

Heterogeneity of Chronic Lung Allograft Dysfunction Phenotypes: Spirometric, Histopathologic and Imaging Associations over a 16-Year Experience

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Abstract

Background: The 2019 update by the ISHLT of classification for chronic lung allograft dysfunction (CLAD) includes a mixed and undefined phenotype.

Objectives: Assess (a) frequency of phenotypes (per ISHLT) with emphasis on double lung transplants (DLTx), (b) concordance of spirometry with imaging and transbronchial biopsy findings, (c) mortality rates amongst phenotypes.

Methods: Single-center retrospective study of adult patients. Time from transplant to CLAD and mortality amongst phenotypes compared by ANOVA adjusted for age, gender and BMI.

Results: Of 360 patients, 96 (27%) met criteria for CLAD (57 DLTx). Seventy-four (77%) experienced combined FEV1 and FVC decline. In DLTxs, onset of FEV1 decline (n=12) occurred 10 months earlier than for FVC (n=4) and 5 months before simultaneous onset of FVC and FEV1 decline (n=41). Amongst DLTxs, largest cohort was mixed group (n=30, 53%); RAS phenotype second largest (n=16, 28%). Median onset of CLAD: 30 months for BOS and mixed categories (combined n=34); 48 months for RAS (n=16). Time to death following DLTx: longest in BOS, shortest in RAS, intermediate in mixed phenotypes.

Conclusion: CLAD occurs in more than one-quarter of patients; three-fourths exhibit concurrent decline in FEV1 and FVC. Imaging detects changes when biopsy findings are mild or not identified and often do not reflect spirometric changes.

Keywords: Bronchiolitis Obliterans Syndrome (BOS) • Chronic lung allograft dysfunction • Lung transplantation • Lung allograft dysfunction • Mixed phenotype • Restrictive Allograft Dysfunction (RAS) • Spirometric decline • Undefined phenotype

Introduction

The clinical course of Chronic Lung Allograft Dysfunction (CLAD) following lung transplantation is variable. Most commonly, it has been described as manifesting as a progressive airway obstruction phenomenon, usually as bronchiolitis obliterans syndrome (BOS). A restrictive phenotype of CLAD ("restrictive allograft syndrome", RAS, formerly known as rCLAD) has also recently been recognized, which has been reported to occur in approximately 30% of CLAD patients [1-9]. Median survival in RAS has been reported to be only 6 to 18 months compared with 3 to 5 years with BOS [7,9,10]. FVC loss is

the most important determinant after CLAD onset, independent of other factors such as age, gender, grade of graft dysfunction, prior history of organizing pneumonia, restrictive native lung disease, single lung, prior lymphocytic bronchiolitis, and age at transplant [9,10]. Recently the International Society for Heart and Lung Transplantation (ISHLT) expanded the classification system of CLAD phenotypes in recognizing patients with CLAD who do not fall into the purely BOS or RAS forms of the condition [7]. While spirometry is the biomarker commonly used to recognize the onset and type of CLAD, it may not be reliable enough to identify patients who may be experiencing the early stages of chronic lung rejection. Multidetector CT scanning, more recently microCT scanning [3,4] and parametric response mapping [5] have been used as more precise means of detecting lung disease in LTx patients and are able to detect structural alterations even before physiologic changes occur.

Our primary objective was to assess patterns of spirometric decline (i.e., decline in FEV1 alone vs combined decline in FEV1 and FVC with FEV1/FVC preserved at 0.7 or higher) in patients who underwent single, double, and lobar lung transplants (SLTx, DLTx and Lobar Tx, respectively) in a lung transplant center. We focused on the DLTx cohort, subdivided into phenotypes based on the 2019 ISHLT guidelines for CLAD classification [7]; secondary objectives were to (a) determine to what degree spirometric changes reflect CT imaging and biopsy findings, and (b) to compare mortality rates amongst phenotypes.

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Received 16 February, 2021; **Accepted** 11 March, 2021; **Published** 18 March, 2021

Research Methodology

Patients

Clinical, physiologic, histopathologic and imaging data of patients who received single, double lung or lobar transplants, aged 18-70 years, between April 2000 and August 2016 at the University of Southern California Health Sciences Center were retrospectively screened (S. C.). Study was approved by the Institutional Review Board of the University of Southern California Health Sciences Center, study number HS-16-00563. Patients with malignancy, uncontrolled infection, pulmonary embolic disease, pneumonectomy, neuromyopathic conditions, recurrent sarcoidosis, pleural effusions, weight gain, persistent lung edema due to kidney/heart/liver failure, re-transplants, and patients with unavailable lung function data were excluded. However, when other causes for functional decline intervened, such as acute rejection or infection, but CLAD persisted 3 months beyond resolution of these etiologies, the date of onset of CLAD was determined as the date of the first value of FEV1 <80%, per ISHLT guidelines [7,10]. Because of factors potentially confounding the interpretation of spirometric data in patients with single lung or lobar transplants, we focused on functional decline in patients with double lung transplants, while comparisons with the other types of lung transplants were made where relevant. Patients were listed along with their demographic information, follow-up lung function testing, and surveillance bronchoscopies. All patients received standard immunosuppression, spirometric follow-up, and surveillance bronchoscopies.

Spirometry

Spirometry was performed according to American Thoracic Society/European Society guidelines [11]. Obstructive airway disease was defined as an FEV1/FVC of <0.7, while "restrictive" lung disease was defined as reduction in both FEV1 and FVC to below 80% predicted with maintenance of FEV1/FVC at ≥ 0.7 . CLAD was defined as a sustained >20% decline in FEV1 as compared with the average of the two best post-transplant FEV1 measured at least 3 weeks apart in the absence of other clinical confounders [3]. Patients were considered to have FVC loss if at CLAD onset, the FVC/FVCbest was <0.8. The FVCbest was defined as the average of two best post-transplant FVC measurements used in the CLAD calculation. Stable FVC at CLAD onset was defined as the FVC/FVCbest >0.8. The date of transplant (time zero) was the time beyond which all patients were followed. Spirometric data (FVC and FEV1) were initially recorded at 6-month intervals until the most recent measurements available or death. The date of onset of CLAD was recorded for each patient; patients were subdivided according to the onset of decline in FEV1, FVC or both concurrently.

The ISHLT recognizes that in the absence of availability of total lung capacity measurements, use of the FVC/FVCbest <0.8 at CLAD onset is acceptable, although at the time of the 2019 recommendations, the true overlap of patients meeting criteria of FVC loss and those meeting criteria based on TLC loss is not defined because of the lack of plethysmographically obtained lung volumes [7].

Imaging

Chest imaging was available in all 96 patients: 76 of these received computed tomography (CT) imaging; 20 had only chest X-rays because of cardiorespiratory instability or CT scheduling conflicts. CT was obtained at full inspiration and relaxed expiration with slice thicknesses of 1.25 mm for all scans. Images were reviewed independently by 2 radiologists (A.W. and B.G.) to determine the presence of alveolar or interstitial changes (nodular, ground glass or reticular opacities, and septal thickening), or airway changes (air trapping, mosaic attenuation, bronchiectasis or bronchial wall thickening). Table 1 lists the classification of patients who underwent CT imaging based on obstructive and restrictive characteristics. For patients who underwent multiple CT scans, the scan obtained nearest the time of CLAD onset was evaluated for characteristic findings of airway vs parenchymal disease in order to classify the CLAD as obstructive or restrictive in nature. RAS-like opacities (RLO) were defined according to the 2019 consensus of the International Society for Heart

Table 1. Corresponding imaging findings based on spirometric changes in 76 patients who underwent computed chest tomography.

Obstructive (n=21)*	Restrictive (n=29)*
Mosaic attenuation	Atelectasis/scarring
Tree-in-bud	Ground glass opacities
Mucus plugging	Septal thickening
Bronchial wall thickening	Architectural distortion/fibrosis
Bronchiectasis	-
Peribronchial nodules	-

*Mixed CT findings in 15 patients; negative findings in 11.

and Lung Transplantation (ISHLT), namely as "opacities and/or increasing pleural thickening consistent with pulmonary and/or pleural fibrosis and that are likely to cause restrictive physiology rather than airway-based changes consistent with bronchiectasis" [7].

CLAD categories based on 2019 ISHLT classification

BOS was defined as CLAD with spirometry consistent with an obstructive pattern without RLO. Patients were classified as RAS when spirometry was consistent with a restrictive defect and confirmed if this defect was associated with parenchymal lung disease characterized by the presence of RLOs on chest imaging [7,10]. The 2019 ISHLT statement introduced a new mixed phenotype to describe CLAD with a combination of obstructive and restrictive defects on lung function accompanied by RLO on imaging. Another category referred to as an undefined phenotype was characterized by (a) obstruction and RLO without restrictive changes or (b) obstruction and restriction without RLO. We additionally subclassified our patients according to these 2 new categories. Patients with CLAD who did not fit any of these definitions were characterized as unclassified [10].

Transbronchial biopsies

Tissue from donor lungs of patients exhibiting RAS was obtained by transbronchial biopsy, examined for changes characteristic for CLAD and classified by features most closely consistent with bronchiolitis obliterans and organizing pneumonia (W. E. and M. N. K.). Changes consistent with infectious processes (such as bacterial or viral pneumonia) were documented. Histopathologic data obtained within 6 months of spirometry were recorded.

Transbronchial biopsies from lung allografts were of both indicated and surveillance types. For each biopsy, 4 hematoxylin and eosin-stained levels, and single elastic-, trichrome-, AFB-, GMS- and Gram's- stained slides were available for review. Within each biopsy, the following pathologic features were sought: presence (yes/no) of acute cellular rejection (including grade of rejection using the Banff classification) [12] presence (yes/no) of obliterative bronchiolitis, acute pneumonia, organized pneumonia and specific infections (i.e., fungal, viral, bacterial). In addition, the presence and the extent of interstitial fibrosis, peribronchial fibrosis, and peribronchial chronic inflammation and interstitial chronic inflammation were evaluated semiquantitatively as follows: None = 0%; minimal = <2% of parenchyma; mild = 2-10% of parenchyma; moderate = 10-25% of parenchyma; severe = >25% of parenchyma. The biopsies were reviewed in a light microscope by two pathologists (W.E. and M.N.K.). Histopathologic data obtained within 6 months of spirometry were recorded.

Statistical analysis

Group characteristics, spirometric variables, imaging and transbronchial biopsy findings in all patients were analyzed. Continuous variables were expressed as mean (\pm SD) or median (range) depending on normality of distribution, and categorical variables were expressed as frequency and percentage. Analysis of variance (ANOVA) was used to assess differences for demographic and time-independent baseline and time-dependent outcome characteristics across groups adjusted for age, gender, BMI [13]. ANOVA with Wilcoxon rank sum test was employed for comparison of cohorts with nonparametric distribution. All patients were included in the analysis. Comparison tests were two-sided with significance level set at <0.05.

Association between spirometric, imaging and biopsy findings

Data from each testing modality (imaging, spirometry, and biopsy) were related to one another in pairs, such that spirometry results (that is, obstructive versus restrictive) were compared to imaging results; imaging results were compared to biopsy results; and biopsy results were compared to spirometry (obstructive versus restrictive) results. For the purpose of this analysis, only patients (of any kind of lung transplant) who had the relevant recorded tests performed within 100 days of each other were considered for each analysis (e.g. imaging and spirometry performed in period of less than 100 days). Sensitivity and specificity were calculated for each pairing, with imaging used to compare with spirometry and biopsy results, and spirometry used to compare with imaging and biopsy results otherwise.

Results

Patient characteristics

During the 16-year time span 360 patients underwent lung transplantation, all of whom underwent lung function testing. Of these patients, 121 (33%) exhibited spirometric criteria for CLAD. Twenty-five additional patients were eliminated from analysis because of exclusionary features listed in the COHORT diagram (Figure 1), most of which were uncontrollable infections, leaving 96 (27%) for analysis. Cystic fibrosis (30%) and COPD (30%, including alpha-1 antitrypsin cases) were the leading indications for transplantation. Fifty-seven (59%) of the remaining 96 patients underwent double lung transplantation, while the remainder underwent single lung (32%) or lobar (8%) transplantations.

Table 2 lists anthropometric and clinical data for the 96 patients diagnosed with CLAD. All patients were receiving standard immunosuppressive therapy, including mycophenolate, tacrolimus or sirolimus, and prednisone (in variable doses, depending on their rejection status). Twenty-nine (30%) patients received azithromycin, mainly throughout the entire course of followup. Seventeen (18%) patients received extracorporeal photopheresis (ECP), mostly late in their post-transplant course, ranging from 3 to 20 cycles. Five (5.2%) patients received both azithromycin and ECP.

Patterns of lung function decline

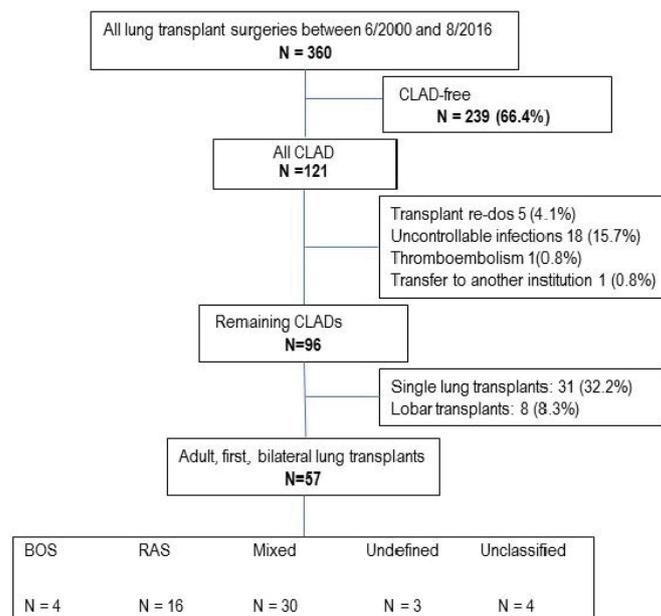
Seventy-four of the 96 (77%) patients experienced a sustained combined FEV1 and FVC decline; 22 (23%) exhibited a sustained decline in FEV1 alone. Figure 1 shows that this parallel decline was sometimes step-wise, but in general, progressive. Nine patients in this group who initially exhibited the restrictive pattern of functional decline developed an obstructive pattern late in their courses following transplant, five experiencing a rapid clinical decline. All of these patients showed initial response to immunosuppressive therapy; all but one eventually succumbed to their condition at the time of this writing.

When CLAD in the DLTx group was subdivided according to the updated ISHLT categories [7] with addition of the unclassified category defined by Levy et al. [10], the largest cohort was the mixed group (n=30, 53%) and the RAS phenotype comprising the second largest (n=16, 28%) (Figure 1). All 46 of these patients exhibited RLOs on CT imaging.

Table 3 shows time of onset of decline in FEV1 or FVC initially or both concurrently in the double lung transplants (n=57) following surgery and the time elapsed developing CLAD. The onset of FEV1 decline alone (n=12) occurred at a median of 6 months earlier than in patients with onset of FVC alone (n=3) and at the same time as those with simultaneous onset of FVC and FEV1 decline (n=42) (Figure 2). When subclassified according to the recent ISHLT categories (Table 4), median onset of CLAD was 30 months for both the BOS and mixed categories (combined n=34), while it was 48 months for the RAS cohort (n=16).

Transbronchial biopsy findings

The pathology slide evaluation encompassed 76 patients (79% of all CLAD cases) and a total of 353 biopsies, ranging from 1 to 12 biopsies per patient



Abbreviations: BOS: Bronchiolitis Obliterans Syndrome; CLAD: Lung Allograft Syndrome; CONSORT: Consolidated Standards of Reporting Trials; RAS: Restrictive Allograft Syndrome.

Figure 1. CONSORT diagram of patients studied.

Table 2. Anthropometric and clinical characteristics in all 96 patients.

Variables	FEV1 and FVC Decline (n=75)	FEV1 Decline Only (n=21)	Double Lung Tx (n=57)	Single Lung Tx (n=31)	Lobar Lung Tx (n=8)
Mean Age (year) at Transplant	46	45	42	60	28
Male (n=44)	38	6	29	13	3
Female (n=52)	37	15	28	18	5
Native Lung Disease					
Cystic Fibrosis	22 (29%)	7 (33%)	21 (37%)	n/a	8 (100%)
COPD	18 (24%)	8 (38%)	8 (14%)	18 (58%)	n/a
Pulmonary Fibrosis	23 (31%)	5 (24%)	17 (30%)	11 (36%)	n/a
Bronchiectasis	5 (7%)	1 (5%)	6 (10%)	n/a	n/a
Alpha 1-Anti-trypsin	3 (4%)	0	1 (2%)	2 (6%)	n/a
Pulmonary Hypertension	4 (5%)	0	4 (7%)	n/a	n/a
Note: Bronchiectasis group is non-cystic fibrosis; COPD: Chronic Obstructive Pulmonary Disease not associated with alpha-1 anti-trypsin deficiency. n/a, cystic fibrosis patients underwent only double lung or lobar transplants.					

Table 3. Double lung transplants (n=57): Onset of decline in FEV1 or FVC followed by subsequent decline in both FEV1 and FVC, or concurrent start of decline in FEV1 and FVC following transplant, and time elapsed developing CLAD (in months).

Variables	Onset decline after transplant	Time elapsed to CLAD
FEV ₁ (n=12)	12 (6-36)*	36 (6-36)
FVC (n=3)	18 (6-42)	72 (66-90)
Concurrent (n=42)	12 (6-72)	30 (12-156)

*Values represent median (range) months.

(average, 4.6 biopsies/patient). Eight biopsies (2.3%) lacked alveolar tissue and were deemed inadequate for evaluation of the lung parenchyma, leaving 345 biopsies for study. These biopsies contained 1-9 alveolar fragments (average, 4.0 alveolar fragments/biopsy). Sixty-one biopsies contained 1-3 bronchioles, but in only 4 biopsies (6.5%) in 3 patients (3.9%) was there microscopic evidence of obliterative bronchiolitis.

The most common microscopic abnormality was the presence of peribronchial fibrosis (Figure 3): One hundred and twenty-two (35%) biopsies

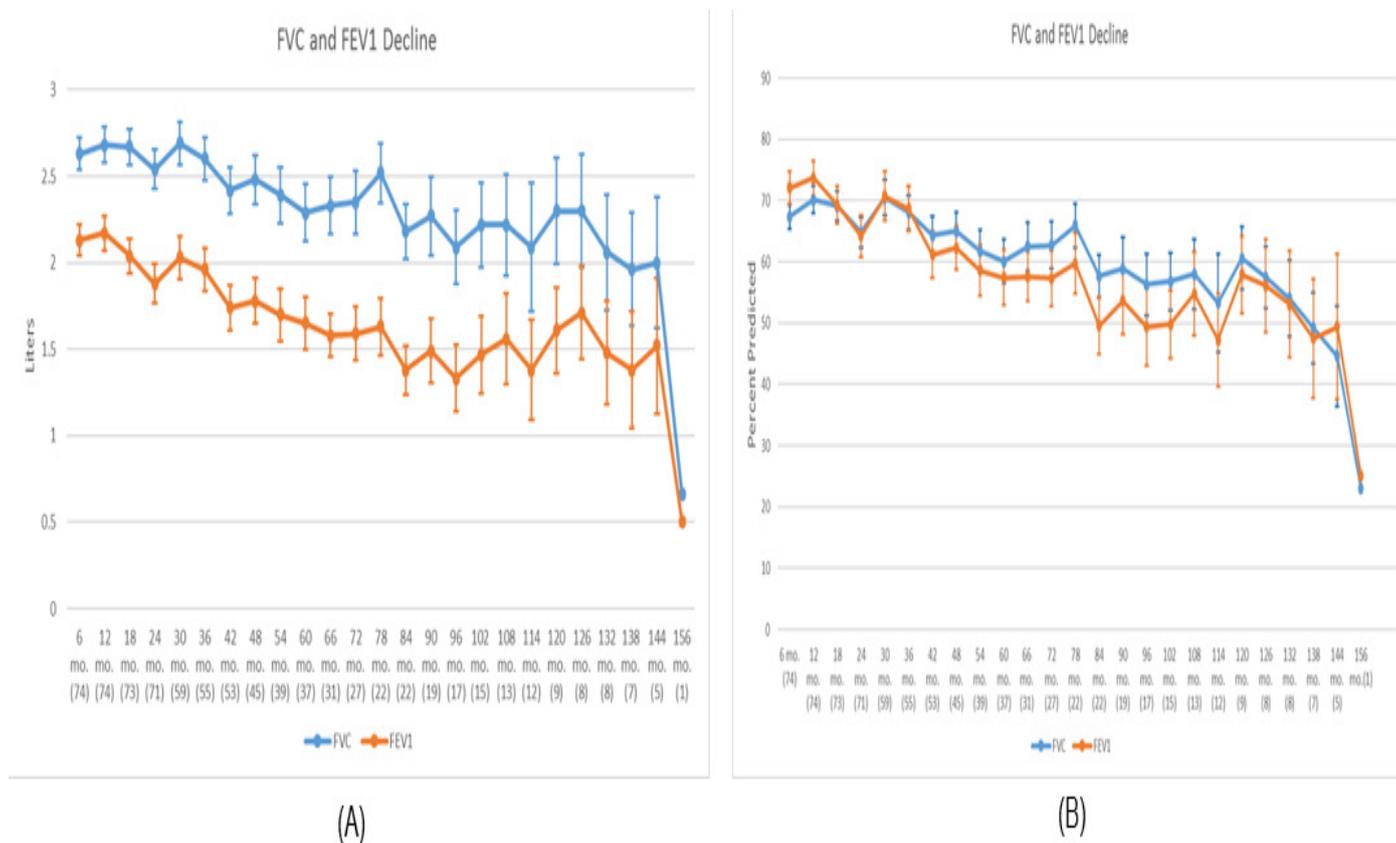


Figure 2. All patients exhibiting concurrent FEV1 and FVC decline, (A) in liters, (B) as % predicted (n=74 of 96 CLAD at the outset). Values represent mean ± SEM. The initial time point for FVC represents the rCLAD onset at which the FVC/FVCbest has already fallen below 0.8, where FVCbest is the average of the two FVC measurements that were paired with two best post-transplant FEV1 used in the CLAD calculation. Numbers in parentheses at bottom indicate patients remaining alive at time points. Data were not censored at the last spirometry in order to show the consistent pattern in FEV1 and FVC decline until death.

Table 4. Double lung transplants: Onset of CLAD, time from transplant to death and time elapsed between CLAD onset and death according to CLAD type (in months).

Type of CLAD	Time to CLAD	Time to Death	CLAD onset to death†
BOS (n=4)	30 (12-54)	32.5 (23-42) (n=2)*	12 (11-238) (n=2)
RAS (n=16)	48 (12-102)	75 (15-181) (n=11) §	20 (3-114) (n=11)
Mixed (n=30)	30 (12-90)	47 (3-154) (n=20) §§	19 (5-80) (n=20)
Undefined (n=3)	12 (12-12)	157 (25-157) (n=3)	26 (21-47) (n=3)
Unclassified (n=4)	73.5 (18-156)	46.5 (34-59) (n=2)	9 (5-32) (n=2)

Values represent median (range) months.

*Significant from BOS and mixed groups, p<0.025, ANOVA.

§ Significant from BOS group, p<0.02, ANOVA.

§§ Significant from RAS group, p <0.05, ANOVA.

†CLAD onset to death is not the same as the difference between time to CLAD and time to death as medians were computed on the basis of individual differences between times to CLAD and death, rather than as the difference between the medians for each cohort.

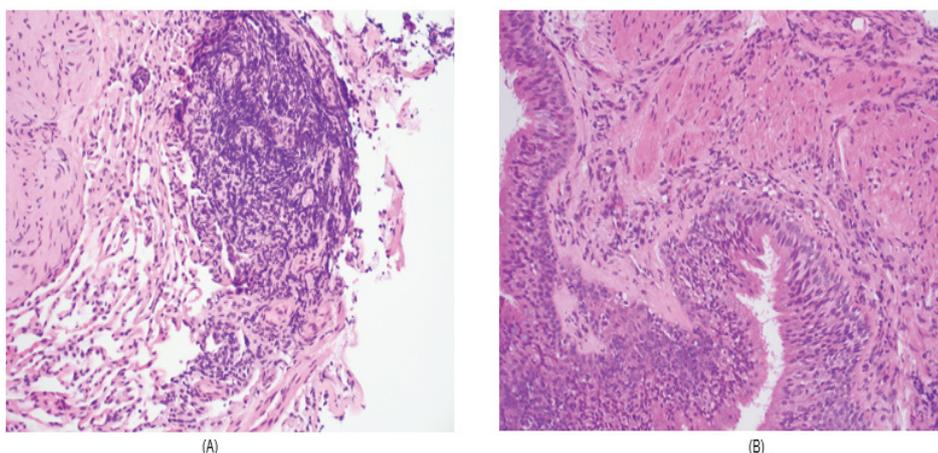


Figure 3. (A) Peribronchial inflammation and (B) Peribronchial fibrosis. Transbronchial biopsy; hematoxylin and eosin, 20x.

had peribronchial fibrosis in the mild to severe range, including 93 biopsies with mild, 28 with moderate and one with severe peribronchial fibrosis. One other biopsy showed peribronchiolar fibrosis. Some degree of peribronchial chronic inflammation was also common (Figure 3), but only 120 biopsies (34%) showed peribronchial chronic inflammation in the mild to severe range, including 87 biopsies with mild and 33 with moderate or severe inflammation. Twenty-nine (8.4%) biopsies in 25 patients (32.9%) showed some degree of interstitial fibrosis, including 16 biopsies with mild, 4 with moderate and 1 with severe interstitial fibrosis. Acute cellular rejection was present in 85 biopsies (25%) in 43 patients (56.6%). The overwhelming majority of these were grade A1 (61 biopsies), followed by A2 (20 biopsies) and A3 (4 biopsies). One biopsy had light microscopic changes suspicious for a humoral component of rejection.

Relation of spirometry to imaging findings

Twenty-four patients had spirometry and imaging data on record within 100 days of one another. For each of these patients, a single biopsy was correlated with the single imaging result taken closest in time. Imaging results were classified as showing the following: airway disease, parenchymal (interstitial or airspace filling) disease, both airway and parenchymal disease, or neither airway nor parenchymal disease. Of the 24 patients, 3/24 (12.5%) exhibited obstructive changes on spirometry (FEV1/FVC <70%) and 21/24 (87.5%) exhibited restrictive changes (FEV1/FVC ≥70%). On imaging, 21% exhibited airway disease, 17% exhibited parenchymal disease, 39% exhibited neither disease process, and 25% exhibited both disease processes. Sensitivity and specificity were determined by considering true positive results as those that showed restrictive changes on spirometry and correlated with the presence of either “parenchymal disease” or “both parenchymal disease and airway disease” on imaging. Using this method, spirometry demonstrated a sensitivity of 80% and a specificity of 7%.

Relation of imaging to biopsy findings

Twenty-eight patients had biopsy and imaging data on record within 100 days of one another. For each of these patients, a single biopsy was correlated with the single imaging result taken within closest proximity in time.

Each category of pathologic finding was considered separately: organized pneumonia, interstitial fibrosis, interstitial chronic inflammation, peribronchial fibrosis, and peribronchial chronic inflammation. Results were only considered positive if pathology indicated mild, moderate, or severe with regards to severity of each category; pathology findings of minimal or

none were considered negative. Table 5 details the number of patients with positive/negative results in each category of pathology with respect to each possible imaging finding. Sensitivity and specificity of each pathology category was calculated to determine how well positive results on biopsy correlated to imaging results of “interstitial disease” or “both interstitial and airway disease”. As seen, pathology findings such as organizing pneumonia, interstitial chronic inflammation, interstitial fibrosis and peribronchial fibrosis generally showed high specificity (ranging 67%-100%) but low sensitivity (ranging 3.6%-34%).

Relation of spirometry to biopsy findings

Thirty-two (33%) patients had biopsy and spirometry data on record within 100 days of one another. For each of these patients, a single biopsy was correlated with the single spirometry result taken within closest proximity in time. Each category of pathology was considered separately, as before. Table 6 details the number of patients with positive/negative results in each category of pathology with respect to each possible imaging finding. Using spirometry base comparison, sensitivity and specificity of each pathology category was calculated to determine how well positive results on biopsy correlated to spirometry results of “restrictive changes”. Once again, positive biopsies showed good specificity when present but poor sensitivity.

Mortality rates amongst CLAD subcohorts

When all 96 patients are considered, the one-, two- and five-year median mortality rates in patients with FEV1 decline alone were 0%, 0% and 48%, respectively, and in those with combined FEV1 and FVC decline (with FEV1/FVC maintained at ≥70%) were 0%, 4% and 49%, respectively (p<0.01, between one and five years and two and five years, ANOVA). The median survival after CLAD onset for patients with concurrent decline in FEV1 and FVC (restrictive pattern), and FEV1 alone was 58 months and 63 months, respectively (NS).

Two thirds of the 57 DLTx patients died during the 16-year study period. When subdivided according to the recent ISHLT classification, the shortest median survival following transplant was in the BOS group (32.5 mos, n=4, Table 4). Eleven patients with RAS died at a median of 75 mos after their transplants (significant from BOS, p<0.02). The 30 patients with mixed phenotype (defined by combined declines in FEV1/FVC and FVC and presence of RLOs) exhibited an intermediate time to death of 47 mos after transplant (significant from RAS, p<0.05). The RAS and mixed phenotypes (both of which were defined by the presence of RLOs) died a median 19 and 20 mos after onset of CLAD. The

Table 5. Correspondence between imaging results and biopsy findings in 28 patients.

Organizing pneumonia (n=28, seen in 3 patients on biopsy, not seen in 25)*			
11 absent + (interstitial or both)	2 present + (interstitial or both)	14 absent + (airway or neither)	1 present + (airway or neither)
Sensitivity	0.15		
Specificity	0.93		
Interstitial chronic inflammation (n=28, seen in 3 patients on biopsy, not seen in 25)*			
10 absent + (interstitial or both)	2 present + (interstitial or both)	15 absent + (airway or neither)	1 present + (airway or neither)
Sensitivity	0.17		
Specificity	0.94		
Interstitial fibrosis (n=28, seen in 3 patients on biopsy, not seen in 25)*			
11 absent + (interstitial or both)	2 present + (interstitial or both)	14 absent + (airway or neither)	1 present + (airway or neither)
Sensitivity	0.15		
Specificity	0.93		
Peribronchial fibrosis (n=28, seen in 6 patients on biopsy, not seen in 22)*			
10 absent + (interstitial or both)	3 present + (interstitial or both)	12 absent + (airway or neither)	3 present + (airway or neither)
Sensitivity	0.23		
Specificity	0.80		
Peribronchial chronic inflammation (n=28, seen in 9 patients on biopsy, not seen in 19)*			
10 absent + (interstitial or both)	3 present + (interstitial or both)	9 absent + (airway or neither)	6 present + (airway or neither)
Sensitivity	0.23		
Specificity	0.60		

*Imaging findings for 28 patients: 4 patients with airway disease on imaging, 9 with interstitial disease, 4 with both, and 11 with neither

Table 6. Correspondence between biopsy results and spirometric results in 32 patients.

Organizing pneumonia (n=32, seen in 2 patients on biopsy, not seen in 30)*			
27 absent + restrictive	1 present + restrictive	2 absent + obstructive	1 present + obstructive
Sensitivity	0.036	-	-
Specificity	0.67	-	-
Interstitial chronic inflammation (n=32, seen in 5 patients on biopsy, not seen in 27)*			
25 absent + restrictive	4 present + restrictive	2 absent + obstructive	1 present + obstructive
Sensitivity	0.14	-	-
Specificity	0.67	-	-
Interstitial fibrosis (n=31, seen in 1 patient on biopsy, not seen in 30)*			
28 absent + restrictive	1 present + restrictive	3 absent + obstructive	0 present + obstructive
Sensitivity	0.034	-	-
Specificity	1	-	-
Peribronchial fibrosis (n=32, seen in 7 patients on biopsy, not seen in 25)*			
22 absent + restrictive	7 present + restrictive	3 absent + obstructive	0 present + obstructive
Sensitivity	0.24	-	-
Specificity	1	-	-
Peribronchial chronic inflammation (n=32, seen in 11 patients on biopsy, not seen in 21)*			
18 absent + restrictive	11 present + restrictive	3 absent + obstructive	0 present + obstructive
Sensitivity	0.33	-	-
Specificity	1	-	-

*Spirometric results for 32 patients: 29 with restrictive changes, and 3 with obstructive changes.

unclassified group exhibited the shortest median time between CLAD onset and death (2 of 4 patients, 9 mos, $p < 0.05$).

We found no significant differences in mean survival between patients who received extracorporeal photopheresis (in obstructive and restrictive CLAD combined) and those who did not (mean \pm SD 69 \pm 43 months and 67 \pm 39 months, respectively, NS), nor between those who received azithromycin and those who did not (73 \pm 43 months and 64 \pm 49 months, respectively, NS).

Discussion

The main findings of this study are: (a) three-quarters of patients with CLAD exhibited a parallel decline in FEV1 and FVC, that is a restrictive pattern of dysfunction with the FEV1/FVC ratio remaining >0.7 throughout their course, (b) amongst patients who underwent DLTx, the largest single cohort was the mixed group, comprising more than half of patients, (c) patients with the BOS and mixed phenotypes (exhibiting both obstructive and restrictive decline and RLOs) comprised more than double the number of RAS patients, and (d) the most prominent findings on surveillance transbronchial biopsies was peribronchial inflammation or fibrosis.

The number of patients who received lung transplants during the period of study (360 over 16 years) averaged 23/year, with approximately 30/year up to 2005 and 15/year after 2005. The proportion of patients diagnosed with CLAD (33% of all lung transplant cases over 16 years) is similar to that reported by Sato et al. [6] (33% over 12.5 years) and by Todd et al. [8] (38% over 12 years), but fewer than that reported by Levy et al. [10] (34% over 6 years), Saito et al. [14] (24% over 8.5 years), Sato et al. [15] (29% over 7 years), and lower than the 50% over 5 years reported by the Registry for ISHLT [16]. Variations in prevalence of CLAD are likely related to differing definitions of CLAD, comorbid conditions altering the course of lung function decline, and changes in post-transplant management of patients over time.

Spirometric decline

We traced the decline in lung function with spirometry alone as plethysmographic lung volume determinations were not available for most patients, and besides would have been altered by the inclusion of single lung and lobar transplants in the analysis; air trapping may result in reduction in FVC and FEV1, with preservation or increase in the TLC. CT scanning detected RLOs in two-thirds of patients who exhibited a concomitant FEV1 and FVC decline, while less than one-fourth of this group exhibited no CT changes. The

decline in lung function in many RAS and mixed phenotype patients occurred in a stair-step manner, similar to that reported by Sato et al. [6] and Verleden et al. [17] who showed that some patients exhibited stabilization or even transient improvement following immunosuppressive therapy or extracorporeal photopheresis, but the effects of treatment were not consistent.

While RAS (as defined by the ISHLT) constituted 28% of double lung transplant patients, similar to the approximately 30% reported by others [1,6-10], the overall prevalence of RLOs in the combined RAS, mixed and undefined patients was considerably higher (82%). Thus, the new classification clearly extended the imaging findings of "restrictive" pleuropulmonary changes to patients that failed to show an RAS pattern as defined only by spirometric criteria. Levy et al. [10] reported a combined RAS, mixed and undefined cohort as comprising only 25% of all their CLAD patients. The higher prevalence of RLOs in our study likely include patients with fibrotic pleuropulmonary changes resulting from healed inflammatory processes followed by continuation of a persistent pattern of functional decline that defines CLAD. Inclusion of patients who experience a continued decline in lung function following acute rejection or infection is accepted in the definition of CLAD by the ISHLT, provided there is appropriate treatment of the acute complication [7,17]. As such, there may need to be a re-consideration of the presence of RLOs resulting from healed inflammatory processes resembling changes identified with RAS; admittedly, such cases would be difficult to distinguish from true RAS, even on biopsy or explants.

Relation of spirometric changes to imaging findings

Multidetector CT scanning detected changes in two-thirds of our RAS patients who did not exhibit spirometric decline, while less than one-fourth of those with concomitant FEV1 and FVC decline exhibited no CT changes. Our findings are similar to the majority of RAS patients reported by Sato et al. [6] and Verleden et al. [3,4,17,18] who exhibited infiltrates (ground glass opacities, reticular infiltrates and even honeycombing), pleural and septal thickening, and volume loss on thoracic imaging, although we did not discern an upper lobe distribution. Sato et al. [6] reported primarily an upper lobe distribution of fibroelastosis, while Verleden et al. [3,18] also described basal or diffuse opacities on CT.

Relation between imaging and histopathologic changes

While some of our rCLAD patients exhibited parenchymal changes not involving the airways on CT imaging, over one-third of biopsies exhibited peribronchial fibrosis in a mild to severe extent, mostly mild. Such

histopathologic changes may represent an early stage of a progressive fibrotic constriction of small airways described in CT imaging and histologic studies [4,5]. Using parametric response mapping to quantify functional small airway and parenchymal disease (PRMPD) in patients who had undergone DLTx, Belloli et al. [5] found that patients with concurrent FEV1 and FVC decline had significantly higher PRMPD than control subjects. In an elegant examination by micro-CT of lungs donated from patients with RAS, Verleden et al. [4] showed extensive reduction and narrowing of visible pre-terminal bronchioles located between generation 6 and 11 of airway branching in RAS, even more than in bronchiolitis obliterans syndrome. While our findings are similar, they should be viewed with caution, as the ability of transbronchial biopsy to sample peripheral airways is limited [19-22]; indeed, only 61 of 353 (17%) biopsies exhibited bronchioles.

Relation between spirometric decline and histopathologic changes

We found a variable association between spirometric and histopathologic findings on transbronchial biopsy. Over one-third of our patients exhibited peribronchial inflammation and/or peribronchial fibrosis, changes previously described in patients with RAS [4,5]. Such peribronchial changes may represent early stages of RAS. None of our patients exhibited infiltrative parenchymal changes (such as fibroelastosis) despite spirometric criteria for RAS. Random distribution of parenchymal disease likely resulted in sampling error related to the small sample sizes.

Mortality amongst CLAD subcohorts

Overall mortality rates in our study are similar to 1- and 3-year rates listed by the Scientific Registry of Transplant Recipients for all lung transplants between July 2009 and December 2016 (10% at 1 year and 31% at 3 years) [23]. They are slightly better than those reported by the ISHLT in 2014: 21%, 36% and 47% at 1, 3 and 5 years, respectively [16]. In contrast to earlier reports describing worse outcomes with RAS [16,24], amongst the double lung transplants alone, the median time between transplant and death in patients with RAS was more than twice as long as that of the BOS group, although the numbers are too small to make a definitive statement. Factors that would account for these findings include fewer episodes of acute rejection, more effective immunosuppressive therapy, and fewer episodes of humoral rejection [25,26]. The median time to death following DLTx in the mixed phenotype was intermediate to that of the BOS and RAS phenotypes, not surprising as this cohort comprised a combination of obstructive and restrictive features on spirometry in association with RLOs.

The strength of this study includes the length of time over which the study was conducted (16 years), as compared to shorter periods described by others [5,6,8,10,14,15]. The study also represents the first systematic attempt to assess concordance between spirometric, imaging and biopsy results, using the 2019 ISHLT classification as a guide. Its major limitation is that it was retrospective, with challenges in matching findings due to differences in timing of diagnostic studies. Another consideration is that patient selection methods and post-transplant management have evolved over that time, especially following changes in the patient allocation process by the Organ Procurement and Transplantation Network in 2005 [27]. The pleuro-parenchymal effects of acute rejection and infection during the earlier years may have resulted in lasting effects as compared to currently more effective management of these complications. A further limitation is that transbronchial biopsies were obtained from random sites, mainly for the purpose of assessing for bronchial obstruction (BOS) and infection in patients who exhibited lung function decline. This method can easily miss patchy parenchymal involvement. Transbronchial lung cryobiopsy has been proposed as an alternative bronchoscopic technique for histological sampling in patients with ILD, potentially combining the higher yield of surgical lung biopsy with the lower complication rate of transbronchial forceps biopsy [19].

In defining the onset of CLAD, we used the first measurement demonstrating FEV1 decline of $\geq 20\%$ from baseline, as stipulated by the 2019 ISHLT consensus document. As did Levy et al. [10], we determined the CLAD phenotype based on continued changes in FEV1 and FVC following

the initial decline in FEV1, as well as imaging findings, which sometimes were not available until 3 months or more after onset of FEV1 decline. As Todd et al. [8] points out in an editorial accompanying the study by Levy et al. [10], the delayed availability of information following the onset of CLAD (as defined by decline in FEV1) challenges ISHLT consensus definitions relevant to clinical findings. Separating the onset of CLAD from defining phenotype may also contribute to bias in survival analysis, a factor that probably contributed to our mortality findings that differed from those of previous studies.

Finally, the definition of phenotypes is relevant to treatment options for both BOS and rCLAD, but the discreet separation of the mixed phenotype from these conditions is based on incomplete data (functional, imaging and histological) and may remain controversial for some time. The magnitude and significance of differences between CLAD syndromes is currently not fully clear. Given the overlap in risk factors, that obstructive bronchiolitis (OB) lesions can sometimes be detected in both syndromes, and the possible evolution of one syndrome to another, there is likely at least some degree of overlap between BOS and rCLAD [6,17]. An important aspect in this practice is early detection of CLAD and the development of tools and biomarkers more sensitive than spirometry and HRCT. Adequately powered clinical trials can help resolve issues, given the disappointing outcomes following lung transplantation as compared to other solid organ transplantations. For example, until recently BOS (by physiologic or TBBX evidence) was considered to be responsive to ECP. This may now only be true for early BOS and not later stages of BOS. The recognition of different phenotypes indicates that the future will probably lie in individualized therapies that may further improve survival.

In light of the recent ISHLT classification of CLAD phenotyping, our findings should be viewed as hypothesis-generating with respect to the association of spirometric changes to imaging and histopathologic findings. Larger studies (ideally prospective) would provide additional information in this regard.

Conclusion

CLAD (as defined by spirometry) can be detected in one-third of LTx patients having undergone LTx. Approximately three-quarters of patients with CLAD exhibited a simultaneous decline in FEV1 and FVC. Spirometry is insensitive in detecting the presence of structural changes found on biopsy and imaging studies, and conversely may be abnormal in the absence of such findings. CT imaging detects structural changes when biopsy findings are mild or not identified (possibly because of sampling error); conversely, most pathologic findings occur in patients who also exhibit imaging findings. In this study, onset of RAS occurred later than in the BOS and mixed phenotypes. Patients with BOS and mixed phenotype had shorter survival times than those with RAS were roughly equal, etc. Classifying phenotypes based on the recent ISHLT guidelines refines the clinical presentation and course of CLAD beyond just BOS and RAS, but results in considerable overlap in its natural history and outcome, and raises questions of its relevance to clinical outcomes and management.

Acknowledgements

The authors thank Dr. Timothy Floreth and Dr. Kamyar Afshar for their helpful comments.

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How to cite this article: Silvia Coronel, Shamili Allam, Neha Teekappanavar, and Ahmed Abdelhalim, et al. "Heterogeneity of Chronic Lung Allograft Dysfunction Phenotypes: Spirometric, Histopathologic and Imaging Associations over a 16-Year Experience." *J Pulm Respir Med* 10 (2021): 522.