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Short Telomeres, Telomeropathy, and Subclinical Extrapulmonary Organ Damage in Patients With Interstitial Lung Disease

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BACKGROUND: Human telomere disease consists of a wide spectrum of disorders, including pulmonary, hepatic, and bone marrow abnormalities. The extent of bone marrow and liver abnormalities in patients with interstitial lung disease (ILD) and short telomeres is unknown.

METHODS: The lung transplant clinic established a prospective protocol to identify short telomeres in patients with ILD not related to connective tissue disease or sarcoidosis. Patients with short telomeres underwent bone marrow biopsies, liver biopsies, or both as part of the evaluation for transplant candidacy.

RESULTS: One hundred twenty-seven patients met ILD categorization for inclusion. Thirty were suspected to have short telomeres, and 15 had the diagnosis confirmed. Eight of 13 (53%) patients had bone marrow abnormalities. Four patients had hypocellular marrow associated with macrocytosis and relatively normal blood counts, which resulted in changes to planned immunosuppression at the time of transplant. Four patients with more severe hematologic abnormalities were not listed because of myelodysplastic syndrome (two); monoclonal gammopathy of unclear significance (one); and hypocellular marrow, decreased megakaryocyte lineage associated with thrombocytopenia (one). Seven patients underwent liver biopsies, and six had abnormal liver pathology. These abnormalities did not affect listing for lung transplant, and liver biopsies are no longer routinely obtained.

CONCLUSIONS: Subclinical bone marrow and liver abnormalities can be seen in patients with ILD and short telomeres, in some cases in the absence of clinically significant abnormalities in peripheral blood counts and liver function tests. A larger study examining the implication of these findings on the outcome of patients with ILD and short telomeres is needed.

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ABBREVIATIONS: CTD = connective tissue disease; FIP = familial interstitial pneumonia; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; MCV = mean corpuscular volume; MGUS = monoclonal gammopathy of unknown significance; NK = natural killer; TERT = telomerase reverse transcriptase; TERC = telomerase RNA complex

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Human telomere disease consists of a wide spectrum of disorders, including pulmonary, hepatic, and bone marrow abnormalities (eg, aplastic anemia, acute leukemia).[1] Mutations in genes controlling telomere length have incomplete penetrance and can induce single or multi-organ disease, associated with different phenotypes and varying degrees of severity.[1,2] Short telomeres and telomerase mutations are important risk factors for familial and sporadic forms of idiopathic pulmonary fibrosis (IPF).[3,4] Approximately 15% of patients with familial interstitial pneumonia (FIP) have mutations in telomerase reverse transcriptase (TERT) or telomerase RNA complex (TERC).[5] Moreover, about 25% of patients with sporadic IPF have short telomeres in peripheral blood leukocytes, despite no detectable telomerase mutations,[6] suggesting that other genetic or nongenetic causes could lead to shortened telomeres. Usual interstitial pneumonia, the histologic hallmark of IPF, is found in 85% of patients with interstitial lung disease (ILD) and short telomeres.[7] However, other ILDs[7] as well as the combined pulmonary fibrosis emphysema[8,9] syndrome have also been reported in association with telomerase mutations and short telomeres.

Prior studies of the manifestations of short telomeres have examined kindreds of affected subjects and found that telomere length and genetic mutations of genes controlling telomere length were associated with aplastic

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anemia and pulmonary and liver disease.[3,10,11] However, the extent of subclinical bone marrow and/or liver disease in patients with ILD and short telomeres has not been previously investigated.

In 2011, a subject suspected of having short telomeres underwent a lung transplant at our institution, which was complicated by severe bone marrow and liver failure. This led our program to establish a comprehensive plan to evaluate subjects with ILD for potential telomeropathy, as defined by short telomeres and any organ dysfunction known to be associated with functional mutations in genes encoding telomerase.[12] Here, we report the results of our evaluation and its effectiveness at assessing for telomeropathy and subclinical organ dysfunction in a cohort of patients with ILD undergoing evaluation for lung transplantation.

Material and Methods

Subjects

In September 2011, the lung transplant program at Brigham and Women's Hospital established clinical guidelines designed to increase the index of suspicion for short telomeres and associated disease(s) in patients referred for consideration of candidacy. Here, we report the results of this intervention. All patients with ILD and two or more visits to the program were included in the study cohort. Patients with sarcoidosis or connective tissue disease (CTD)-associated ILD were excluded. Patients with ILD were suspected to have short telomeres if they had any of the following:

- WBC count, hematocrit level, or platelet count below the lower limit of normal[13]
- Mean corpuscular volume (MCV) above the upper limit of normal[14,15]
- Abnormal liver function tests[11]
- Abnormal coagulation profile
- History or evidence of hepatosplenomegaly on abdominal ultrasonography
- Family history of interstitial pneumonia, self-reported early graying, aplastic anemia, or liver disease

Individuals with suspected short telomeres then underwent telomere length testing. They were diagnosed with short telomeres if telomere length was shorter than the 10th percentile of the reference population. Patients with short telomeres were referred for bone marrow and liver biopsies to further evaluate their candidacy for lung transplantation.

Demographic and laboratory characteristics of those who were suspected of having short telomeres and those who were not are listed in e-Table 1. An analysis of the results of the implementation of this protocol was conducted with approval from the Institutional Review Board (Protocol# 2011-P-002391/1).

Diagnosis of ILD and Clinical Information

A review of medical records including CT scanning and existing surgical lung biopsy results was used to determine the diagnosis of ILD. Hematology, chemistry, and pathology results were obtained through a review of the computerized medical records.

Telomere Length by Flow Fluorescence In Situ Hybridization

Telomere length analysis in peripheral blood lymphocytes was performed with a Clinical Laboratory Improvement Amendments-approved test performed at Repeat Diagnostics Laboratory, Vancouver, Canada.[16,17] The control population for this test consisted of 835 subjects identified only by age and sex; no other demographic or medical information was available.[17] The age distribution of the control population was as follows: 0 to 40 years, 398; 41 to 50 years, 80; 51 to 70 years, 155; and >70 years, 202 control subjects. Telomere length was defined as short if it was under the 10th percentile of age-matched control subjects, which represented the statistical outlier of telomere length compared with the control population (details of the study population are in e-Table 1 of Reference 14).

Sequencing and Mutation Analysis

Seven subjects consented to genetic testing. Sequencing and mutational analysis for *TERT* and *TERC* were performed in a Clinical Laboratory Improvement Amendments-approved laboratory.

Statistical Analysis

Results are expressed as mean $\pm$ SD for n number of samples. Analysis of difference between groups was conducted using an analysis of variance, *t* test, or Fisher exact test as appropriate. A two-sided *P* value $<.05$ was used for statistical significance. All analysis was done using GraphPad Prism software (GraphPad 5.0).