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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, NAF1*) 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 $\geq$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 $\geq$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 $\geq$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 m^2 for $\geq$3 months. Elevated liver function tests (LFTs) were defined as aspartate transaminase $\geq$100 U/liter, alanine transaminase $\geq$100 U/liter, or alkaline phosphatase $\geq$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