affect the results. However, we considered a 3-month risk period because it was hypothesized that adverse clinical events occurring soon after viral infection were more likely to be associated with that infection.

In conclusion, CARV causing LRTID is an independent risk factor leading to the development of CLAD in LuTRs recipients. Given the important goal of improving the long-term survival of LuTRs by reducing the onset of CLAD, future efforts should include earlier CARV diagnosis, as well as the development of antiviral treatment and preventive measures including the development of vaccines against CARVs.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. I. L. A. has a Rio Hortega contract in the 2016 Strategic Action Health call from Instituto de Salud Carlos III of the Spanish Health Ministry for the years 2017–2018. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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