Characterizing the Airway Inflammatory Milieu in Post-Transplantation Lung Biopsies of Cystic Fibrosis Patients: A Single Institution Study of Acute Cellular Rejection

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Introduction

Patients with cystic fibrosis (CF) frequently require lung transplantation despite aggressive therapy to combat increased mucostasis, poor bacterial clearance, and severe airway inflammation. More than 33% of all lung transplant recipients are treated for acute rejection in the first year alone.

The diagnosis of pulmonary allograft rejection from surveillance transbronchial biopsies has been standardized with an international grading scheme adopted by the International Society for Heart and Lung Transplantation (ISHLT). Acute cellular rejection remains based on perivascular and interstitial mononuclear infiltrates; however, since its introduction in 1990, modifications to the grading scheme (1996, 2007) have been made to accommodate the influence of airway (bronchiolar) inflammation.

Accurately grading allograft rejection in the CF transplantation population is uniquely challenging because of the potential for continued airway inflammation from possible chronic post-transplantation microbial colonization. Current ISHLT rejection scoring guidelines give little recommendation to the findings of acute inflammation. The presence of neutrophils and eosinophils in surveillance biopsies have long been associated with microbial infection (deferring grading).

It is the aim of this study to characterize the acute inflammation in the post-transplantation lungs of CF patients in association with final diagnosis ISHLT rejection scoring. Our goal is to better predict allograft rejection in this population.

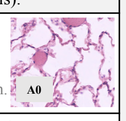
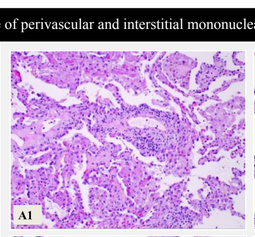
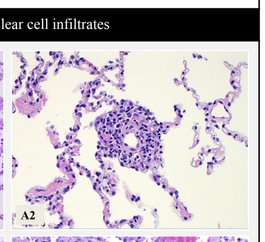
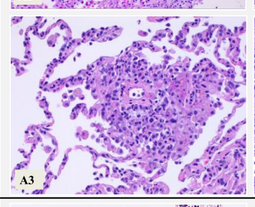
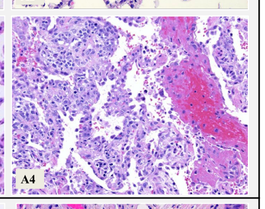
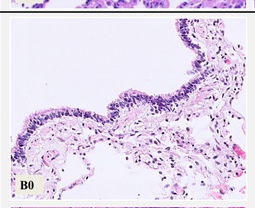
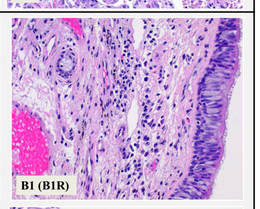
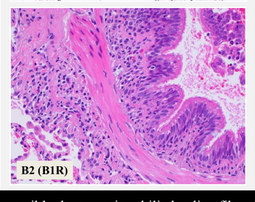
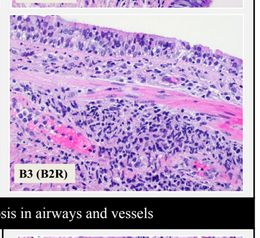
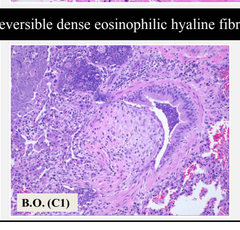
1996	2007 "R"	ISHLT WORKING FORMULATION FOR THE STANDARDIZATION OF NOMENCLATURE IN THE DIAGNOSIS OF PULMONARY ALLOGRAFT REJECTION	
ACUTE REJECTION: diagnosis is based exclusively on the presence of perivascular and interstitial mononuclear cell infiltrates			
*A: **Acute Rejection***: perivascular mononuclear cell infiltrates, accompanied sub-endothelial infiltration (endothelialitis/intimitis).			
A0	*A0*	*Normal Pulmonary Parenchyma* No Hemorrhage, No Necrosis, No Mononuclear Cell Infiltration.	 A0
A1	*A1*	*Minimal Acute Cellular Rejection* Some Circumferential Infiltration	 A1
A2	*A2*	*Mild Acute Cellular Rejection* Significant Circumferential Infiltration	 A2
A3	*A3*	*Moderate Acute Cellular Rejection* Alveolar Septa Infiltration.	 A3
A4	*A4*	*Severe Acute Cellular Rejection* Alveolar Fibrin, Hyaline Membranes, Reorganizing Airspaces.	 A4
*B: **Airway Inflammation***: Lymphocytic Bronchitis/ Bronciolitis			
B0	*B0*	*No Airway Inflammation* No evidence of bronchiolar inflammation.	 B0
B1	*B1R*	*Low Grade Lymphocytic Bronchiolitis* Mononuclear Cells within Bronchiole Submucosa (Scattered, Patchy, or Circumferential). Spares respiratory epithelium. No epithelial damage, or intra-epithelial infiltration.	 B1 (B1R)
B2			 B2 (B1R)
B3	*B2R*	*High Grade Lymphocytic Bronchiolitis* Mononuclear cells expand submucosa and home to epithelial basement membrane and into overlying respiratory epithelium. Epithelial cell necrosis, apoptosis, metaplasia observed. If severe, fibrino-purulent exudate.	 B3 (B2R)
B4			
CHRONIC REJECTION: manifest by fibrous scarring, presumed irreversible dense eosinophilic hyaline fibrosis in airways and vessels			
*C: **Chronic Airway Rejection***: Obliterative Bronchiolitis		*D: **Chronic Vascular Rejection***: Accelerated Graft Vascular Sclerosis	
B.O. (C1)	*Obliterative Bronchiolitis* Hyaline submucosal fibrosis; luminal occlusion	**D1**	*Chronic Vascular Sclerosis* Fibrointimal thickening
B.O. (C1)		*D1*	 B.O. (C1)  D1

Table 1: In 1990 an international scheme for the grading of pulmonary allograft rejection was adopted by the International Society for Heart and Lung Transplantation (ISHLT) and was modified in 1996 and 2007. Acute rejection is currently based on perivascular and interstitial mononuclear infiltrates.

Methods

We examined 173 transbronchial biopsies from 18 post-transplantation lungs within CF patients at Loyola University Medical Center between 2006 and 2016. Acute cellular rejection statuses were collected based on the 1996 ISHLT grading scheme for lung rejection surveillance. Neutrophilic and eosinophilic inflammation were examined and were quantified by a grading scheme of 0-4, with 0 as absent and 4 as extensive infiltration. Microbiological isolation data were obtained from BAL specimens obtained at the time of biopsy. Spirometry data were collected within two weeks of biopsy. Radiograph disease scoring was performed on chest x-rays obtained before biopsy. Scoring was performed via the following three schemes: Chrispin-Norman Scoring[4], Brasfield Scoring[6], and Severe Acute Respiratory Infection Scoring[5]. Results were analyzed by paired analysis with one-way ANOVA.

Conclusions

- **The presence of neutrophils and eosinophils, in large airways especially, may be an important indication of higher grade rejection in CF patients.**
- **Microbiological isolation status alone is not a significant predictor of graft rejection, nor does it correlate significantly with acute inflammation found in transbronchial surveillance biopsy.**
- ISHLT Grading Scheme may benefit from recommending specific evaluation of eosinophilic and neutrophilic inflammation in rejection surveillance grading.
- ISHLT Grading Scheme may benefit from recommending the evaluation of large airways in lung transplantation rejection surveillance.
- *Follow up studies include correlation with chronic lung allograft dysfunction, cytokine isolation studies, and survival data.*

References

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Results: Neutrophil/Eosinophil Grade is Directly Related to Rejection Score

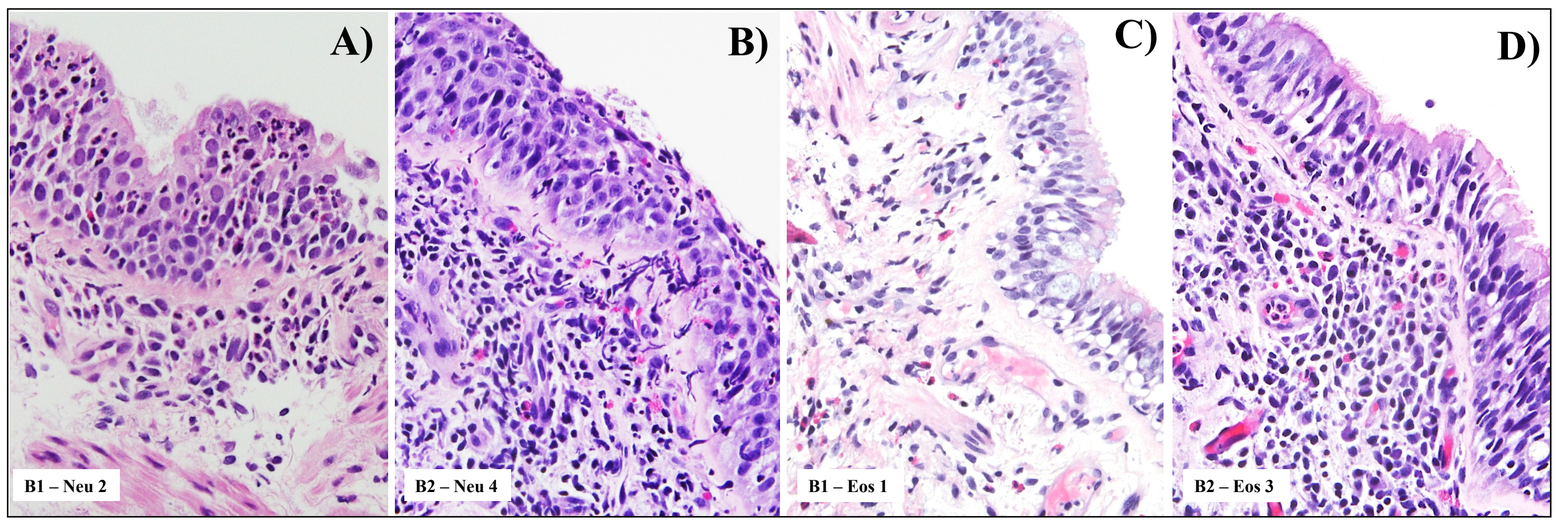
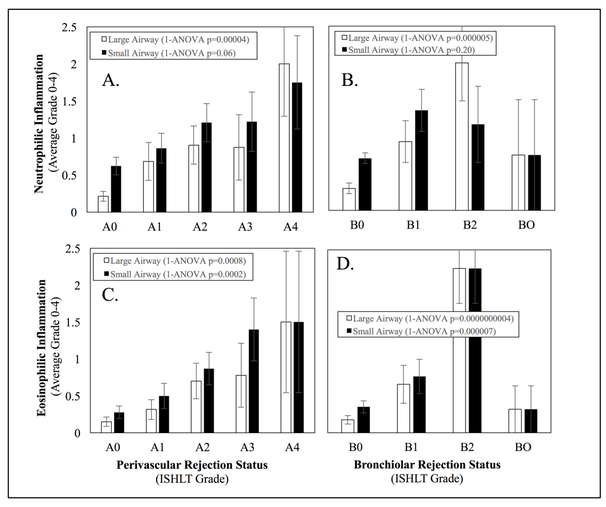


Figure 1: *(Above)* Representative transbronchial surveillance biopsy hematoxylin and eosin (H&E) stained tissue sections from (A) final diagnosis B1 low grade lymphocytic bronchiolitis with moderate neutrophilic infiltration and (B) final diagnosis B2 high grade lymphocytic bronchiolitis with considerable neutrophilic infiltration. (C) Final diagnosis B1 low grade lymphocytic bronchiolitis with minimal eosinophilic infiltration and (D) final diagnosis B2 high grade lymphocytic bronchiolitis with considerable eosinophilic infiltration.

Figure 2: *(Right)* There is significantly more neutrophilic (A-B) and eosinophilic (C-D) inflammation present in higher grade rejection surveillance transplanted lung specimens of CF patients, particularly in large airways.



Results: Microbiological Isolation is Independent of Rejection Status

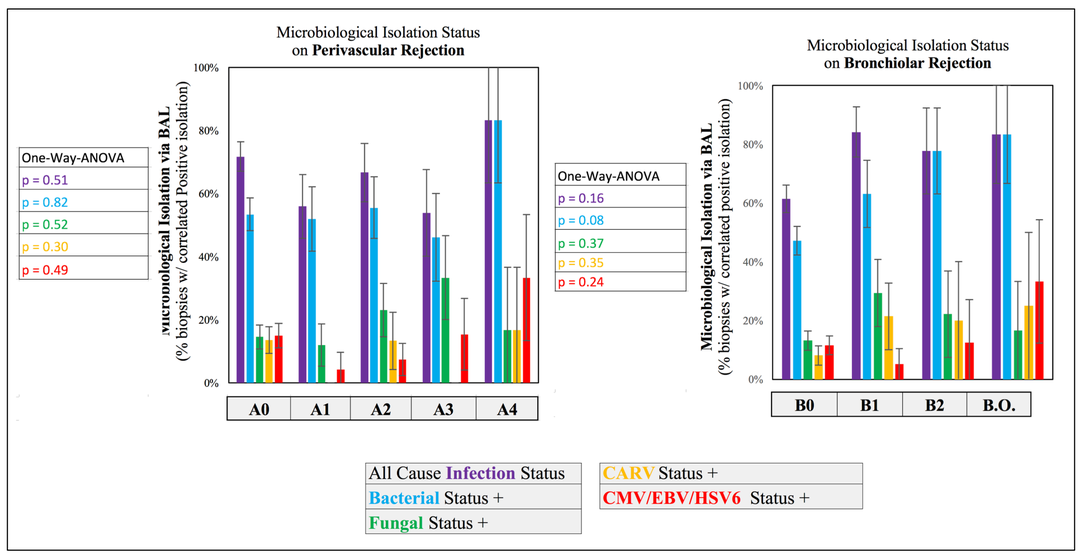
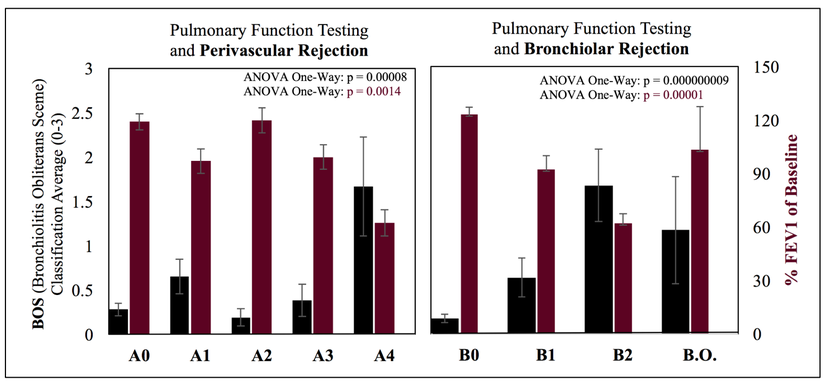


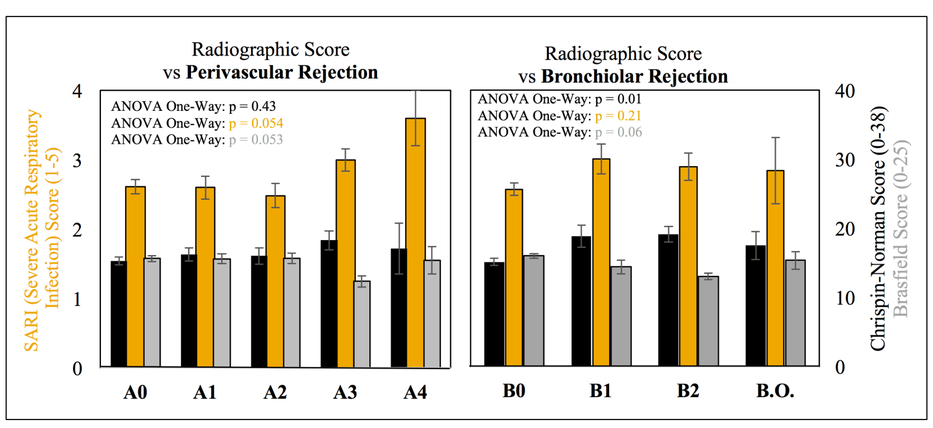
Figure 3: *(Left)* There is no significant correlation between any of the following differentiated microbiological isolation statuses and perivascular rejection status (left) or bronchiolar rejection status (right): (purple) all cause positive isolation status, (blue) bacterial positive isolation status, (green) fungal positive isolation status, (yellow) community acquired respiratory virus (CARV) positive isolation status, and (red) herpesviridae positive isolation status.

Results: Rejection Predicts Bronchiolitis Obliterans Syndrome (%FEV1)

Figure 4: *(Right)* Pulmonary function testing performed within 1 week of transbronchial surveillance biopsy was used to calculate a Bronchiolitis Obliterans Score (BOS) (0-3) based on %FEV1 of initial transplanted lung baseline spirometry. As expected, %FEV1 of baseline significantly decreases with worsening perivascular and bronchiolar rejection status. Similarly, BOS significantly increases with worsening perivascular and bronchiolar rejection status. This indicates the predictive utility of ISHLT lung allograft rejection grading in our patient population, and that our specimens were likely scored accurately.



Results: Radiographic Scoring is Not Prognostic for Perivascular Rejection



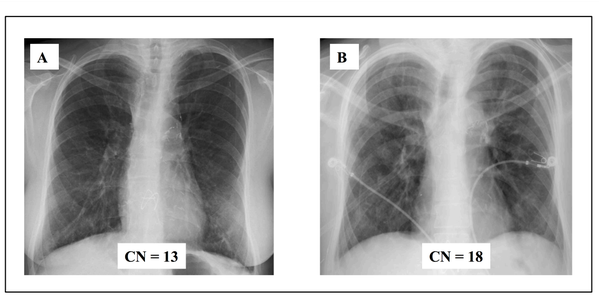
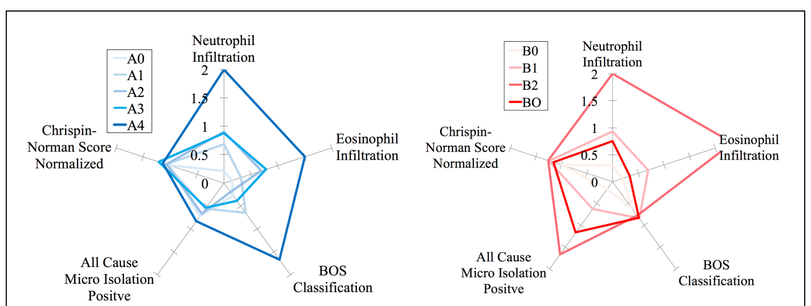


Figure 5: *(Above)* Representative Chrispin-Norman Scoring[4] at different intervals of surveillance (A→B) at time of surveillance trans-bronchial biopsy (before procedure).

Figure 6: *(Above Left)* Three radiographic chest scoring schemes for structural lung changes and consolidation pattern were examined: Chrispin-Norman Scoring[4], Brasfield Scoring[6], and Severe Acute Respiratory Infection Scoring[5]. There is no significant correlation between any of the three scoring schemes and perivascular rejection status. There is a significant correlation between increasing Chrispin-Normal Score and bronchiolar rejection status.

Results: Isolated Acute Inflammation is Indicative of Worsening Rejection

Figure 7: *(Right)* Data from Figures 1-6 are synthesized via radar analysis. Chrispin-Normal Score[4] averages were normalized to fit axis. Variables which correlate significantly with perivascular rejection status include neutrophilic infiltration, eosinophil infiltration, and BOS classification. Notably, positive microbiological isolation status and radiographic scoring do not significantly distinguish perivascular rejection status. Variables which correlate significantly with bronchiolar rejection status include neutrophilic infiltration, eosinophil infiltration, BOS classification, and Chrispin-Normal Scoring (not microbiological isolation status).



Standard Error	A0	A1	A2	A3	A4
Neutrophil Infiltration	0.066	0.254	0.257	0.441	0.707
Eosinophil Infiltration	0.064	0.134	0.242	0.434	0.957
BOS Classification	0.071	0.195	0.096	0.180	0.558
All Cause Micro Isolation Positve	0.047	0.101	0.092	0.137	0.200
Chrispin-Norman Score Normalized	0.037	0.063	0.080	0.089	0.240

Standard Error	B0	B1	B2	BO
Neutrophil Infiltration	0.071	0.284	0.516	0.750
Eosinophil Infiltration	0.059	0.270	0.494	0.333
BOS Classification	0.047	0.219	0.408	0.601
All Cause Micro Isolation Positve	0.047	0.086	0.147	0.167
Chrispin-Norman Score Normalized	0.033	0.107	0.076	0.133