PAPER ID: PMC4706632

TITLE:
Symptomatic Respiratory Virus Infection and Chronic Lung Allograft Dysfunction

ABSTRACT:
Background. Chronic lung allograft dysfunction (CLAD) is a major cause of allograft loss post-lung transplantation. Prior studies have examined the association between respiratory virus infection (RVI) and CLAD were limited by older diagnostic techniques, study design, and case numbers. We examined the association between symptomatic RVI and CLAD using modern diagnostic techniques in a large contemporary cohort of lung transplant recipients (LTRs). Methods. We retrospectively assessed clinical variables including acute rejection, cytomegalovirus pneumonia, upper and lower RVI, and the primary endpoint of CLAD (determined by 2 independent reviewers) in 250 LTRs in a single university transplantation program. Univariate and multivariate Cox models were used to analyze the relationship between RVI and CLAD in a time-dependent manner, incorporating different periods of risk following RVI diagnosis. Results. Fifty patients (20%) were diagnosed with CLAD at a median of 95 weeks post-transplantation, and 79 (32%) had 114 episodes of RVI. In multivariate analysis, rejection and RVI were independently associated with CLAD (adjusted hazard ratio [95% confidence interval]) 2.2 (1.2–3.9), P = .01 and 1.9 (1.1–3.5), P = .03, respectively. The association of RVI with CLAD was stronger the more proximate the RVI episode: 4.8 (1.9–11.6), P < .01; 3.4 (1.5–7.5), P < .01; and 2.4 (1.2–5.0), P = .02 in multivariate analysis for 3, 6, and 12 months following RVI, respectively. Conclusions. Symptomatic RVI is independently associated with development of CLAD, with increased risk at shorter time periods following RVI. Prospective studies to characterize the virologic determinants of CLAD and define the underlying mechanisms are warranted.

Cohort ::: METHODS:
We retrospectively assessed a cohort of 250 consecutive adult patients who received a first lung transplant at the University of Washington Medical Center (UWMC) between January 2007 and May 2012. Clinical variables and endpoints were collected for 1 year after the most recently transplanted patient, allowing a possible maximum duration of follow-up of 5.4 years for the first patient included and a minimum of 1 year for the last patient included. The University of Washington has a comprehensive electronic health record system, and patient demographics and clinical and laboratory information were collected via medical record review using standardized data collection forms by trained personnel who were blinded to the primary endpoint (CLAD). The University of Washington Institutional Review Board approved the study.

Cytomegalovirus Prevention ::: Transplantation Protocols ::: METHODS:
Patients who were either recipient or donor seropositive for cytomegalovirus (CMV) received 3 months of intravenous (IV) ganciclovir or oral valganciclovir followed by chronic acyclovir prophylaxis. CMV recipient and donor seronegative patients received chronic acyclovir prophylaxis. Monitoring for CMV viremia was done by blood quantitative polymerase chain reaction (PCR) after discontinuation of prophylaxis, weekly for 1 month, every 2 weeks for 2 months, and then monthly through year 1, and when CMV disease was clinically suspected.

Immunosuppression and Treatment of Rejection ::: Transplantation Protocols ::: METHODS:
Routine induction therapy was given with basiliximab. Maintenance immunosuppression consisted of prednisone, a calcineurin inhibitor (generally tacrolimus), and an antimetabolite (generally mycophenolate mofetil or mycophenolic acid).

Assessment and Treatment of Rejection ::: Transplantation Protocols ::: METHODS:
No routine surveillance bronchoscopy to evaluate for rejection was performed during the study period. Patients with clinically suspected rejection underwent bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial biopsy. An episode of acute rejection was defined if a biopsy of lung tissue demonstrated grade A1 or B1R or higher rejection or if the patient received high-dose methylprednisolone. Biopsies were assessed according to The International Society for Heart & Lung Transplantation guidelines [12]. Standard treatment for acute rejection was methylprednisolone 1 g IV × 3 days. For refractory rejection, antithymocyte globulin was generally used, although a second course of methylprednisolone may have been used based on clinician discretion.

Follow-up of Lung Transplant Recipients ::: Transplantation Protocols ::: METHODS:
The standard protocol for outpatient follow-up after initial hospital discharge was weekly for 4 weeks, every 2 weeks for 1 month, every 2–3 months until 12 months post-transplantation, and then every 3–12 months depending on distance from transplantation center and involvement of local pulmonologist. Formal spirometry was performed at all clinic visits and if clinically indicated. All patients were instructed to perform home spirometry and to notify the team if there was a ≥10% decrease in forced expiratory volume in 1 second (FEV1).

Respiratory Virus Testing ::: Transplantation Protocols ::: METHODS:
RVI testing was done only for evaluation of compatible clinical signs and symptoms or radiographic abnormalities; no surveillance testing in asymptomatic patients was performed. Nasal swabs, washes, sputum, or BAL specimens were tested for respiratory virus RNA using either direct fluorescent antibody (FA) and culture or a laboratory-developed PCR assay that tests for the following 12 viruses: respiratory syncytial virus (RSV), parainfluenza 1–4, influenza A and B, adenovirus, coronavirus, rhinovirus, metapneumovirus, and bocavirus, as previously described [13–18]. The PCR platform was the same throughout the entire study period, and routine testing of all respiratory samples included PCR.

Determination of Exposures and Outcomes ::: Transplantation Protocols ::: METHODS:
A respiratory viral episode was defined as upper (nasal swab or nasal wash) or lower (sputum, BAL) respiratory specimen positive for a respiratory virus by culture, FA, or PCR. Patients with both upper and lower respiratory tract specimens that were positive were categorized as having a lower respiratory tract infection (LRTI). In patients who tested positive for subsequent RVI after their initial infection, a new viral episode was defined if at least 1 month had passed after the initial positive specimen for a specific respiratory virus pathogen or if there was at least 1 test negative for the initial respiratory virus followed by a newly positive test for the same virus.
CLAD was diagnosed based on routine serial spirometry monitoring. BOS was defined as having an FEV1 ≤ 80% of the patient's prior baseline best FEV1 (average of two best FEV1 values) for at least 3 weeks, and R-CLAD was defined as having a forced vital capacity (FVC) ≤ 80% of the patient's baseline best FVC in addition to meeting the criteria for BOS, both persisting for at least 3 weeks and with no improvement at any measured time in the future [19–21]. Two transplant pulmonologists independently evaluated all patients for CLAD. In the case of differing assessments, they reviewed the primary data and reached a consensus diagnosis. Alternate explanations for the decrease in spirometry, such as airway stenosis, were investigated through chart review. If present at the time of decreased spirometric values, the patient was not considered as having CLAD at that time.
CMV pneumonia was diagnosed based on positive shell vial and/or positive viral culture from a BAL in the setting of compatible symptoms and radiographic findings.

Statistical Analyses ::: METHODS:
We examined the association between symptomatic RVI and CLAD in both univariate and multivariate models using Cox proportional hazards models. Covariates included age, type of transplantation (bilateral or single lung), acute rejection, and CMV pneumonia. We analyzed CMV pneumonia and acute rejection as time-dependent indicator variables that remained “on” after the first episode of CMV pneumonia or acute rejection for the duration of the person's follow-up. RVI was analyzed as a time-dependent step function for the entire duration of follow-up as well as for specific risk periods (3 months, 6 months, and 12 months) following the diagnosis of RVI. We hypothesized that the relationship of RVI with CLAD would be greater with shorter time periods based on the waning of the immune response to RVI over time. The primary outcome was development of CLAD. A secondary outcome was the composite endpoint of CLAD or death. The Kaplan–Meier method was used to estimate and graph the probability of CLAD and its phenotypes BOS and R-CLAD. The proportional hazards assumption was tested using plots of Schoenfeld residuals. All analyses were performed using Stata software, version 12.1 (Stata Corporation, College Station, Texas).

Respiratory Tract Infection ::: Cohort ::: RESULTS:
A total of 114 viral episodes were diagnosed in 79 patients (31.6%). Table 3 details the characteristics of the RVI episodes. Median time to first RVI was 19 weeks (IQR, 9–63 weeks). Nine viral episodes had more than 1 virus isolated, 8 had 2 viruses (all had rhinovirus as 1 of the

viruses), and 1 had 3 viruses for a total of 124 viruses isolated. Figure 2 is a Kaplan–Meier curve showing the time to development of first RVI following transplantation. Among the 50 patients with CLAD, 21 (42%) had 1 or more RVIs preceding CLAD diagnosis compared with 56 (28%) patients who remained CLAD-free during follow-up.

Association of RVI With Outcomes ::: Cohort ::: RESULTS:
We analyzed the relationship between several factors and CLAD in both univariate and multivariate analyses in a time-dependent manner, and modeled periods of risk of 3 months, 6 months, 12 months, and "ever" (ie, at last follow-up) for RVI. In both univariate and multivariate analysis, RVI at all modeled risk periods was independently associated with development of CLAD (Table 4). The association was stronger at shorter modeled periods of risk. We confirmed the previously reported association of acute rejection with development of CLAD (Table 4). Our secondary endpoint, the composite endpoint of CLAD and death, showed a similar trend as the primary endpoint in both univariate and multivariate analysis, with a higher hazard ratio (HR) of RVI at 3 months and progressively declining HR as time from RVI increased (HR, 4.85; 95% confidence interval [CI], 2.76–8.43; $P < .01$ vs HR, 2.75; 95% CI, 1.72–4.37; $P < .01$ at 3 months vs 12 months post-RVI, respectively). We also analyzed the association between gastroesophageal reflux disease (GERD) and CLAD using 2 methods, first requiring a positive pH probe test for GERD diagnosis and second including anyone with a clinical diagnosis of GERD. No statistically significant association was seen in univariate analysis (data not shown) with either definition of GERD; therefore, GERD was not included in the final models. We were also interested in whether 1 CLAD phenotype was more strongly associated with RVI than the other (R-CLAD vs BOS). While the numbers are small, precluding a formal analysis, overall, a higher percentage of R-CLAD patients had at least 1 preceding viral episode as compared with BOS (9/17 [52.9%] vs 12/33 [36.4%], respectively).

DISCUSSION:
In a large contemporary cohort of LTRs who underwent symptom-guided testing for RVI using molecular diagnostic methods, we found high rates of RVI and an independent association between symptomatic RVI and CLAD. The association between RVI and CLAD was stronger with shorter modeled periods of risk but was also present when all follow-up time was considered. We demonstrate for the first time that the strength of the association of symptomatic RVI with CLAD is significantly influenced by time (eg, the proximity of the RVI episode with CLAD diagnosis). The stronger association in the first several months after RVI has important implications for future trials of antiviral agents, including powering trials for an endpoint of CLAD. This study also addresses several important limitations of prior studies, significantly extends our understanding of the role of RVI and CLAD, and provides a strong rationale for the conduct of large prospective studies to characterize the specific virologic determinants and mechanistic links between RVI and CLAD.
Several prior studies have assessed the association between RVI and BOS (Table 1). Magnusson et al found an association between BOS and RVI (odds ratio, 3.0; 95% CI, 1.3–6.8; $P = .008$) in a retrospective cohort study [6]. However, they only considered LRTIs based largely on surveillance BALs and their cohort was small (38 LTRs, 14 with RVIs). While they followed the LTRs for 10 years for outcomes, RVI episodes were only recorded for the first year post-transplantation. Khalifah et al also reported an association between RVI and BOS in univariate and multivariate analyses in a retrospective cohort study [2], but they assessed only 4 viruses using nonmolecular methods, did not distinguish BOS and R-CLAD or use blinded reviewer assessment of CLAD, and included relatively few cases of RVI [22]. Billings et al found no relationship between RVI and CLAD overall, but a subanalysis demonstrated an association with high-grade BOS only for LRTI and had many of the same limitations as that of Khalifah et al [9]. In contrast to the above studies, Milstone et al reported no association; however, the sample size was small (only 4 BOS cases, 17 RVIs) [8]. Thus, these prior studies were relatively heterogeneous and had 1 or more significant limitations that might explain discrepancies with the present study.
Our study had important strengths. We examined a large cohort of patients (among the largest to assess this issue) and diagnosed RVI using modern molecular diagnostic techniques that detected a large number of respiratory viruses. We used time-dependent multivariate analyses that controlled for factors associated previously with CLAD and also modeled differing periods of risk to provide insight into mechanisms the underlie the relationship between RVI and CLAD. Importantly, the primary endpoint (CLAD) was assigned using recently developed consensus

definitions and by 2 blinded transplant pulmonologists. We also acknowledge certain limitations, including the retrospective single-center study design. Despite comprehensive medical records and close follow-up, we cannot exclude missing data. Thus, our rates of RVI should be considered minimal estimates because patients may have been diagnosed outside our medical center. We were not able to assess all the factors that have been linked to CLAD, including pseudomonas pneumonia and/or colonization [22–24] and small conidial forms of Aspergillus [22]. However, there is no consensus that these are proven risk factors for CLAD. Our study size was not large enough to permit assessment of risk of CLAD associated with specific viral organisms, nor was it large enough to assess differences between the 2 CLAD phenotypes, BOS and R-CLAD.

Although we found an independent association of symptomatic RVI with CLAD, it is possible that RVI could simply be a marker for patients who are otherwise predisposed to CLAD, rather than a cause of CLAD. There are several lines of evidence suggesting that RVI might be an underrecognized contributor (rather than marker) in a substantial proportion of CLAD cases. As summarized in Table 1, RVI has been linked to CLAD in several prior studies [2, 6–10, 25–29]. RVI is relatively common in LTRs, and a significant proportion of cases detected by sensitive methods can be asymptomatic. Importantly, both symptomatic and asymptomatic RVI has been linked to CLAD. Additionally, in vitro and animal studies have identified specific biologically plausible mechanisms through which RVI could mediate CLAD [30, 31]. RVI-mediated regulatory T-cell dysfunction leading to augmentation of innate, allo-, and auto-immune pathways are possible mechanistic pathways to explain a causal link between RVI and CLAD. Finally, a small clinical trial of a novel antiviral (siRNA) for a specific RVI (RSV) in LTRs demonstrated reduced risk for CLAD in the group randomized to active RSV therapy [32]. In total, these epidemiologic and experimental data are consistent with a causal association between RVI and CLAD.

In summary, our study addresses many of the limitations of prior studies and adds significantly to the body of evidence linking RVI to development of CLAD. This association is relevant as RVI could represent a distinct, diagnosable, and potentially treatable major cause of allograft dysfunction and loss in LTRs. If the association were confirmed in future prospective studies, at a minimum, it could identify specific patients at increased risk for development of CLAD who could then be entered into trials of preventive or treatment strategies. With the development of new antivirals for several respiratory viruses [32–35], it will be possible to directly test the hypothesis that RVI causes CLAD by means of interventional controlled trials. Prior to embarking on such studies, it is imperative to carefully characterize the virologic determinants and mechanisms that underlie CLAD in order to aid in the design of such interventional trials. While our study strongly suggests an association between symptomatic RVI and CLAD, we propose a large, multicenter, prospective natural history study with careful virologic and clinical assessments and mechanistic studies as a logical next step. A better understanding of the role of respiratory viruses in the pathogenesis of CLAD through future prospective studies is critical in devising rational strategies to improve long-term patient and graft survival in LTRs.