



ORIGINAL CLINICAL SCIENCE

Telomere length in patients with pulmonary fibrosis associated with chronic lung allograft dysfunction and post-lung transplantation survival

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BACKGROUND: Prior studies have shown that patients with pulmonary fibrosis with mutations in the telomerase genes have a high rate of certain complications after lung transplantation. However, few studies have investigated clinical outcomes based on leukocyte telomere length.

METHODS: We conducted an observational cohort study of all patients with pulmonary fibrosis who underwent lung transplantation at a single center between January 1, 2007, and December 31, 2014. Leukocyte telomere length was measured from a blood sample collected before lung transplantation, and subjects were stratified into 2 groups (telomere length < 10th percentile vs \geq 10th percentile). Primary outcome was post-lung transplant survival. Secondary outcomes included incidence of allograft dysfunction, non-pulmonary organ dysfunction, and infection.

RESULTS: Approximately 32% of subjects had a telomere length < 10th percentile. Telomere length < 10th percentile was independently associated with worse survival (hazard ratio 10.9, 95% confidence interval 2.7–44.8, $p = 0.001$). Telomere length < 10th percentile was also independently associated with a shorter time to onset of chronic lung allograft dysfunction (hazard ratio 6.3, 95% confidence interval 2.0–20.0, $p = 0.002$). Grade 3 primary graft dysfunction occurred more frequently in the < 10th percentile group compared with the \geq 10th percentile group (28% vs 7%; $p = 0.034$). There was no difference between the 2 groups in incidence of acute cellular rejection, cytopenias, infection, or renal dysfunction.

CONCLUSIONS: Telomere length < 10th percentile was associated with worse survival and shorter time to onset of chronic lung allograft dysfunction and thus represents a biomarker that may aid in risk stratification of patients with pulmonary fibrosis before lung transplantation.

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Since implementation of the current lung allocation score system, pulmonary fibrosis has become the leading indication

for lung transplantation.^{1,2} Patients with idiopathic pulmonary fibrosis (IPF) account for the largest proportion of patients awaiting, and dying while awaiting, lung transplantation. Despite rigorous pre-transplant evaluation and selection, post-lung transplant median survival is 5.7 years for all recipients and only 4.7 years for recipients with pulmonary fibrosis.² One of the main limitations to survival after lung

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transplantation is chronic lung allograft dysfunction (CLAD). Treatment of CLAD is challenging because its underlying pathogenesis is not well understood. Immunosuppressive medications are used to prevent and treat rejection, but these medications are commonly associated with many side effects.

Telomeres consist of nucleotide repeats (TTAGGG) located on the end of chromosomes that serve to protect these ends during cell replication. Telomeres normally shorten with age, but excessive shortening can lead to activation of DNA damage-signaling pathways resulting in cellular senescence.³ Pathogenic rare variants in several different genes in the telomere pathway (*TERT*, *TERC*, *PARN*, *RTEL1*, *NAFI*) are found in patients with familial pulmonary fibrosis.^{4–7} Heterozygous mutations in these genes are associated with short telomere lengths and a rapidly progressive form of pulmonary fibrosis, which is most commonly characterized as IPF.⁸ Small observational studies have found that patients with pulmonary fibrosis with *TERT* or *TERC* mutations have high rates of bone marrow failure, infection, renal dysfunction, and allograft dysfunction after lung transplant.^{9–11} However, these studies were limited by small numbers of patients and the absence of a comparison cohort.

Although pathogenic mutations are rare, short telomere lengths are relatively common in patients with pulmonary fibrosis. Age-adjusted telomere lengths <10th percentile of normal are found in approximately 40% of patients with familial pulmonary fibrosis and approximately 25% without a family history of lung fibrosis.^{12,13} As the side effects of immunosuppression medications overlap the broad clinical spectrum of short telomere syndromes, it can be difficult to identify the clinical phenotypes that are specifically related to intrinsic patient characteristics. In this study, we sought to characterize clinical outcomes associated with age-adjusted telomere length in patients with pulmonary fibrosis who underwent lung transplantation. We hypothesized that short telomere lengths would be associated with shorter post-transplant survival times and higher rates of allograft dysfunction, non-pulmonary organ dysfunction, and infection.

Methods

This prospective observational cohort study included patients from the University of Texas Southwestern Medical Center (Dallas, TX). All patients provided written informed consent and provided a sample of blood on enrollment. Patients were recruited without regard to family history or any clinical manifestation of a short telomere syndrome. Each patient underwent lung transplantation between January 1, 2007, and December 31, 2014. Patients were excluded if they did not have a pre-transplant diagnosis of pulmonary fibrosis, underwent transplantation elsewhere, or were enrolled after transplantation.

Clinical information was retrospectively extracted from the electronic medical record. All patients were maintained on a 3-drug immunosuppression regimen including a calcineurin or mammalian target of rapamycin inhibitor (cyclosporine, tacrolimus, or sirolimus), an anti-metabolite (azathioprine or mycophenolate mofetil), and a corticosteroid (prednisone). Protocol-driven patient

assessments included serial laboratory tests, pulmonary function tests, and surveillance bronchoscopies.

Clinical variable definitions

Survival time was calculated from date of transplant to death or censor date (September 30, 2015). Cause of death was adjudicated by transplant pulmonologists (V.K., F.T.). The presence and severity of primary graft dysfunction (PGD) was determined by degree of hypoxemia and by chest radiography at 48 and 72 hours.¹⁴ Grade 3 PGD was defined as a ratio of partial arterial pressure of oxygen to fraction of inspired oxygen of <200 mm Hg and pulmonary infiltrates on chest x-ray.¹⁵ PGD could not be assessed in 18 patients because of missing fraction of inspired oxygen data (8 in the <10th percentile group; 10 in the ≥10th percentile group). Acute cellular rejection (ACR) was determined by histopathologic evaluation of transbronchial biopsy specimens.¹⁶ The ACR score represents the sum of the histopathologic “A” scores divided by the number of biopsies.¹⁷ Clinical rejection was defined as an acute deterioration in allograft function as evidenced by spirometric decline or worsened chest imaging; supporting histopathologic evidence was not required. CLAD was defined as a persistent decline in forced expiratory volume in 1 second <80% of baseline (average of 2 best forced expiratory volume in 1 second values after transplantation) that was not due to infection or ACR.¹⁸ Time to onset of CLAD was calculated from the date of transplant. CLAD was further subdivided into bronchiolitis obliterans syndrome and restrictive-CLAD (R-CLAD), which were defined by a forced vital capacity of ≥80% for bronchiolitis obliterans syndrome or <80% baseline forced vital capacity for R-CLAD at the time of CLAD onset.¹⁹

Infection was defined as the presence of pathogens isolated from sterile sites or presence of virus from nasal or respiratory specimens. Cytopenias were defined as leukopenia (white blood cell count <4,000/μl), anemia (hemoglobin <12.0 g/dl for women and <12.4 g/dl for men), thrombocytopenia (platelet count <150,000/μl), and macrocytosis (mean corpuscular volume >98 fl). Number of transfusions was tabulated, excluding transfusions required within 30 days of transplantation. Acute renal failure was defined as an increase in serum creatinine to ≥1.5 times baseline within a 7-day period; chronic renal failure was defined as reduction in glomerular filtration rate to <60 ml/min/1.73 m² for ≥3 months. Elevated liver function tests (LFTs) were defined as aspartate transaminase ≥100 U/liter, alanine transaminase ≥100 U/liter, or alkaline phosphatase ≥150 U/liter. Cirrhosis was determined by imaging or liver biopsy. The presence of malignancy was based on pathologic specimens. Venous thromboembolism was defined as the presence of either pulmonary embolism or deep venous thrombosis. Pulmonary embolism was diagnosed based on visualization by computed tomography angiography or by high-probability ventilation/perfusion scan. Deep venous thrombosis was diagnosed based on ultrasonography. The total number of immunosuppression and antibiotic prophylaxis drugs was tabulated.

Telomere length measurement

Telomere length was measured using quantitative polymerase chain reaction from genomic DNA isolated from peripheral blood leukocytes using Autopure LS (Qiagen, Valencia, CA).^{12,20,21} Telomere length was represented as a logarithm-transformed relative ratio of telomere to single copy gene; age-adjusted telomere length was calculated using normal

controls and represented as either an observed minus an expected value or the percentile for a given age. Transplant physicians involved in clinical management of patients were blinded to the leukocyte telomere length result. Sequencing of telomere-related genes was not systematically performed. Five subjects with telomere-related gene mutations (all with telomere lengths <10th percentile) were included in other studies.^{8,11}

Statistical analysis

Patient characteristics were compared using Fisher's exact test (categorical variables), *t*-test (normally distributed continuous variables), or Mann-Whitney-Wilcoxon test (non-normally distributed continuous variables). Median survival time and time to onset of CLAD were calculated from Kaplan-Meier curves; groups were compared using the log-rank test. Cox proportional hazards models were used in univariable and multivariable analysis to assess the relative effects of clinically relevant covariates. Survival analysis included grade 3 PGD, ACR score, CLAD, total infection rate, and telomere length (<10th percentile) as covariates. Time to onset of CLAD analysis included grade 3 PGD, ACR score, pulmonary infection rate, and telomere length (<10th percentile) as covariates. In sensitivity analyses, telomere length (observed minus expected value) was treated as a continuous variable. We noted no evidence of non-proportional hazards in the Cox models. All analyses were performed using R version 3.2.2 software (www.R-project.org).

Results

Of 319 patients who underwent lung transplantation at the University of Texas Southwestern Medical Center between January 1, 2007, and December 31, 2014, 136 consented to participate in the study (Figure S1, available in the online version of this article at www.jhltonline.org); 54 of these patients were excluded because of a diagnosis other than pulmonary fibrosis or because consent was obtained after transplantation. There were 82 subjects included in the study and divided into 2 groups: 26 subjects (32%) with leukocyte telomere length <10th percentile and 56 subjects (68%) with telomere lengths ≥10th percentile. More patients in the telomere length <10th percentile group had a clinical diagnosis of IPF and had a lung explant histopathologic diagnosis of usual interstitial pneumonia compared with the ≥10th percentile group.

There was no difference between the 2 groups with regard to patient demographics or severity of lung disease (Table 1). More subjects in the <10th percentile group had a baseline macrocytosis compared with the ≥10th percentile group (10 [39%] vs 7 [12%], *p* = 0.017), but there was no difference in rate of leukopenia, anemia, or thrombocytopenia. Most patients in both groups underwent double-lung transplantation.

Survival

Patients were followed for a mean duration of 5 years after lung transplantation. During the follow-up period, there were 14 (54%) deaths in the telomere length <10th

percentile group compared with 10 (18%) deaths in the telomere length ≥10th percentile group (*p* = 0.0015) (Table 2 and Figure 1). The overall survival time was significantly different between the 2 groups (log-rank test, *p* = 0.019). In univariable analysis, telomere length <10th percentile and total number of infections per year were associated with decreased survival time (hazard ratio [HR] 2.6, 95% confidence interval [CI] 1.1–6.0, *p* = 0.023 for telomere length) (Table 3). In multivariable analysis, telomere length <10th percentile was associated with worse survival (HR 10.9, 95% CI 2.7–44.8, *p* = 0.001), independent of other clinically relevant risk factors, including grade 3 PGD, ACR score, CLAD, or infection rate. Similar results were found after excluding single-lung transplant recipients (*n* = 56, telomere length <10th percentile, HR 17.1, 95% CI 3.2–91.3, *p* = 0.001; data not shown). Telomere length as a continuous variable was significantly associated with survival in univariable and multivariable analysis (HR 0.01, 95% CI 0–0.25, per unit change in telomere length, *p* = 0.005) (Table S1, available in the online version of this article at www.jhltonline.org). No single cause of death was overrepresented in the group of patients with short telomere lengths. Causes of death included CLAD, infection, malignancy, cardiovascular complications, and others (Table S2, available in the online version of this article at www.jhltonline.org).

Lung allograft dysfunction

Patients with telomere length <10th percentile had higher rates of allograft dysfunction compared with patients with telomere lengths ≥10th percentile (Table 2). Data to assess PGD were available for 64 of 82 patients. For this subset of patients, there was a significant difference in the incidence of grade 3 PGD between the 2 groups (28% in the telomere length <10th percentile group vs 7% in the ≥10th percentile group; *p* = 0.034). Likewise, there was a higher incidence of CLAD in the short telomere group (50% in the telomere length <10th percentile group vs 23% in the telomere length ≥10th percentile group; *p* = 0.022) (Figure 2). There was a trend toward a higher incidence of R-CLAD in the short telomere group, although this did not reach significance. Telomere length <10th percentile was an independent predictor for the time to onset of CLAD after adjusting for other covariates, including grade 3 PGD, ACR score, and pulmonary infections per year (HR 6.3, 95% CI 2.0–20.0, *p* = 0.002) (Table 4). Similar results were found after excluding single-lung transplant recipients (*n* = 52, telomere length <10th percentile, HR 7.3, 95% CI 2.2–24.6, *p* = 0.001; data not shown). Telomere length as a continuous variable was significantly associated with time to development of CLAD in the univariable analysis (HR 0.20, 95% CI 0.04–0.98, per unit change in telomere length, *p* = 0.047); after correcting for relevant covariates, there was a trend toward significance (HR 0.11, 95% CI 0.01–1.09, *p* = 0.059) (Table S3, available in the online version of this article at www.jhltonline.org).

Table 1 Pre-Transplant Clinical Characteristics

Characteristics	Total (N = 82)	<10th percentile (n = 26)	≥10th percentile (n = 56)	p-value
Age at transplant, years, mean ± SD	59 ± 9	60 ± 6	58 ± 10	0.51
Male sex, n (%)	57 (69.5)	21 (80.8)	36 (64.3)	0.197
Ethnicity, n (%)				
White	71 (86.6)	26 (100)	45 (80.4)	0.15
Black	5 (6.1)	0	5 (8.9)	
Hispanic	3 (3.7)	0	3 (5.4)	
Asian	3 (3.7)	0	3 (5.4)	
Family history of pulmonary fibrosis, n (%)	22 (27)	8 (31)	14 (25)	0.60
Pulmonary fibrosis diagnosis, n (%)				
IPF	50 (61)	22 (85)	28 (50)	0.003
CTD-ILD	10 (12)	3 (11)	7 (12)	1.0
Chronic HP	8 (10)	1 (4)	7 (12)	0.42
Non-UIP IIP	6 (7)	0	6 (11)	0.17
Unclassifiable fibrosis	5 (6)	0	5 (9)	0.17
Other ^a	3 (4)	0	3 (5)	0.55
Explant pathologic pattern, n (%)				
UIP	63 (76.8)	26 (100)	37 (66.1)	<0.001
Non-UIP	19 (23.2)	0	19 (33.9)	
Former smoker, n (%)	42 (51.2)	14 (53.8)	28 (50.0)	0.81
Pack-years, median (IQR)	16 (10–36)	15 (10–48)	16 (9–31)	0.77
PFTs before transplant, mean ± SD (n) ^b				
FVC absolute	1.90 ± 0.75 (76)	1.93 ± 0.58 (26)	1.88 ± 0.83 (50)	0.46
FVC % predicted	44.6 ± 14.9 (76)	43.5 ± 13.4 (26)	45.2 ± 15.7 (50)	0.81
DLCO absolute	5.8 ± 2.5 (55)	5.4 ± 2.4 (16)	5.9 ± 2.5 (39)	0.46
DLCO % predicted	21.8 ± 10.9 (55)	18.8 ± 10.9 (16)	23.0 ± 10.8 (39)	0.20
Bone marrow dysfunction, n (%)				
Leukopenia ^c	5 (6.1)	1 (3.8)	4 (7.1)	1
Anemia ^d	24 (29.3)	7 (26.9)	17 (30.4)	0.80
Macrocytosis ^e	17 (20.7)	10 (38.5)	7 (12.5)	0.017
Thrombocytopenia ^f	7 (8.5)	2 (7.7)	5 (8.9)	1
Number of lungs, n (%)				
Single	12 (14.6)	3 (11.5)	9 (16.1)	0.74
Double	70 (85.4)	23 (88.5)	47 (83.9)	

CTD-ILD, connective tissue disease-associated interstitial lung disease; DLCO, diffusing capacity of lung for carbon monoxide; FVC, forced vital capacity; HP, hypersensitivity pneumonitis; IPF, idiopathic pulmonary fibrosis; IQR, interquartile range; Non-UIP IIP, non-usual interstitial pneumonia idiopathic interstitial pneumonia; PFTs, primary function tests; UIP, usual interstitial pneumonia.

^aOther diagnoses include combined pulmonary fibrosis and emphysema (n = 1), Hermansky-Pudlak syndrome (n = 1), and sarcoidosis (n = 1).

^bPFTs within 1 year of transplant.

^cLeukopenia defined as white blood cell count <4,000/μL.

^dAnemia defined as hemoglobin level <12.0 g/dL for women and <12.4 g/dL for men.

^eMacrocytosis defined as mean corpuscular volume >98 fL.

^fThrombocytopenia defined as platelet count <150,000/μL.

Other manifestations of organ dysfunction

Non-pulmonary organ dysfunction generally occurred at similar rates across the 2 groups. Although there was a trend that patients with short telomere lengths received more packed red blood cell transfusions per year (Table S2, available in the online version of this article at www.jhltonline.org), there was no significant difference in the incidence of anemia, leukopenia, or thrombocytopenia at 30 days (Table S2, available in the online version of this article at www.jhltonline.org) or at 1 year post-transplant (Table 2). Renal dysfunction was common but did not differ between the 2 groups. Acute and chronic renal failure occurred in 66% and 62% of patients, respectively. Overall, only 2 patients required dialysis, lasting no longer than 3 days.

Patients with short telomeres had higher rates of LFT elevations after transplantation, but the incidence of liver cirrhosis was rare. There was no difference in the rate of pulmonary and non-pulmonary infections or in the type of pathogens (Table S2, available in the online version of this article at www.jhltonline.org).

The incidence rate of venous thromboembolism (both pulmonary embolism and deep venous thrombosis) was similar in the 2 groups (Table S2, available in the online version of this article at www.jhltonline.org). Post-transplant malignancy was diagnosed in 15 (18%) patients, with no difference between telomere length groups. Most common cancers were non-melanoma skin cancers; only 1 patient developed a non-skin malignancy, an adenocarcinoma of unknown primary.

Table 2 Post-Transplant Clinical Characteristics

Characteristics	Total (N = 82)	<10th percentile (n = 26)	≥10th percentile (n = 56)	p-value
Follow-up time, years, mean ± SD	5.0 ± 2.5	5.1 ± 2.7	5.0 ± 2.5	0.85
Death, n (%)	24 (29.3)	14 (53.8)	10 (17.9)	0.0015
Survival, median (95% CI)	7.1 (7.0–)	6.2 (2.3–)	—	0.019
Primary graft dysfunction	n = 64	n = 18	n = 46	
Grade 3 PGD, n (%)	8 (12.5)	5 (27.8)	3 (6.5)	0.034
ACR	n = 82	n = 26	n = 56	
ACR score, median (IQR) ^a	0.33 (0–0.50)	0.27 (0–0.40)	0.37 (0–0.57)	0.099
CLAD	n = 82	n = 26	n = 56	
Time to onset of CLAD, median (95% CI)	5.3 (3.8–)	2.7 (1.5–)	—	0.0054
CLAD present, n (%)	26 (31.7)	13 (50.0)	13 (23.2)	0.022
BOS present, n (%)	16 (19.5)	7 (26.9)	9 (16.1)	0.37
R-CLAD present, n (%)	10 (12.2)	6 (23.1)	4 (7.1)	0.066
Bone marrow dysfunction ^b	n = 74	n = 23	n = 51	
Leukopenia, n (%) ^c	20 (27.0)	7 (30.4)	13 (25.5)	0.78
Anemia, n (%) ^d	42 (56.8)	14 (60.9)	28 (54.9)	0.80
Macrocytosis, n (%) ^e	41 (55.4)	16 (69.6)	25 (49.0)	0.13
Thrombocytopenia, n (%) ^f	15 (20.3)	4 (17.4)	11 (21.6)	0.76
Infectious complications	n = 82	n = 26	n = 56	
Total infections per year, median (IQR)	0.96 (0.22–2.29)	1.04 (0.44–1.87)	0.90 (0.15–2.40)	0.72
Pulmonary infections per year, median (IQR)	0.61 (0.03–1.84)	0.61 (0.25–1.28)	0.60 (0–1.86)	0.95
Non-pulmonary complications	n = 82	n = 26	n = 56	
Acute renal failure, n (%)	54 (65.9)	16 (61.5)	38 (67.9)	0.62
Chronic renal failure, n (%)	50 (61.7)	18 (72.0)	32 (57.1)	0.23
RRT required, n (%)	2 (2.4)	1 (3.8)	1 (1.8)	0.54
Elevated LFTs, n (%)	37 (45.1)	17 (65.4)	20 (35.7)	0.017
Cirrhosis, n (%)	1 (1.2)	0	1 (1.8)	1

ACR, acute cellular rejection; BOS, bronchiolitis obliterans syndrome; CI, confidence interval; CLAD, chronic lung allograft dysfunction; IQR, interquartile range; LFTs, liver function tests; PGD, primary graft dysfunction; R-CLAD, restrictive-chronic lung allograft dysfunction; RRT, renal replacement therapy.

^aACR score calculated by adding all pathologic “A” scores and dividing by number of transbronchial biopsies.

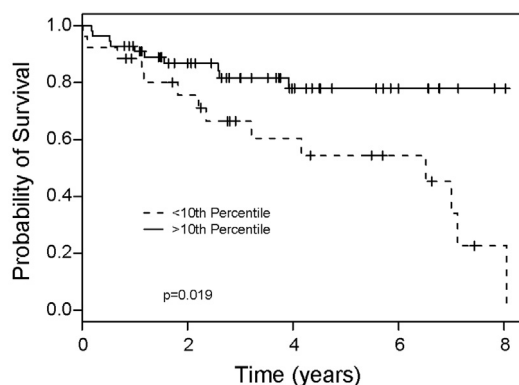
^bCell counts assessed at 1 year post-transplant.

^cLeukopenia defined as white blood cell count <4,000/μL.

^dAnemia defined as hemoglobin <12 g/dl for women and <12.4 g/dl for men.

^eMacrocytosis defined as mean corpuscular volume >98 fL.

^fThrombocytopenia defined as platelets <150,000/μL.



Number at risk:

<10th percentile	26	17	10	6	1
≥10th percentile	56	38	19	8	1

Figure 1 Survival time post-lung transplantation in patients with pulmonary fibrosis stratified by leukocyte telomere lengths depicted in a Kaplan-Meier survival plot. Patients with leukocyte telomere lengths <10th percentile have worse survival than patients with telomere length ≥10th percentile (log-rank test, $p = 0.019$).

Medication regimen

All patients were maintained on a triple-drug immunosuppression regimen after lung transplantation (Table 5). However, patients in the <10th percentile group were exposed to a median of 5 drugs (within the 3 drug classes) compared with a median of 3 drugs in the ≥10th percentile group ($p = 0.005$). There was no difference in rate of basiliximab induction or prophylactic antibiotic use between the 2 groups.

Discussion

We compared clinical outcomes of patients who underwent lung transplantation for pulmonary fibrosis stratified by telomere length. Although previous studies have investigated post-lung transplantation outcomes for patients with mutations in telomere-related genes,^{9–11} the association of recipient leukocyte telomere length to patient outcomes has not been extensively studied. We found that a leukocyte telomere length <10th percentile of normal was associated with shorter post-transplantation survival as well as higher

Table 3 Univariable and Multivariable Analysis of Survival

Variables	Univariable analysis			Multivariable analysis (<i>n</i> = 64)	
	<i>n</i>	HR (95% CI)	<i>p</i> -value	HR (95% CI)	<i>p</i> -value
Telomere <10th percentile	82	2.62 (1.14–6.02)	0.023	10.9 (2.68–44.8)	0.001
Grade 3 PGD	64	1.6 (0.36–7.21)	0.53	1.93 (0.37–10.0)	0.43
ACR score	82	0.23 (0.05–1.08)	0.063	2.28 (0.31–16.9)	0.42
CLAD	82	1.2 (0.52–2.75)	0.67	7.10 (1.10–46.3)	0.041
Total infections/year	82	1.23 (1.12–1.36)	<0.001	1.63 (1.34–1.98)	<0.001

ACR, acute cellular rejection; CI, confidence interval; CLAD, chronic lung allograft dysfunction; HR, hazard ratio; PGD, primary graft dysfunction.

The reported HRs are for the presence of binary predictors (presence of telomeres <10th percentile, grade 3 PGD, or CLAD) or for a 1-unit difference in quantitative predictors (ACR score or total infections/year).

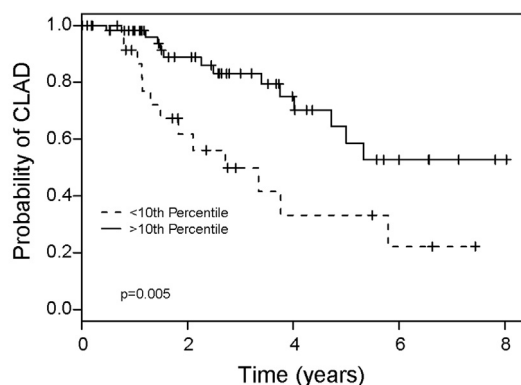
incidence of grade 3 PGD and shorter time to onset of CLAD. Use of the 10th percentile cutoff, representing a degree of telomere shortening shared by most (>80%) telomere-related mutation carriers,⁶ allows for dichotomization of the full cohort for comparison of clinical outcomes.

Only 1 prior study has investigated the effect of telomere length on transplant outcomes.²² This study is similar in that the cohort was stratified by telomere length, and the group with shorter lengths represented approximately one-third of the total. However, this study differs in a number of ways. First, this study measured telomere length from recipient blood leukocytes, not from explanted lung tissue, to generate control-adjusted and age-adjusted percentiles. Second, this study included 100%, not approximately 40%, of subjects who underwent lung transplantation for pulmonary fibrosis to increase the homogeneity of patient responses to lung transplantation. Third, the enrollment period (7 years) was more than double the prior study, so long-term outcomes, such as survival, could be assessed. Fourth, we found higher rates of PGD and CLAD in the

group of patients with telomere lengths <10th percentile. Finally, the prior study did not show an association between recipient telomere length and survival as the current study does.

The results of this study also differ from prior studies of patients with pulmonary fibrosis harboring a heterozygous pathogenic mutation in 1 of the telomerase genes.^{9–11} We found no significant difference in blood cell counts after lung transplantation; however, the overall incidence of leukopenia (27%), anemia (57%), and thrombocytopenia (20%) was common for the entire cohort. We also did not find a significant increase in renal dysfunction associated with telomere length; instead, both acute and chronic renal dysfunction was commonly found across the entire cohort and was not specifically associated with telomere length. Similarly, we did not find an association between incident malignancy or infection and leukocyte telomere length. The only short telomere-associated phenotype seen at a higher incidence in the patients with short telomere lengths was an elevation of LFTs. Elevation of LFTs likely led to more immunosuppression medication changes for patients with shorter telomere lengths, as this group had, on average, exposure to more medications than the group with longer telomeres.

We found a significant increase in PGD and CLAD in patients with shorter telomere lengths. PGD accounts for most early morbidity and mortality after transplant^{23–25} and has been linked to various donor, recipient, and surgical characteristics.^{26–28} Recipient telomere lengths may potentially explain the previously reported association between reperfusion injury and native fibrotic, as opposed to obstructive, lung disease.²⁹ Similar to PGD, the pathogenesis of CLAD is multifactorial. CLAD results in non-reversible allograft damage after insults such as infection,^{30,31} gastroesophageal reflux,^{32,33} elevated donor-specific antibodies,^{34–36} and episodes of ACR.^{17,37} CLAD subtypes include an obstructive defect (bronchiolitis obliterans syndrome) and a restrictive defect (R-CLAD). Although it did not reach statistical significance, short telomere lengths were more closely associated with R-CLAD. This finding is notable because the pulmonary pathologic manifestations of R-CLAD, including diffuse alveolar damage,^{38,39} acute fibrinous organizing pneumonia,⁴⁰ and pleuroparenchymal fibroelastosis,⁴¹ have been described for patients with telomere-related gene muta-



Number at risk:

<10 th percentile	26	11	4	2	0
≥10 th percentile	56	34	16	6	1

Figure 2 Time to development of CLAD in patients stratified by leukocyte telomere lengths depicted in a Kaplan-Meier plot. Patients with leukocyte telomere length <10th percentile have a shorter time to onset of CLAD compared with patients with telomere lengths ≥10th percentile (log-rank test, *p* = 0.005). CLAD is defined as the persistent decline in forced expiratory volume in 1 second (FEV₁) <80% of the individual baseline FEV₁ (average of the 2 best FEV₁ values after transplantation) that cannot be attributed to concurrent infection or ACR.

Table 4 Univariable and Multivariable Analysis of Time to Onset of Chronic Lung Allograft Dysfunction

Variables	Univariable analysis			Multivariable analysis (<i>n</i> = 58)	
	<i>n</i>	HR (95% CI)	<i>p</i> -value	HR (95% CI)	<i>p</i> -value
Telomere <10th percentile	82	2.85 (1.32–6.17)	0.0077	6.3 (2.0–20.0)	0.002
Grade 3 PGD	64	1.32 (0.17–10.0)	0.78	0.39 (0.05–3.4)	0.39
ACR score	82	1.09 (0.35–3.41)	0.89	4.6 (0.85–24.7)	0.076
Pulmonary infections/year	82	1.51 (1.17–1.95)	0.0018	1.8 (1.1–2.7)	0.011

ACR, acute cellular rejection; CI, confidence interval; HR, hazard ratio; PGD, primary graft dysfunction.

The reported HRs are for the presence of binary predictors (presence of telomeres <10th percentile or grade 3 PGD) or for a 1-unit difference in quantitative predictors (ACR score or total infections/year).

tions.⁸ Although type II alveolar epithelial cell senescence contributes to the development of pulmonary fibrosis,^{42,43} this mechanism cannot be responsible for CLAD in these patients after lung transplantation. We could not fully assess for an association between CLAD and donor-specific antibodies, as the latter were not measured for the entire cohort.

The association between short telomere lengths and CLAD does not prove causality, but it suggests that the immune dysregulation and/or the injuries related to infections associated with telomere shortening may be linked to CLAD in the post-transplant period. Shortened telomere lengths in T cells are associated with the loss of expression of the CD28 co-stimulatory molecule and results in broad reprogramming of CD28[−] T cells and an altered adaptive immunity⁴⁴ and reduced clonal expansion.⁴⁵ Shortened telomere lengths may also contribute to the risk or persistence of pulmonary infections that may trigger the onset of CLAD. Short telomere lengths are associated with higher rates of upper respiratory viral infections in healthy subjects,⁴⁶ and individuals with short telomere lengths may be less tolerant to infections when they occur.⁴⁷ Further study is needed to investigate the underlying pathogenesis of CLAD in patients with short telomere lengths.

Despite an extensive pre-transplant recipient selection process, the ability to predict complications and survival after lung transplantation is limited. Telomere length is a biomarker that could aid in risk stratification of patients with

IPF at the time of their diagnosis not only to predict risk of progressive disease and the need for lung transplantation^{20,48} but also to predict risk of post-transplant complications such as PGD and CLAD. Although the overall survival of patients with telomere length <10th percentile was significantly lower than survival of patients with telomere length ≥10th percentile, the patients with short telomere length still had an acceptable survival time compared with national benchmarks.

The present study has several limitations. First, this was a single-center study. Overall, the percentage of patients with familial pulmonary fibrosis (27%) was higher than has been described at other centers⁴⁹ and may lead to an enrichment of patients with short telomere lengths. Second, because we limited inclusion to patients with pulmonary fibrosis, these results cannot be extrapolated to patients with other indications for lung transplant. Finally, we were unable to assess donor demographic information or donor telomere lengths with post-transplant outcomes.

In conclusion, telomere length represents a potential biomarker that can easily be assessed during the pre-transplant evaluation to identify patients at increased risk for post-transplant complications such as PGD and CLAD. The results from this single-site study are compelling and warrant replication in a larger, multicenter cohort. If replicable, these results may lead to additional investigations probing the link between lung transplant recipient short leukocyte telomere lengths, allograft dysfunction, and survival.

Table 5 Use of Immunosuppressive Medications After Lung Transplantation

Medications	Total (<i>N</i> = 82)	<10th percentile (<i>n</i> = 26)	≥10th percentile (<i>n</i> = 56)	<i>p</i> -value
	<i>n</i> = 67	<i>n</i> = 21	<i>n</i> = 46	
Basiliximab induction, <i>n</i> (%)	41 (67)	13 (62)	28 (61)	1
Triple immunosuppressive regimen, <i>n</i> (%)	<i>n</i> = 82	<i>n</i> = 26	<i>n</i> = 56	
Immediately post-transplant	80 (100)	25 (100)	55 (100)	1
At 1 year post-transplant	74 (100)	23 (100)	51 (100)	1
At 3 years post-transplant	43 (100)	13 (100)	30 (100)	1
At 5 years post-transplant	19 (100)	6 (100)	13 (100)	1
At 7 years post-transplant	5 (100)	1 (100)	4 (100)	1
Total number immunosuppressive medications used, median (IQR) ^a	5.0 (3.0–5.0)	5.0 (4.25–6.0)	3.0 (3.0–5.0)	0.005
Total number prophylactic antimicrobial drugs used, median (IQR)	2.0 (2.0–3.0)	2.0 (2.0–3.0)	2.0 (2.0–2.0)	0.21

IQR, interquartile range.

^aExposure to medications including prednisone, azathioprine (Imuran), mycophenolate (Cellcept), cyclosporine, FK-506/tacrolimus, and sirolimus.

Disclosure statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

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Supplementary data

Supplementary data are available online at www.jhltonline.org.

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