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ORIGINAL ARTICLE

Significance of phenotype change after chronic lung allograft dysfunction onset

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SUMMARY

Definitions for chronic lung allograft dysfunction (CLAD) phenotypes were recently revised (2019 ISHLT consensus). Post-CLAD onset phenotype transition may occur as a result of change in obstruction, restriction, or RAS-like opacities (RLO). We aimed to assess the prevalence and prognostic implications of these transitions. This was a single-center, retrospective cohort study of bilateral lung transplants performed in 2009–2015. CLAD phenotypes were determined per ISHLT guidelines. CLAD phenotype transition was defined as a sustained change in obstruction, restriction or RLO. We specifically focused on phenotype changes based on RLO emergence. Association of RLO development with time to death or retransplant were assessed using Kaplan–Meier and Cox proportional hazards models. Among 211 patients with CLAD, 47 (22.2%) experienced a phenotype transition. Nineteen patients developed RLO. Development of RLO phenotype after CLAD onset was associated with a shorter time to death/retransplant when considering the entire CLAD patient cohort (HR = 4.00, CI 2.74–5.83, $P < 0.001$) and also when restricting the analysis to only patients with a Non-RLO phenotype at CLAD onset (HR 9.64, CI 5.52–16.84, $P < 0.0001$). CLAD phenotype change based on emergence of RAS-like opacities implies a worse outcome. This highlights the clinical importance of imaging follow-up to monitor for phenotype transitions after CLAD onset.

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Key words
chronic lung allograft dysfunction, lung transplantation, phenotype change

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Introduction

Chronic lung allograft dysfunction (CLAD) affects 50% of lung transplant patients by 5 years and is the leading cause of death following lung transplantation (LTx) [1]. Within the last decade, different phenotypes of CLAD have been described. Initially, the restrictive allograft syndrome (RAS) was recognized as distinct from the bronchiolitis obliterans syndrome (BOS) because of its worse prognosis [2,3]. The heterogeneous nature of CLAD was recently highlighted by the May 2019 International Society of Heart and Lung Transplant (ISHLT) consensus document [4], standardizing the nomenclature of CLAD and its clinical phenotypes based on

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combinations of obstruction, restriction and presence of RAS-like opacities (RLO) [4]. The diagnostic criteria for BOS and RAS remained largely the same [2,5,6]. The mixed and undefined phenotypes were further added.

Applying the new ISHLT guidelines to our lung transplant recipient population, we have recently confirmed that, similar to prior publications, RAS and mixed phenotypes are associated with worse prognosis compared with BOS [3,4,7]. Furthermore, we demonstrated that the presence of RLO at CLAD onset was associated with worse survival.

The clinical evolution after CLAD onset is overall an important area of study. After CLAD onset, a transition from the initial phenotype to a different phenotype may occur, as previously shown by Sato *et al* [2] and Verleden *et al* [8]. This transition may present as either a change in obstruction, restriction or the emergence of RLO. The nature of these changes is not clearly understood, especially in light of the new phenotype classification.

We, therefore, aimed to comprehensively assess phenotype transitions after CLAD onset and their implications on post-CLAD allograft survival, with a specific focus on the role of RLO emergence as a potentially key prognostic factor. We hypothesized that development of RLO after CLAD onset portents poor outcome.

Patients and methods

Patients

This was a single-center, retrospective cohort study of consecutive adult, first bilateral lung transplants performed between January 1, 2009, and December 31, 2015 (see consort diagram in Fig. 1). The study was approved by the Institutional Research Ethics Board. All patients diagnosed with CLAD were included. Follow-up data were obtained from electronic medical records and computerized databases and censored on December 20, 2019.

Clinical data review

We used a computerized database and electronic patient record to identify all patients who developed CLAD and to extract epidemiological and clinical information, including CLAD onset date, pulmonary function tests (PFTs), and chest computed tomography (CT). FEV1/forced vital capacity (FVC) ratio and total lung capacity (TLC) trajectories from the entire post-CLAD timeline until death/retransplant or censoring. CT images were reviewed to determine the presence of persistent opacities. Acute rejection score (A score) was obtained by summing all pathologic rejection A-grades from transbronchial biopsies and dividing them by the number of evaluable biopsies. Immunologic testing for donor-specific antibodies (DSA), bronchoalveolar lavage (BAL) cultures, and histologic acute cellular rejection A-grades was assessed after CLAD onset to evaluate possible immunologic triggers of phenotype change. The Toronto Lung Transplant Program protocol [9] dictates posttransplant surveillance PFTs to be done weekly in the first three months and monthly thereafter. Chest CTs were routinely performed at 3,6,9,12,18, and 24 months posttransplant. Additional PFTs and chest CTs were done when clinically indicated. Reviews to determine CLAD phenotype transitions were performed by two independent pulmonologists. Disagreements in interpretation of clinical data were resolved by a third reviewer and/or consensus-based discussion.

CLAD definitions

Initial CLAD phenotype determination was based on clinical data closest to the date of CLAD onset, within 3 months after CLAD onset for PFT and 6 months for radiographic studies, as guided by the ISHLT consensus [4] and as described previously [7]. FEV1/FVC<70% was used to define obstruction, and TLC≤90% of baseline was used to define restriction. Opacities on imaging were classified as RLO according to the ISHLT consensus document: "opacities and/or increasing pleural thickening consistent with a diagnosis of pulmonary and/or pleural fibrosis and that are likely to cause the restrictive physiology, rather than airway-based changes consistent with bronchiectasis" [4]. CLAD phenotype definitions were based on the ISHLT consensus document (Table S1) [4]. Briefly, BOS was defined as CLAD with obstruction without RLO, RAS as CLAD with restriction and RLO, mixed phenotype as obstruction with restriction and RLO. The undefined group consisted of patients with obstruction and RLO but no restriction or combined obstruction and restriction without RLO. Patients who did not satisfy the criteria for any of the above 4 distinct phenotypes were labeled as unclassified (Table S1). For the purpose of this study, undefined and unclassified phenotypes were additionally sub-phenotyped radiographically based on the presence or absence of RLO.

CLAD phenotype transitions

To ensure a systematic approach, phenotype transition was identified at the earliest occurrence of a sustained

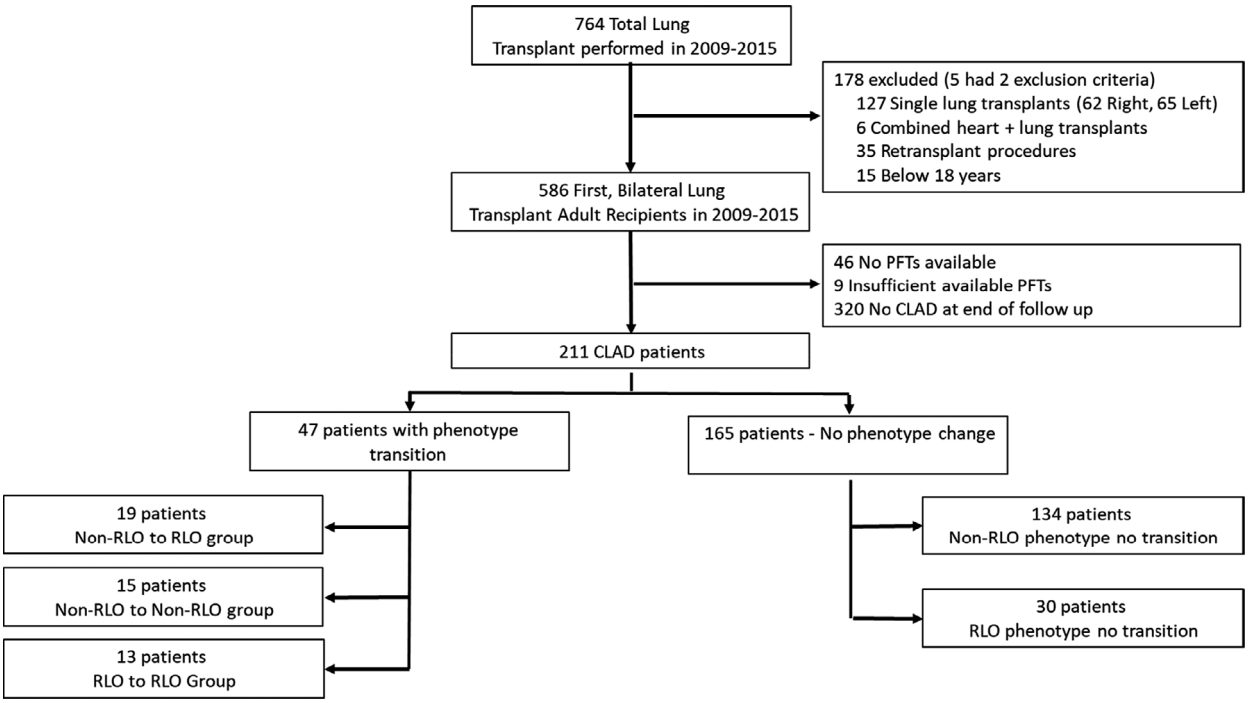

Figure 1 Study cohort flowchart. PFTs = Pulmonary function tests; RLO = RAS-like opacities.

(for at least 3 months) change in at least one of the three determinants of CLAD phenotypes: obstruction, restriction or RLO.

To assess the role of RLO emergence across all phenotype transitions, patients with phenotype change were then grouped into 3 phenotype transition groups according to the presence or absence of RLO: transition from Non-RLO to RLO phenotype (Non-RLO to RLO group), or based on a change in ventilatory defect transition between different Non-RLO phenotypes (Non-RLO to Non-RLO group) or different RLO phenotypes (RLO to RLO group). Grouping process is depicted in Fig. 2.

Statistical analysis

Clinical characteristics were assessed as counts and percentages for categorical variables, and as standard measures (median and interquartile range (IQR)) for continuous variables. To compare baseline characteristics between groups, we used Wilcoxon rank-sum test for continuous variables and Chi-squared test for categorical variables. Univariable and multivariable Cox proportional hazards models were used for evaluating the effect of phenotype change to an RLO-based phenotype, modeled as a time-dependent variable from CLAD onset to death/retransplant. Variables selected *a priori* for model inclusion were age, sex, native lung disease, and donor-recipient cytomegalovirus (CMV) serostatus match based on their previous association with post-lung-transplant outcomes [10–13]. For illustration purposes, we provide Kaplan–Meier survival curves. All analyses were conducted using R statistical software version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as a two-tailed P value less than or equal to 0.05.

Results

Study cohort

Of 584 adult, first, bilateral LTx recipients transplanted between January 1, 2009, and December 31, 2015, 211 patients (36.1%) developed CLAD by the time of data censoring. The median time from transplant to CLAD onset was 758 days (IQR 380–1395), and the median time from CLAD onset to death or retransplant was 463 days (IQR 244–746).

Among the 211 subjects with CLAD, median age at the time of transplant was 54 (IQR 37–62) and 98 (46.4%) were females. The most common presenting phenotype at time of CLAD onset was BOS (134 patients), followed by the undefined phenotype (24, of these 13 without RLO and 11 with RLO), RAS (21), and mixed (8). Twenty-four patients who developed CLAD did not meet any of the CLAD phenotype

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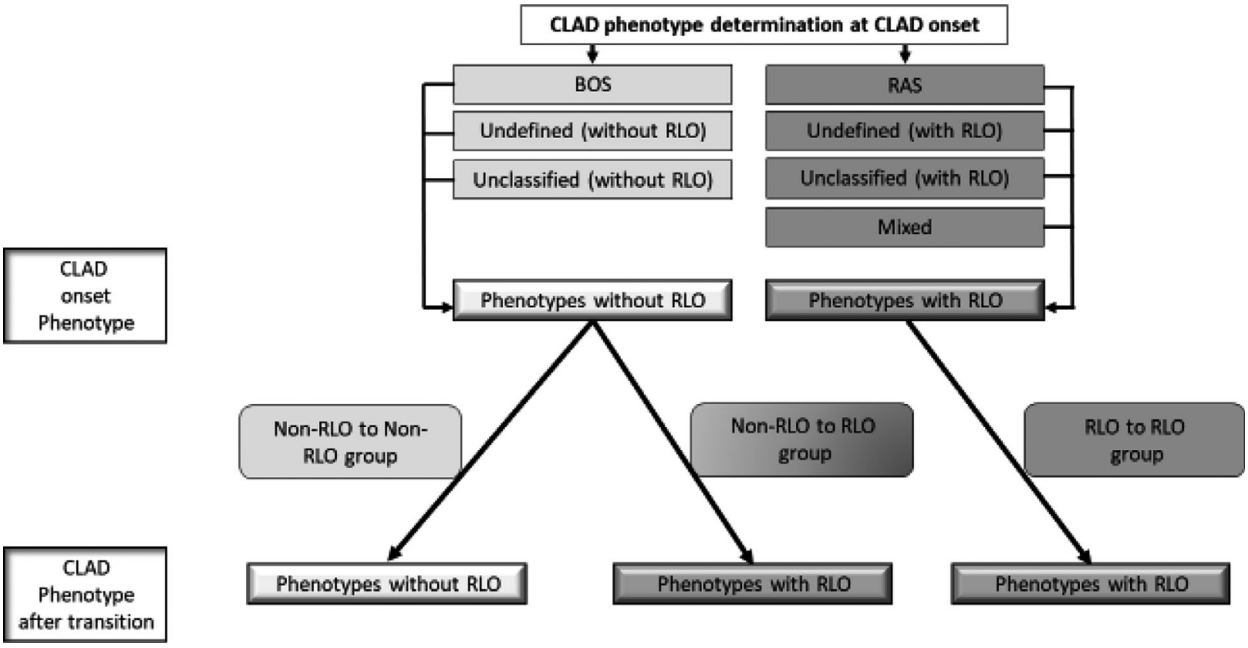

Figure 2 CLAD phenotype transition grouped radiologically according to the presence of RAS-like opacities (RLO). BOS = Bronchiolitis obliterans syndrome; RAS = Restrictive allograft syndrome; RLO = RAS-like opacities.

criteria and remained unclassified: of these, 3 had RLO at CLAD onset (see consort diagram in Fig. 1).

Phenotype transition

Among the 211 CLAD patients, 47 (22.2%) had a phenotype change after CLAD onset and these transitions included almost all combinations of phenotypes (Table 1). Specifically, of all BOS patients in our cohort, 23 (17%) underwent a transition: 9 to RAS, 1 to mixed, 10 to undefined, and 3 to unclassified. Eight patients with RAS (38%) underwent a transition: 4 to mixed, 3 to undefined, and 1 to unclassified. Only 1 patient with a mixed phenotype (13%) underwent a transition to RAS. Ten patients with undefined phenotype (42%) underwent a transition: 5 to BOS, 2 to mixed, and 3 to unclassified. Five unclassified patients (21%) transitioned to BOS (1), mixed, (2), RAS (1), and undefined (1). Radiologic examples of such phenotype transitions are shown in Fig. 3.

Phenotype grouping based on RLO

In order to better assess the role of RLO emergence during phenotype transitions, which we hypothesized would be the most clinically significant feature of phenotype change, we further subdivided the undefined and unclassified phenotypes based on the presence or absence of RLO. We then grouped the phenotypes based on RLO at CLAD onset and RLO at transition (Table 2 is an expansion of Table 1 to illustrate this concept). Of the 47 patients who underwent CLAD phenotype transition, 19 patients without RLO at CLAD onset had a transition to RLO (Non-RLO to RLO group). The other transitions were based solely on a change in obstruction or restriction with 15 patients transitioning between Non-RLO phenotypes (Non-RLO to Non-RLO group) and 13 patients transitioning between RLO phenotypes (RLO to RLO group). There were no patients who transitioned form RLO to Non-RLO. Median time from CLAD onset to transition was 312 days (IQR 175–669). Of the 164 patients who did not have a phenotype change, 30 had RLO.

Clinical characteristics

Clinical characteristics comparing patients with or without phenotype change, categorized based on the presence or absence of RLO, are summarized in Table 3. There were no statistically significant differences between groups in age ($P = 0.074$), sex ($P = 0.687$), native lung disease ($P = 0.410$) or CMV mismatch rate ($P = 0.744$). Moreover, physiologic and pathologic parameters did not differ significantly in baseline FEV1 ($P = 0.272$), baseline FVC ($P = 0.556$), baseline TLC ($P = 0.447$) and acute rejection by mean A score ($P = 0.143$). Time from transplant to CLAD onset was

Table 1. Transitions between CLAD phenotypes post-CLAD onset.

Transition to (47) Primary phenotype (n = 211)	BOS (6)	Undefined total (14)	unclassified total (7)	Mixed (17)	RAS (3)	No transition
BOS (134)	-	10	3	9	1	111
Undefined total (24)	5	-	3	2		14
unclassified total (24)	1	1	-	2	1	19
Mixed (8)				-	1	7
RAS (21)		3	1	4	-	13

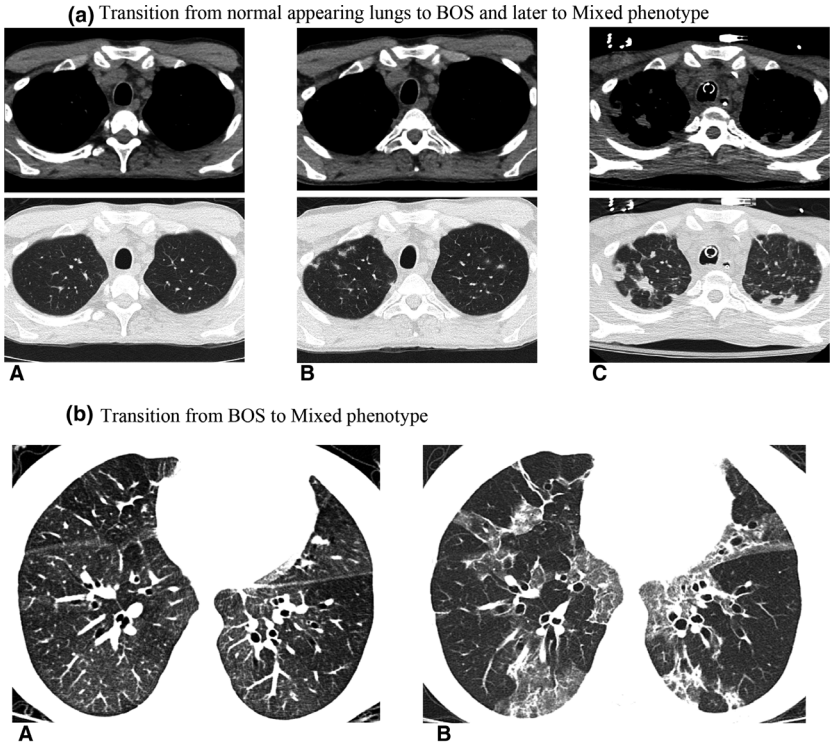

Figure 3 Radiology examples of phenotype transition from Non-RLO- to RLO-based phenotype. (a) Transition from normal appearing lungs to BOS and later to mixed phenotype. (A) Normal appearing lungs following double-lung transplantation. (B) The same patient was later diagnosed with CLAD and BOS type based on spirometry and minimal opacities. Centrilobular clustered nodules were seen. (C) The appearance of RAS-like opacities on lung windows with pleural thickening on mediastinal windows suggested the phenotype change from BOS to MIXED. Due to respiratory failure, patient required mechanical ventilation. (b) Transition from BOS to mixed phenotype. Double-lung transplant with CLAD, BOS type, showing air trapping (A), changing to MIXED phenotype with worsening bronchiectasis and new ground-glass opacities (B).

longest for patients without phenotype transition without RLO ($P < 0.001$). Additionally, the groups differed in the frequency of PFT measurements post-CLAD onset, which was highest in the Non-RLO to RLO group ($P < 0.001$), probably because of the clinical deterioration. Time from CLAD onset to transition was longest in the Non-RLO to RLO group compared with other transition groups ($P < 0.001$).

CLAD patients without phenotype change

Consistent with previously published data, for CLAD patients who did not have a post-CLAD onset transition, the presence of RLO at CLAD onset was associated with a significantly shorter time from CLAD onset to death/retransplant compared with the absence of RLO (HR = 4.31; CI 2.69–6.89; $P < 0.0001$ and HR = 4.20; CI 2.58–6.82; $P < 0.0001$ in univariable and multivariable analysis, respectively).

Potential trigger events preceding phenotype change

In order to explore potential etiologies of phenotype change, we assessed de novo DSA, rejection, and infection events in the time period between CLAD onset and phenotype change (Table 3). Within the entire study

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Table 2. Transitions between CLAD phenotypes post-CLAD onset, grouped based on RAS-like opacities (RLO).

		Phenotypes without RLO (15)			Phenotypes with RLO (32)				
Transition to (n = 47) Primary CLAD phenotype (n = 211)		BOS (6)	Undefined without RLO (4)	Unclassified without RLO (5)	Unclassified with RLO (2)	Undefined with RLO (10)	Mixed (17)	RAS (3)	No transition
Phenotypes without RLO phenotypes (168)	BOS (134)	-	3	3		7	9	1	111
	Undefined without RLO (13)	5	-	2			1		5
	Unclassified without RLO (21)	1	1	-			1		18
Phenotypes with RLO (43)	Unclassified with RLO (3)				-		1	1	1
	Undefined with RLO (11)				1	-	1		9
	Mixed (8)						-	1	7
	RAS (21)				1	3	4	-	13

cohort, 25 patients had evidence of de novo DSA following CLAD onset. Among the 47 patients who experienced a phenotype change, 8 patients developed de novo DSA following CLAD onset. Of these 8 patients, 5 developed de novo DSA between CLAD onset and phenotype change and belonged to the following categories: 2 (12%) in the Non-RLO to RLO group, 2 (22%) in the RLO to RLO group, and one (11%) in Non-RLO to Non-RLO group.

A1 or higher-grade histologic acute cellular rejection on bronchoscopic transbronchial biopsies was observed post-CLAD onset in 42 of all study patients. Twelve of them were among patients with phenotype change and occurred between CLAD onset and phenotype transition. The Non-RLO to RLO group (47% of patients in this group) had the highest rate of acute cellular rejection among all groups (P = 0.016).

Positive BAL cultures for significant respiratory pathogens were found in 104 patients (49%) post-CLAD onset. The rate was highest among the Non-RLO to RLO group (74%), P = 0.023. Among phenotype change patients, 28 had positive BAL documented infection episodes post-CLAD onset, 20 of them happened between CLAD onset and phenotype change, also mainly in the Non-RLO to RLO group (11 patients, 58%).

Prognostic significance of RLO development after CLAD onset

A total of 19 patients changed their phenotype from a Non-RLO phenotype (17 BOS, 1 undefined, and 1 unclassified) to a phenotype with RLO (1 RAS, 11 mixed, and 7 undefined with RLO) (Table 2). The most common transition among this group was from BOS to mixed (9 patients). Median time from CLAD onset to transition was 140 days (CI 77–383).

The Non-RLO to RLO phenotype transition group had the largest FEV1 drop of 15.4% within 3 months post-CLAD onset, which was similar to the 14.4% FEV1 drop in the RLO group without transition. Other groups had a much more gradual FEV1 decline of 4%–7% during this early post-CLAD period.

All 19 patients either died (8 patients) or underwent retransplantation (11 patients) during follow-up. Survival and retransplantation rates for all patient groups at censoring date are delineated in Table S2.

We hypothesized that Non-RLO to RLO phenotype transition has an important negative impact on survival in our cohort. We, therefore, performed three complementary analyses to explore the role of this transition:

First, we wanted to visualize post-CLAD onset survival in all five groups, that is, RLO and Non-RLO phenotypes without transition as well as all 3 transition groups. We also wanted to visualize the survival after CLAD phenotype transition in those patients who had a phenotype change. For illustration purposes, Kaplan–Meier curves are shown in Fig. 4a and b and suggest that RLO emergence is associated with worse survival after CLAD onset. We did not perform any statistical analyses to compare these Kaplan–Meier curves because of the inherent survival bias in the phenotype transition groups.

Next, we set off to formally assess the association between RLO emergence and post-CLAD onset survival. We used a Cox proportional hazards model with RLO as a time-dependent variable in all 211 patients with CLAD.

Table 3. Patient characteristics, grouped according to RAS-like opacities (RLO).

	Non-RLO		Non-RLO transition to RLO	RLO from CLAD onset		
	No transition (Non-RLO) *N* = 134	Transition between Non-RLO Group *N* = 15	Transition Non-RLO to RLO *N* = 19	No Transition (with RLO) *N* = 30	Transition between RLO Group *N* = 13	*P* value*
Age at transplant, median (IQR)	56 (43–62)	59 (37–62)	48 (28–58)	44 (31–60)	48 (29–59)	0.074
Sex, female, *n* (%)	62 (46)	6 (40)	10 (53)	12 (40)	8 (61)	0.687
Native lung disease, *n* (%)						0.410
COPD/emphysema	39 (29)	4 (27)	4 (21)	5 (17)	4 (31)	
Pulmonary fibrosis	46 (34)	7 (46)	4 (21)	10 (33)	4 (31)	
CF/bronchiectasis	25 (19)	4 (27)	8 (42)	11 (37)	4 (31)	
Other (sarcoidosis, PHTN, BO, scleroderma)	24 (18)	0	3 (18)	4 (13)	1 (7)	
CMV Mismatch, *n* (%)	33 (25)	2 (13)	6 (32)	9 (30)	3 (23)	0.744
Baseline PFTs (Median, IQR)						0.272
FEV_1 (L)	2.43 (1.8–3.0)	3.1 (2.2–3.5)	2.55 (2.2–3.5)	2.46 (1.9–2.8)	2.45 (1.6–3.5)	
FVC (L)	3.10 (2.3–3.8)	3.45 (2.8–4.4)	3.13 (2.8–4.2)	3.02 (2.5–3.77)	2.91 (1.8–3.9)	0.556
TLC (L)	4.7 (3.9–5.7)	5.9 (4–6.7)	5.1 (4.3–5.8)	4.91 (4–5.6)	4.6 (3.2–6.5)	0.447
FEV_1 at CLAD onset (%baseline) (Median, IQR)	72.8 (66.7–76.8)	70.1 (64.5–75.9)	72.7 (61.8–77.1)	69 (58.7–75.7)	71.2 (65.4–77.9)	0.579
FEV_1 closest to 3 months post CLAD onset (% baseline) (Median, IQR)	66 (55.2–73.3)	68.6 (52.1–72.9)	55.3 (50–67.7)	56.2 (46.6–66.1)	70 (52.9–75.2)	0.018
Decline in FEV_1 (%baseline) over 3 months after CLAD onset (Median, IQR)	4.9 (0.1–14.2)	7.2 (−2.8–17.4)	15.4 (2.1–25.5)	14.4 (6.1–20.7)	4.6 (−2.8–11.8)	0.06
FEV_1 at time of phenotype change (%baseline) (Median, IQR)	n/a	58.1 (43.3–63.6)	33.4 (21.9–50)	n/a	56.9 (49.4–72.7)	0.001
Acute Rejection Score over the entire post-transplant time: (A Score, mean)	0.32	0.21	0.44	0.28	0.38	0.143
De novo DSA after CLAD onset (# of patients/# of patients with at least one antibody measurement, %)	14/79 (17.7%)	3/12 (25%)	3/18 (16.7%)	3/22 (13.6%)	2/12 (16.7%)	0.950
De novo DSA between CLAD onset and phenotype change (# of patients/# of patients with at least one antibody measurement, %)	n/a	1/9 (11.1%)	2/17 (11.8%)	n/a	2/9 (22.2%)	0.732
Histologic evidence of acute cellular rejection after CLAD onset (# of patients/# of patients with at least one available TBB, %)	20/134 (14.9%)	1/15 (6.7%)	9/19 (47.4%)	8/30 (26.6%)	4/13 (30.8%)	0.006
Histologic evidence of acute cellular rejection between CLAD onset and phenotype change (# of patients/# of patients with at least one available TBB, %)	n/a	1/15 (6.7%)	9/19 (47.4%)	n/a	2/13 (15.4%)	0.016

Table 3. Continued.

	Non-RLO		Non-RLO transition to RLO	RLO from CLAD onset		
	No transition (Non-RLO) *N* = 134	Transition between Non-RLO Group *N* = 15	Transition Non-RLO to RLO *N* = 19	No Transition (with RLO) *N* = 30	Transition between RLO Group *N* = 13	*P* value*
BAL with significant infectious pathogen after CLAD onset (# of patients/# of patients with at least one available BAL, %)	56/134 (41.8%)	7/15 (46.7%)	14/19 (73.7%)	20/30 (66.6%)	7/13 (53.8%)	0.023
BAL with significant infectious pathogen between CLAD onset and phenotype change (# of patients/# of patients with at least one available BAL, %)	n/a	6/15 (40%)	11/19 (57.9%)	n/a	3/13 (23.1%)	0.143
Time from CLAD onset to first post-CLAD PFT, days, median (IQR)	29 (15,49.75)	18 (11,57.5)	21 (10.5,33)	21 (14,32.75)	28 (19,35)	0.422
Time from CLAD onset to Last PFT before death or censoring, days, median (IQR)	692 (303, 1298)	755 (497, 1499)	629 (466, 1297)	217.5 (96, 407)	576 (343, 977)	<0.001
Post-CLAD onset number of PFT measurements						
FEV_1	12 (5,20)	14 (7.5,29)	21 (9,26)	5.5 (3.25,9.5)	12 (8,16)	<0.001
FVC	11.5 (5,20)	14 (7.5,29)	21 (9,26)	5.5 (3.25,9.5)	12 (8,16)	0.001
TLC	6 (2,10.75)	6 (4,10.25)	9 (6,12.5)	4 (2,5.5)	6 (4,8)	0.051
Time from transplantation to CLAD Onset, days, median (IQR)	924 (399 – 1529)	813 (486–1715)	497 (276–1243)	641 (281–1144)	564 (404–1018)	<0.001
Time from CLAD onset to transition, days, median (IQR)	n/a	213 (143–971)	591 (332–820)	n/a	168 (118–273)	<0.001
Time from transition to death or retransplant, days, median (IQR)	n/a	333 (137–422)	140 (77–383)	n/a	240 (130–489)	0.006
Time form CLAD onset to death or retransplant, days median (IQR)	423 (201–760)	537 (339–599)	771 (521–1429)	359 (150–492)	481 (273–650)	<0.001

#, number; BO, Bronchiolitis obliterans; CF, Cystic fibrosis; CMV, Cytomegalovirus; COPD, Chronic o^bstructive pulmonary disease; FEV_1, Forced expiratory volume in 1 second; FVC, Forced vital capacity; IQR, interquartile range; n/a, not applicable; PFTs, Pulmonary function tests; PHTN, Pulmonary arterial hypertension; RLO, RAS-like opacities; TBB, Trans Bronchial Biopsies.

*Chi-Square test was used for categorical data and Kruskal–Wallis test for continuous outcomes. All 5 groups were compared, with the exceptions where only the 3 transition groups were compared.

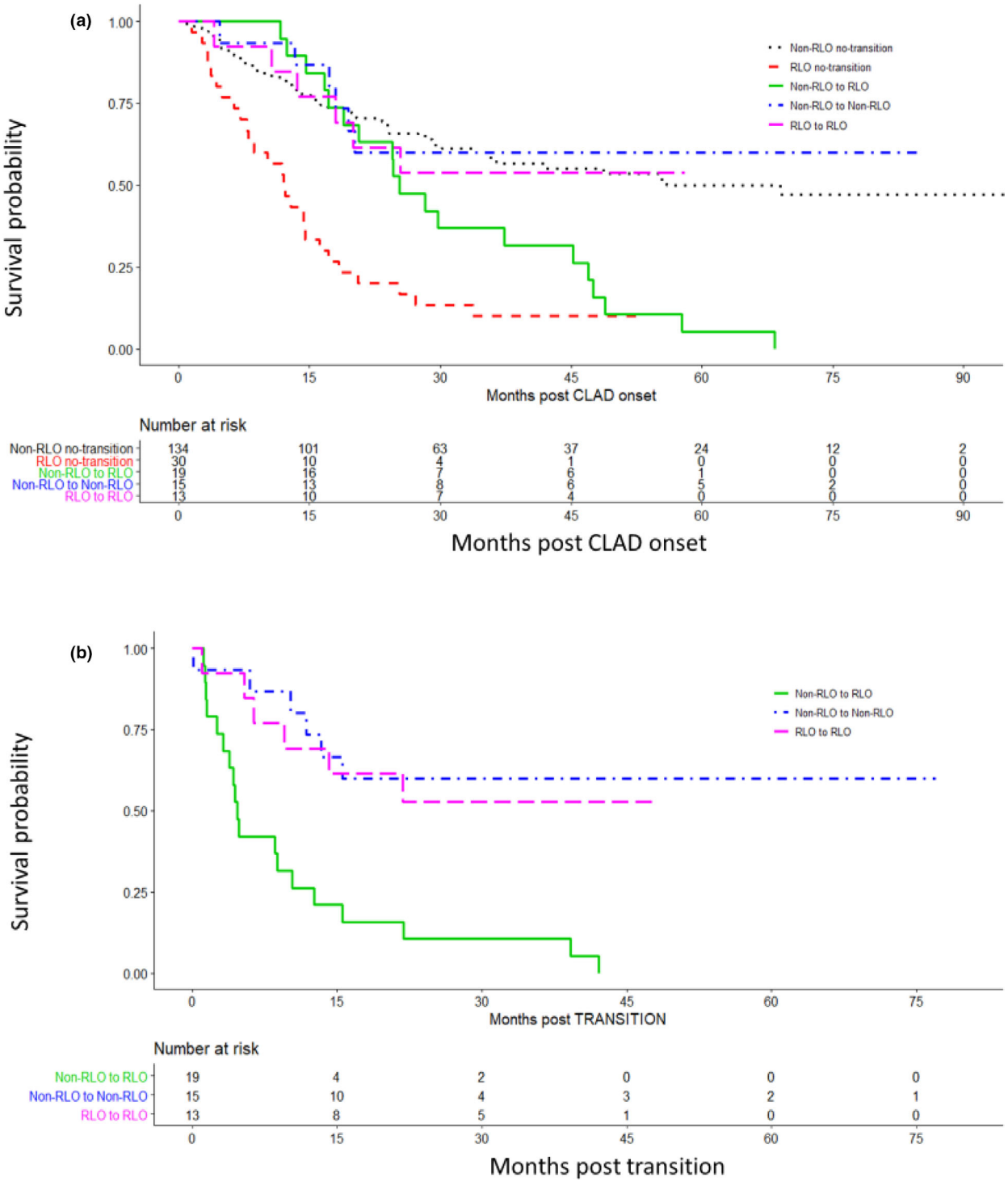

Figure 4 Kaplan–Meier curves showing time to death or retransplant. (a) Survival curves for all groups, from CLAD onset. Non-RLO no transition – patients started with Non-RLO phenotype without transition; RLO no transition – patients started with RLO phenotype without transition; Non-RLO to RLO - patients transitioned from Non-RLO phenotype to RLO phenotype; Non-RLO to Non-RLO – transition between 2 different Non-RLO phenotypes; RLO to RLO - transition between 2 different RLO phenotypes. Worse outcome seen in Non-RLO to RLO and RLO-RLO groups. (b) Survival curves from transition time for the 3 groups with phenotype change: Non-RLO to RLO, Non-RLO to Non-RLO, and RLO to RLO. Worse outcome after transition time seen in Non-RLO to RLO group (patients transitioned from Non-RLO phenotype to RLO phenotype).

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Table 4. Assessment of RLO phenotype as a prognostic variable in a time-dependent analysis in all CLAD patients. Non-RLO phenotype used as reference. Univariable and multivariable Cox proportional hazards models are shown.

Univariable			Multivariable			
HR	CI	*P* value		HR	CI	*P* value
4.05	2.8–5.86	<0.01	RLO status	4.00	2.74–5.83	<0.01
			Age (at transplant)	0.99	0.977–1.00	0.14
			Sex (female as reference) male	1.04	0.71–1.54	0.83
			Underlying disease (COPD as reference)			
			Cystic fibrosis	1.06	0.51–2.20	0.88
			Pulmonary fibrosis	0.96	0.56–1.64	0.58
			Other	1.19	0.65–2.19	0.57
			CMV mismatch*	1.72	1.15–2.57	<0.01

COPD, Chronic obstructive pulmonary disease; CMV, Cytomegalovirus; RLO, RAS-like opacities.
*CMV mismatch (D+/R-) vs other combinations.

Table 5. Assessment of RLO phenotype as a prognostic variable in a time-dependent analysis in only those patients with a Non-RLO phenotype at CLAD onset. Non-RLO phenotype used as reference. Univariable and multivariable Cox proportional hazards models are shown.

Univariable			Multivariable			
HR	CI	*P* value		HR	CI	*P* value
9.7	5.6–16.79	<0.0001	9.	9.64	5.52–16.84	<0.0001
			Age (at transplant)	0.98	0.001–1.89	0.06
			Sex (female as reference) male	0.98	0.23–0.81	0.94
			Underlying disease COPD as reference)			
			Cystic fibrosis	0.83	0.40–0.47	0.65
			Pulmonary fibrosis	0.79	0.31–0.74	0.46
			Other	0.95	0.12–0.36	0.9
			CMV mismatch*	1.4	0.24–1.38	0.17

COPD, Chronic obstructive pulmonary disease; CMV, Cytomegalovirus; RLO, RAS-like opacities.
*CMV mismatch (D+/R-) vs other combinations.

The appearance of RLO-based phenotype was associated with a shorter time to death or retransplantation from the time of CLAD onset, as depicted in Table 4 (univariable: HR 4.05, CI 2.8–5.86, $P < 0.001$; multivariable: HR 4.00, CI 2.74–5.83, $P < 0.001$) (Table 4).

Then, we wanted to focus on the role of RLO emergence after CLAD onset only in patients who started with a Non-RLO phenotype. In a Cox proportional hazards model with RLO as a time-dependent variable in the subset of all patients who were classified as Non-RLO at CLAD onset, the transition to RLO-based phenotype was associated with a shorter time to death/retransplant from the time of CLAD onset, as depicted in Table 5. (Univariable: HR 9.7, CI 5.6–16.79, $P < 0.0001$; multivariable: HR 9.64, CI 5.52–16.84, $P < 0.0001$) (Table 5).

Discussion

In this study, we used the new 2019 ISHLT CLAD classification scheme to describe phenotype changes after CLAD onset. We provide detailed descriptions of the various phenotype transitions and demonstrate that a shift from a phenotype without RAS-like opacities to a phenotype with RAS-like opacities, mainly BOS to mixed or undefined, predicts shorter survival. While the transition from BOS to mixed has been described previously [8], we provide a more granular and comprehensive description of all phenotype transitions, applying strictly the 2019 ISHLT phenotyping guidelines. For analysis purposes, we focused on the change from phenotypes without RAS-like opacities (Non-RLO phenotypes) to phenotypes with RAS-like opacities (RLO

phenotypes), as we hypothesized that this transition would have the worst prognosis.

In our study, the proportion of patients who transitioned between CLAD phenotypes was 22.2 percent (47/211 patients with CLAD). The most common phenotype transition was from BOS to mixed (9 patients) followed by BOS to undefined with RLO (7 patients); these two transitions combined, making 34% of all phenotype transitions, and 7.5% of all CLAD cohort comprised most of the Non-RLO to RLO group. This finding is comparable to the phenotype transition rate reported by Sato *et al* [2]. A recent study published by Verleden *et al* [8] has shown similar results, with 9% of the entire CLAD cohort (11% of BOS cohort) evolving from an initial BOS phenotype to a mixed phenotype. In our cohort, there were no transitions of phenotype that involved a resolution of RLO. This makes sense as RLO represent irreversible fibrotic changes that classically manifest as pleuroparenchymal fibro-elastosis (PPFE) on pathologic examination.

We explored potential triggers of phenotype transition by analyzing DSA, histologic acute cellular rejection, and BAL infectious pathogens after CLAD onset. Between CLAD onset and phenotype change, the Non-RLO to RLO group had the highest rate of positive significant BAL cultures (57.8%) and grade A1 or higher rejection on transbronchial biopsies (47.4%). There were also 2 patients (11%) in this group who developed de novo DSA during that time. These events may partly explain or contribute to driving a transition to the more aggressive phenotype with RAS-like opacities. Future studies with larger cohorts are required to validate these findings.

We also assessed the rapidity of FEV1 decline after CLAD onset in different groups of patients. Compared with the other phenotype transition groups, the most significant FEV1 drop was documented in the Non-RLO to RLO group (15.4 percent) in the first 3 months post-CLAD onset. A similarly high rate of early FEV1 decline post-CLAD onset was seen in the RLO group without transition, supporting the concept that RAS-like opacity development is a particularly rapid and aggressive process of allograft fibrosis.

We then set off to assess survival in the different patient groups. Recent data published by Verleden *et al* [8] demonstrated similar survival for CLAD patients who had RAS "ab initio" and BOS patients with a later transition to a mixed phenotype. However, as acknowledged by the authors, this analysis was confounded by survival bias. To avoid this survival bias, we decided to model transition to an RLO phenotype as a time-dependent variable. Using this approach, in our study, the emergence of an RLO-based phenotype was associated with a significantly worse survival, suggesting that RLO represent a poor prognostic indicator even if they develop after CLAD onset.

It has been previously shown that in patients with RAS phenotype the median survival is only 6 to 18 months, compared with 3 to 5 years for BOS [3,7]. Our data further expand on this finding. The presence of RLO is associated with shorter time from CLAD onset to death or retransplant, as demonstrated in our recent publication by Levy *et al* [7]. Now, we clearly show that emergence of RLO at any time, at or after CLAD onset, is associated with worse survival in our time-dependent analysis (Table 4 $P < 0.001$ and Table 5 $P < 0.0001$). Hence, our findings suggest that CLAD phenotype change involving post-CLAD onset emergence of RLO is the most important outcome determinant and may even identify these patients as distinct "rapid deteriorators," which was a subgroup of interest proposed in the 2019 consensus document [4]. In most patients who had a transition from a Non-RLO- to RLO-based phenotype, the transition occurred within half-a-year post-CLAD onset (median of 140 days). Accordingly, we would recommend a follow-up chest CT at 3 months intervals during the first year following CLAD onset.

Current treatment options for CLAD are disappointingly scarce. A better understanding of different CLAD phenotype transitions on allograft survival post-CLAD onset is of paramount importance. It can help guide the surveillance protocol for monitoring disease progression and may allow tailoring the therapeutic regimen in future. Whether these transitions represent a true biologic and physiologic change or simply a delayed onset of the definitive phenotype is not yet clear. Further studies aiming to identify a "signatory" biologic marker typical for the specific phenotypes are underway and may shed some light on this question. This may also help to enhance our understanding on the true nature of these transitions [14]. We did not identify any specific differences in baseline clinical characteristics between patients that underwent various phenotype changes, except the fact that patients who transitioned to an RLO phenotype had earlier post-transplant onset of CLAD. However, post-CLAD onset analysis identified acute cellular rejection and infection episodes as potential triggers of the transition from Non-RLO to RLO phenotypes. Ongoing aggressive prevention of such triggers may be beneficial in patients who develop CLAD.

This study has a few noteworthy limitations: first, it is limited by the large number of distinct types of transitions, leading to small numbers of patients in each specific transition group. Therefore, statistical power to assess each detailed group was insufficient. We addressed this issue by combining the types of transitions based solely on the presence or absence of RLO. Second, frequency of PFT measurements varied between patients, mostly because of patient location, access to healthcare, severity of disease or worsening status. This may have influenced assessment of transition time. Third, the retrospective nature of this single-institution experience may limit generalizability of the results.

Conclusions

A transition of CLAD patients from a phenotype with RAS-like opacities to a phenotype without RAS-like opacities, including but not limited to a BOS to RAS/mixed transition, portends worse outcome. Our findings indicate the clinical importance of follow-up imaging studies in CLAD patients. We suggest vigilant imaging surveillance at 3 months intervals for CLAD patients to monitor for the emergence of RAS-like opacities, mainly in the first year post-CLAD onset. Additionally, it will be important to record these changes in future CLAD studies as the effects of specific therapeutic approaches may vary by CLAD phenotype.

Authorship

All authors have contributed substantially to the conception, data gathering, analysis and interpretation of the data. All authors had participated in the drafting and / or revising it and approved its final version to be published. All authors have agreed to be accountable for all aspects of the work.

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Conflict of interest

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Phenotypes of chronic lung allograft dysfunction.

Table S2. End-of-follow-up survival and retransplantation rates.

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