[ Diffuse Lung Disease Original Research ]

CHEST

# Clinical Impact of Telomere Length Testing for Interstitial Lung Disease

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**BACKGROUND:** Shortened telomere length (TL) is a genomic risk factor for fibrotic interstitial lung disease (ILD), but its role in clinical management is unknown.

**RESEARCH QUESTION:** What is the clinical impact of TL testing on the management of ILD?

**STUDY DESIGN AND METHODS:** Patients were evaluated in the Columbia University ILD clinic and underwent Clinical Laboratory Improvement Amendments-certified TL testing by flow cytometry and fluorescence in situ hybridization (FlowFISH) as part of clinical treatment. Short TL was defined as below the 10th age-adjusted percentile for either granulocytes or lymphocytes by FlowFISH. Patients were offered genetic counseling and testing if they had short TL or a family history of ILD. FlowFISH TL was compared with research quantitative polymerase chain reaction (qPCR) TL measurement.

**RESULTS:** A total of 108 patients underwent TL testing, including those with clinical features of short telomere syndrome such as familial pulmonary fibrosis (50%) or extrapulmonary manifestations in the patient (25%) or a relative (41%). The overall prevalence of short TL was 46% and was similar across clinical ILD diagnoses. The number of short telomere clinical features was independently associated with detecting short TL (OR, 2.00; 95% CI, 1.27-3.32). TL testing led to clinical treatment changes for 35 patients (32%), most commonly resulting in reduction or avoidance of immunosuppression. Of the patients who underwent genetic testing (n = 34), a positive or candidate diagnostic finding in telomere-related genes was identified in 10 patients (29%). Inclusion of TL testing below the 1st percentile helped reclassify eight of nine variants of uncertain significance into actionable findings. The qPCR test correlated with FlowFISH, but age-adjusted percentile cutoffs may not be equivalent between the two assays.

**INTERPRETATION:** Incorporating TL testing in ILD impacted clinical management and led to the discovery of new actionable genetic variants. CHEST 2024; ■(■):■-■

**KEY WORDS:** genetic counseling; genomics; idiopathic pulmonary fibrosis; precision medicine; pulmonary fibrosis; telomere

**ABBREVIATIONS:** ACMG = American College of Medical Genetics and Genomics; cHP = chronic hypersensitivity pneumonitis; CLIA = Clinical Laboratory Improvement Amendments; CTD = connective tissue disease; FlowFISH = flow cytometry and fluorescence in situ hybridization; FPF = familial pulmonary fibrosis; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; qPCR = quantitative polymerase chain reaction; TL = telomere length; UILD = unclassifiable interstitial lung disease; VUS = variant of uncertain significance

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## Take-Home Points

**Study Question:** What is the clinical impact of telomere length testing on the management of interstitial lung disease (ILD)?

**Results:** Telomere length testing improved genetic testing interpretation and impacted clinical treatment for 32% of patients with ILD, most often resulting in reduction or avoidance of immunosuppression after identification of short telomeres.

**Interpretation:** Clinical telomere length testing for patients with ILD is feasible, actionable, and impactful for clinical treatment.

Fibrotic interstitial lung diseases (ILDs) are a heterogeneous group of chronic scarring disorders of the lungs associated with poor prognosis.[1] Telomere shortening has emerged as a shared genomic risk factor for many different forms of fibrotic ILD, including idiopathic pulmonary fibrosis (IPF),[2-6] chronic hypersensitivity pneumonitis (cHP),[7-9] connective tissue disease-related ILD (CTD-ILD),[10] and unclassifiable ILD (UILD).[10,11] Although Clinical Laboratory Improvement Amendments (CLIA) program-certified measurements of telomeres are available, little is known about the clinical impact of telomere length (TL) testing for patients with fibrotic ILD in a real-world setting.

Telomeres are six-nucleotide repeats that serve as protective caps at the end of chromosomes and shorten with each cycle of cell replication. Inherited genetic mutations in telomere-related genes can lead to accelerated age-adjusted telomere shortening, a progressive pulmonary fibrosis phenotype, and reduced survival.[2,7,10,12,13] In addition, emerging evidence suggests that short TL itself is a pharmacogenetic risk factor that is predictive of adverse events associated with immunosuppression exposure for patients with either IPF[14] or non-IPF fibrotic ILD.[8,15] Despite its potential as a clinical tool, use of TL as a biomarker for fibrotic ILD has been limited to research applications.

In this single-center observational study, we report the diagnostic and clinical impact of incorporating CLIA-certified TL measurement in the workup of patients with fibrotic ILD. We describe the prevalence of short TL in patients with fibrotic ILD and explore its resultant impact on clinical treatment.

## Study Design and Methods

### Study Design and Subjects

This retrospective observational study was approved by the institutional review board at Columbia University Medical Center (IRB AAAS0753), and each patient provided written informed consent to allow collection of clinical data and biospecimens for genetic research. Subjects were enrolled from those evaluated by the Columbia University Medical Center/NewYork-Presbyterian ILD clinic. Patient ILD diagnoses were made according to consensus guidelines[16] with multidisciplinary discussion. Patient demographics, medications, pulmonary function test results, CT imaging reports, and laboratory test results were abstracted from medical records. Familial pulmonary fibrosis (FPF) was defined as having at least one first- or second-degree relative with fibrotic ILD. Clinical features of short telomere syndrome (short telomere features), including the presence of FPF and a personal or familial history of extrapulmonary manifestations of a short telomere syndrome (eg, premature graying before the age of 30 years, cryptogenic cirrhosis, unexplained cytopenias, myelodysplastic syndrome, and acute myeloid leukemia),[17] were abstracted from medical records. Characteristics of patients seen at the clinic during the same time period but not enrolled in the study were obtained from electronic health records, using SlicerDicer. Q7

### Telomere Length Testing

Telomere length testing was conducted via flow cytometry and fluorescence in situ hybridization (FlowFISH) by the CLIA-certified Johns Hopkins Molecular Diagnostics Laboratory.[18] The decision to send TL testing was left to the discretion of the treating provider (Fig 1). Short telomeres were defined as having age-adjusted TL < 10th percentile[14,15] in either granulocyte or lymphocyte populations. All but one patient (n = 107; 99%) underwent research TL testing via quantitative polymerase chain reaction (qPCR), using previously described methods.[6]

### Clinical Management Changes Based on TL Testing

Clinical management changes after TL testing focused on change in pharmacologic treatment strategy. Pharmacologic change after identification of short TL includes cessation/de-escalation or avoidance of immunosuppressants (ie, prednisone, mycophenolate, or azathioprine) for cases in which immunosuppressants are commonly used (ie, cHP,[19] UILD,[20] and CTD-ILD) or early initiation of antifibrotics before confident diagnosis of IPF or progressive pulmonary fibrosis.[16] With





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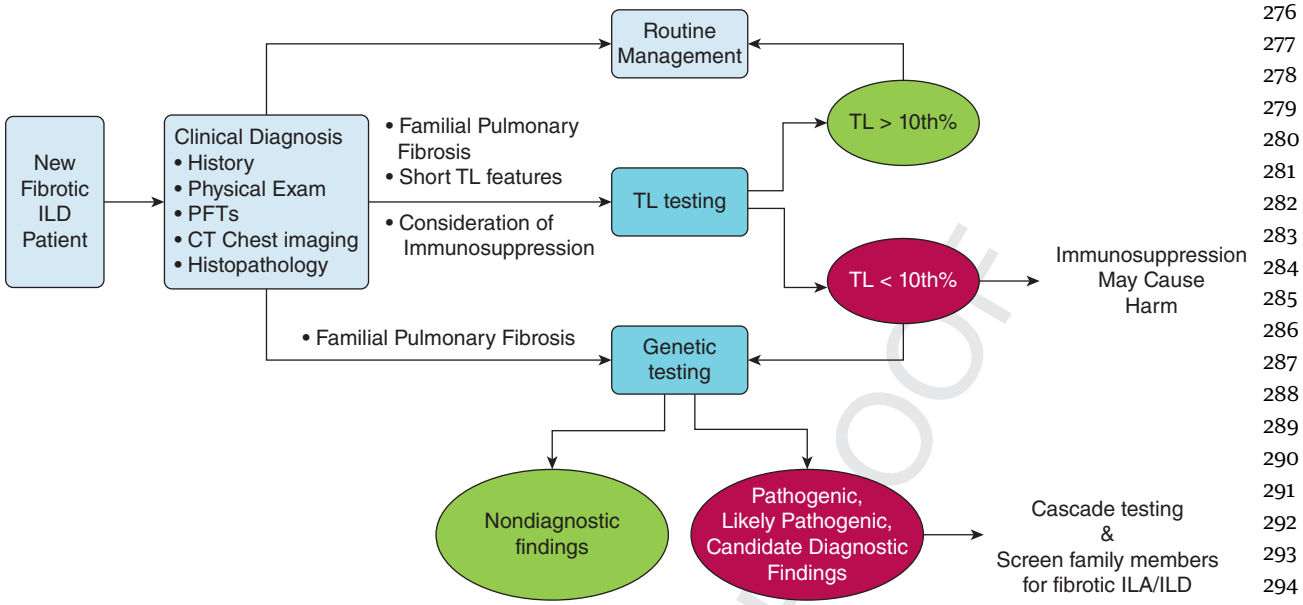


Figure 1 – *Proposed approach for incorporation of genomic and genetic testing into interstitial lung disease (ILD) management. A clinical diagnosis of fibrotic ILD is established on the basis of history, physical examination, pulmonary function testing, CT chest imaging, and histopathology. Patients undergo routine treatment if receiving a fibrotic ILD diagnosis with consensus role for or against immunosuppression without having familial pulmonary fibrosis (at least one first- or second-degree relative with fibrotic ILD) or features of short telomere length (personal or family member with graying before the age of 30 years, cryptogenic cirrhosis, unexplained cytopenia, or bone marrow failure including myelodysplastic syndrome and leukemia). Telomere length testing is suggested for all others. Although this study used a telomere length cutoff of < 10th percentile as suggestive of increased risk of adverse effects from immunosuppression, these risks exist along a spectrum and would be found to a greater degree with more extreme telomere shortening, especially < 1st percentile. Sequential telomere length testing followed by genetic testing, as opposed to simultaneous testing, may be a preferred strategy as detection of a telomere length < 1st percentile can help reclassify variants of uncertain significance to pathogenic/likely pathogenic variants and can prioritize vendors that use a gene panel that is more inclusive of telomere-related genes. Genetic testing for patients with familial pulmonary fibrosis in the absence of telomere shortening is indicated to rule out surfactant-related or mitotic spindle gene mutations. ILA = interstitial lung abnormalities; PFT = pulmonary function testing; TL = telomere length.*

identification of normal TL, management change included empiric initiation/escalation of immunosuppression. Adjudication of management changes was performed by consensus opinion of two ILD pulmonologists (treating and independent clinician). Discordant cases were reviewed by a third ILD pulmonologist who served as tiebreaker. The Cohen κ statistic was calculated for each pair of pulmonologists and the range of κ statistics is reported.

### Genetic Counseling and Testing

For patients with short TL or FPF, genetic counseling was offered before genetic testing and return of results[21] (Fig 1). All genetic tests were sent to CLIA-certified laboratories that classified variants according to American College of Medical Genetics and Genomics (ACMG) criteria.[22-24] Variants were manually reclassified to account for TL data as supporting evidence of pathogenicity if < 10th percentile (PP4 moderate criteria) or < 1st percentile (PP4 strong criteria) in genes linked to both fibrotic ILD and telomere dysfunction (*TERT*, *TERC*, *RTEL1*, *PARN*, *NAF1*, *DKC1*, *TINF2*, *NOP10*, *NHP2*, *ZCCHC8*, and *ACD*).[21] All gene panels included *TERT*, *TERC*, *RTEL1*, *PARN*, *DKC1*, and *TINF2*. Almost all panels included *NOP10* (94%), *NHP2* (94%), and *ACD* (94%), and most panels included *NAF1* (74%) and *ZCCHC8* (68%). A *positive diagnostic finding* was defined as a pathogenic or likely pathogenic variant. A *candidate diagnostic finding* was defined as a variant of uncertain significance (VUS) in a telomere-related gene in a patient with granulocyte or lymphocyte TL < 1st percentile given the specificity of this cutoff for pathogenic telomere gene mutations.[18,25] If genetic testing yielded a positive or candidate diagnostic finding, cascade genetic and TL testing was offered to family members. All variant classifications were submitted to ClinVar.[26]

### Statistical Analysis

Baseline variables for short vs normal TL groups were compared by Student *t*-test for continuous variables and by $\chi^2$ or Fisher exact test for categorical variables. Analysis of variance was used to assess differences in means among multiple groups. Correlation between TL as a continuous variable by different cell populations (lymphocytes vs granulocytes) or different methods (FlowFISH vs qPCR) were assessed by measuring the Pearson correlation.

For our primary analysis, we quantified clinical management changes stratified by clinical diagnosis. We performed $\chi^2$ testing to compare the proportion of patients with treatment changes across clinical ILD diagnoses. To assess interrater variability, we calculated the Cohen κ statistic for each ILD pulmonologist and report a range of κ values.

We performed univariable and multivariable logistic regression to determine clinical features associated with short TL. For multivariable analyses, we adjusted for age,[27] sex,[28] race/ethnicity,[27] IPF diagnosis,[10] as well as variables identified in univariable analyses. We also performed sensitivity analyses by varying the definition of short telomeres as (1) < 1st percentile in granulocytes or lymphocytes or (2) < 10th percentile by qPCR.

We assessed the sensitivity and specificity of qPCR TL measures for detecting FlowFISH TL at different percentile cutpoints (< 10th and < 1st percentile). We also compared the sensitivity and specificity of qPCR vs FlowFISH for detecting actionable genetic findings or identifying patients with short telomere features. To assess agreement of categorization between qPCR and FlowFISH assays, we computed the Cohen κ statistic between the two assays at < 1st and < 10th percentile cutpoints.

All *P* values less than .05 were considered significant. Statistical analyses were performed with R statistical software, version 4.4.0 (R Foundation).

## Results

Between November 2021 and June 2023, 534 new patients with fibrotic interstitial lung disease were evaluated by the Columbia/NewYork-Presbyterian ILD clinic. Of these, 125 patients (23%) completed CLIA-certified TL testing and 108 consented to enroll in the present study (e-Fig 1). Enrolled patients were predominantly male (54%) with a median age of 68 (interquartile range [IQR], 62-74) years. Patients received diagnoses of UILD (n = 31), cHP (n = 29), IPF (n = 27), CTD-ILD (n = 16), and other (n = 5) (Table 1). Features of short telomeres were prevalent in our cohort, including FPF (50%), and extrapulmonary manifestations of short telomeres in the patient (25%) or family member (41%) (Table 1). During the same time period, patients with ILD without TL testing (n = 409) had a similar median age of 70 (IQR, 62-77) years and proportion with an IPF diagnosis (20%) but included fewer male patients (41%) (e-Table 1).

### *Telomere Length Measurement*

Age-adjusted FlowFISH TL determinations in granulocytes and lymphocytes were highly correlated ($R = 0.77$; $P < .001$). Overall, observed minus expected age-adjusted TL measures in kilobases were lower in granulocytes than lymphocytes (e-Fig 2). Some patients had discordant TL < 10th percentile in only one cell population (26%); most patients had concordant TL measures in both cell populations > 10th percentile (54%) or < 10th percentile (22%).

### *Prevalence and Clinical Predictors of Short TL*

The overall prevalence for short TL in our cohort was 46% with 50 of 108 patients with age-adjusted TL < 10th percentile in either lymphocytes or granulocytes. There was no difference in prevalence of short TL by age, sex, or clinical ILD diagnosis (Table 1). Patients with short TL had a different racial/ethnic makeup ($P = .004$) and greater number of short telomere features ($P = .02$) (Fig 2, e-Fig 3). No single short telomere feature was enriched in patients with FlowFISH TL < 10th percentile (Table 1) or < 1st percentile (e-Table 2). In univariable analysis, a definite usual interstitial pneumonia (UIP) pattern or CT honeycombing was significantly associated with short TL. Because of collinearity with CT honeycombing, we excluded radiographic UIP in adjusted analyses. In multivariable analyses, having multiple short telomere features significantly increased the odds of identifying short TL (OR, 2.00; 95% CI, 1.27-3.32; $P < .01$) adjusting for age, sex, non-Hispanic White race/ethnicity, smoking pack-years, honeycombing on CT imaging, and IPF diagnosis (Table 2). CT honeycombing, non-Hispanic White race/ethnicity, and fewer smoking pack-years were associated with short TL in adjusted analyses. The number of short telomere features was also independently associated with TL < 1st percentile in either lymphocytes or granulocytes by FlowFISH (e-Table 3) and with TL < 10th percentile by qPCR (e-Fig 4, e-Table 4).

### *Clinical Impact of TL Testing*

There was good agreement of clinical impact as assessed by independent reviewers (κ, 0.73-0.81). We identified 35 cases (32%) in which management changes were made after TL testing (Table 3). The most common change involved reduction in immunosuppressants after identifying short TL (n = 22; 20%). Empiric initiation of





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**TABLE 1** ] Baseline Clinical and Genetic Characteristics

| Characteristic | All (n = 108) | Short Telomere (< 10th Percentile)[a] (n = 50) | Nonshort Telomere (≥ 10th Percentile)[a] (n = 58) | *P* Value[b] |
|---|---|---|---|---|
| Age (median, IQR) | 68 (62, 74) | 66 (60, 72) | 68 (62, 74) | .82 |
| Male, No. (%) | 58 (54%) | 28 (56%) | 30 (52%) | .80 |
| Race/ethnicity, No. (%) | | | | **.004** |
| White | 67 (62%) | 38 (76%) | 29 (50%) | |
| Hispanic | 24 (22%) | 11 (22%) | 13 (22%) | |
| Black | 4 (4%) | 0 | 4 (7%) | |
| Asian | 9 (8%) | 1 (2%) | 8 (14%) | |
| Other | 4 (4%) | 0 | 4 (7%) | |
| ILD diagnosis, No. (%) | | | | .81 |
| IPF | 27 (25%) | 14 (28%) | 13 (22%) | |
| cHP | 29 (27%) | 12 (24%) | 17 (29%) | |
| UILD | 31 (29%) | 16 (32%) | 15 (26%) | |
| CTD-ILD | 16 (15%) | 6 (12%) | 10 (17%) | |
| Other[c] | 5 (5%) | 2 (4%) | 3 (5%) | |
| Telomere length (kb), (mean ± SD) | | | | |
| GTL, age-adjusted observed − expected | −0.97 ± 0.86 | −1.58 ± 0.54 | −0.54 ± 0.58 | **< .001** |
| LyTL, age-adjusted observed − expected | −0.69 ± 0.99 | −1.43 ± 0.75 | −0.2 ± 0.72 | **< .001** |
| CT UIP pattern, No. (%) | | | | |
| Alternative diagnosis | 56 (52%) | 25 (50%) | 31 (53%) | .85 |
| Indeterminate for UIP | 17 (16%) | 8 (16%) | 9 (16%) | 1 |
| Probable UIP | 17 (16%) | 4 (8%) | 13 (22%) | .06 |
| Definite UIP | 18 (17%) | 13 (26%) | 5 (9%) | **.02** |
| CT features, No. (%) | | | | |
| Ground-glass opacities | 28 (26%) | 13 (26%) | 15 (26%) | 1 |
| Air trapping | 28 (26%) | 13 (26%) | 15 (26%) | 1 |
| Traction bronchiectasis | 80 (74%) | 40 (80%) | 40 (69%) | .27 |
| Honeycombing | 32 (30%) | 22 (44%) | 10 (17%) | **.003** |
| Pathologic UIP pattern,[d] No. (%) | 18 (67%) | 12 (80%) | 6 (50%) | .10 |
| Short telomere features, No. (%) | | | | |
| Familial pulmonary fibrosis | 54 (50%) | 28 (56%) | 26 (45%) | .33 |
| Personal extrapulmonary signs of short TL | | | | |
| Premature graying | 24 (22%) | 15 (30%) | 9 (16%) | .10 |
| Cirrhosis | 3 (3%) | 2 (4%) | 1 (2%) | .59 |
| Hematologic disease | 4 (4%) | 3 (6%) | 1 (2%) | .33 |
| Any | 27 (25%) | 17 (34%) | 10 (17%) | .07 |
| Familial extrapulmonary signs of short TL | | | | |
| Premature graying | 33 (31%) | 13 (26%) | 20 (34%) | .40 |
| Cirrhosis | 14 (13%) | 11 (22%) | 3 (5%) | **.02** |

*(Continued)*





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**TABLE 1** ] *(Continued)*

| Characteristic | All (n = 108) | Short Telomere (< 10th Percentile)[a] (n = 50) | Nonshort Telomere (≥ 10th Percentile)[a] (n = 58) | *P* Value[b] |
|---|---|---|---|---|
| Hematologic disease | 6 (6%) | 5 (10%) | 1 (2%) | .09 |
| Any | 44 (41%) | 22 (44%) | 22 (38%) | .56 |
| Total number of short telomere features (mean ± SD) | 1.28 ± 1.02 | 1.54 ± 1.16 | 1.05 ± 0.83 | **.02** |

cHP = chronic hypersensitivity pneumonitis; CTD-ILD = connective tissue disease-related interstitial lung disease; GTL = granulocyte telomere length; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; IQR = interquartile range; LyTL = lymphocyte telomere length; TL = telomere length; UILD = unclassifiable interstitial lung disease; UIP = usual interstitial pneumonia.
[a]Telomere length < 10th percentile for either lymphocytes or granulocytes as determined by FlowFISH.
[b]*P* values indicate differences between short and nonshort telomere groups.
[c]Other ILD diagnoses: idiopathic interstitial pneumonia (n = 2), sarcoidosis (n = 2), post-COVID fibrosis (n = 1).
[d]Patients with surgical lung biopsy available (n = 27).

immunosuppressants after identifying normal TL occurred in nine patients (8%). In sum, the initial immunosuppressive strategy was heavily informed by TL testing in 31 patients (29%). The proportion of patients with treatment changes differed by ILD clinical diagnosis ($\chi^2$ $P = .01$); most treatment changes occurred in patients with cHP (52%) and UILD (45%) (e-Fig 5).

We quantified active medications for each patient at the time of TL testing compared with 3 months afterward. Among non-IPF patients 3 months after TL testing, 15 of 45 normal TL patients (33%) were treated with immunosuppressants compared with 6 of 36 short TL patients (16%) (e-Table 5, e-Fig 6).

### *Genetic Counseling and Clinical Genetic Testing*

After TL testing, 46 patients with short TL or FPF were referred for genetic counseling and testing and 34 patients completed genetic testing (Fig 3). Genetic testing identified 13 patients with heterozygous variants in telomere related genes (*TERT*, n = 6; *RTEL1*, n = 3; *PARN*, n = 1; *TERC*, n = 1; *NAF1*, n = 1; *ACD*, n = 1). Most variants (9 of 13; 69%) were initially classified as variants of uncertain significance (VUS); two variants were classified as likely benign and two variants as pathogenic or likely pathogenic (e-Table 6). We applied ACMG pathogenicity criteria,[22-24] using TL shortening as supporting evidence for single genetic etiology (PP4 criteria for pathogenicity; TL < 10th percentile

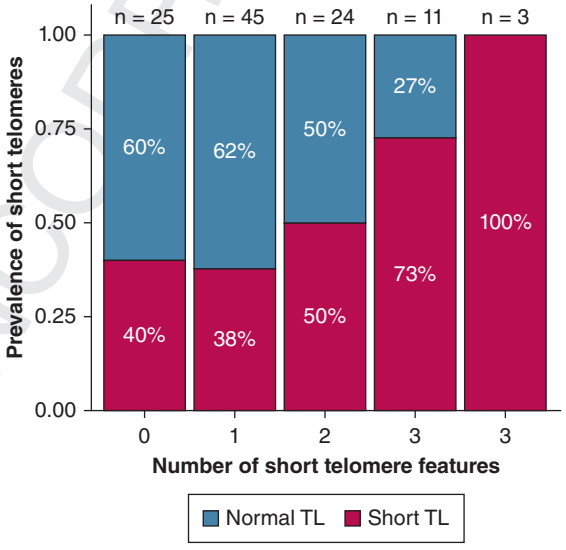


Figure 2 – *Clinical features and short telomeres. For patients with fibrotic interstitial lung disease (ILD) included in this study (n = 108), the prevalence of having a telomere length < 10th percentile for either granulocytes or lymphocytes by FlowFISH is shown for those having the number shown of short telomere features. Short telomere features include having a family history of fibrotic ILD in a related first- or second-degree family member, a personal or family history of gray hair before age 30 years, cryptogenic cirrhosis or liver disease, or unexplained hematologic disease. FlowFISH = flow cytometry and fluorescence in situ hybridization.*







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**TABLE 2** ] Logistic Regression Identification of Clinical Characteristics Associated With Short Telomere Length (FlowFISH < 10th Percentile)

| Characteristic | Univariable Analysis | | | Multivariable Analysis[a] | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *P* Value | OR | 95% CI | *P* Value |
| Age, y | 1.00 | 0.96-1.04 | .82 | 0.98 | 0.93-1.03 | .43 |
| Sex, male | 1.19 | 0.56-2.55 | .66 | 0.84 | 0.34-2.17 | .72 |
| Non-Hispanic White | 3.17 | 1.41-7.45 | **.006** | 5.01 | 1.85-15.2 | **.002** |
| Smoking pack-years | 0.99 | 0.96-1.01 | .36 | 0.97 | 0.93-1.00 | **.04** |
| IPF (yes/no) | 1.35 | 0.56-3.25 | .50 | 0.51 | 0.16-1.49 | .23 |
| Definite UIP on CT chest scan | 3.72 | 1.29-12.4 | **.02** | … | … | … |
| CT honeycombing | 3.77 | 1.60-9.42 | **.003** | 7.64 | 2.54-26.8 | **< .001** |
| No. of STS features[b] | 1.64 | 1.11-2.50 | **.02** | 2.00 | 1.27-3.32 | **.004** |

FlowFISH = flow cytometry and fluorescence in situ hybridization; IPF = idiopathic pulmonary fibrosis; STS = short telomere syndrome; UIP = usual interstitial pneumonia.
[a]All listed variables included in multivariable model.
[b]Clinical features of short telomere syndrome include familial pulmonary fibrosis, personal or familial history of premature graying before age 30 years, unexplained hematologic disease, and cryptogenic cirrhosis.

[PP4 moderate criteria] or < 1st percentile [PP4 strong criteria]) and reclassified two VUS as likely pathogenic variants (see the online supplement). In total, a positive diagnostic finding, defined as a likely pathogenic or pathogenic variant in a telomere-related gene, was found in four patients (*TERT*, n = 2; *PARN*, n = 1; *RTEL1*, n = 1). In addition, we identified a candidate diagnostic finding, defined as a VUS in telomere-related genes with TL < 1st percentile, in six patients (*TERT*, n = 3; *TERC*, n = 1; *NAF1*, n = 1; *ACD*, n = 1). One patient had a nondiagnostic VUS in *TERT* with TL between the 1st and 10th percentile. TL testing enabled reclassification of eight of nine variants originally classified as VUS into actionable positive or candidate diagnostic findings. In total, 10 of 34 patients (29%) had positive or candidate diagnostic findings and were offered cascade testing of relatives.

### *Comparison of Age-Adjusted TL From FlowFISH Assay With qPCR Assay*

There was high correlation between qPCR TL with FlowFISH TL in granulocytes ($R = 0.77$; $P < 2 \times 10^{-16}$) and lymphocytes ($R = 0.79$; $P < 2 \times 10^{-16}$) (e-Fig 7). The qPCR assay identified 24% of the overall cohort as having TL < 10th percentile. Having qPCR TL < 10th percentile was 47% sensitive and 95% specific for FlowFISH TL < 10th percentile, and 87% sensitive and 86% specific for FlowFISH TL < 1st percentile (e-Fig 8). Compared with qPCR < 10th percentile, FlowFISH < 10th percentile in lymphocytes had similar sensitivity and specificity for identifying actionable genetic findings and for identifying patients with short telomere features (e-Fig 9). When comparing agreement between assays, qPCR TL < 10th percentile had the best agreement with FlowFISH TL in lymphocytes < 10th percentile

**TABLE 3** ] Clinical Pharmacologic Changes After Telomere Length Testing

| Type of Treatment Change[a] | cHP (n = 29) | UILD (n = 31) | CTD-ILD (n = 16) | IPF (n = 27) | Other (n = 5) | All (n = 108) |
|---|---|---|---|---|---|---|
| Stop, de-escalate, or avoid immunosuppressants | 10 (34%) | 9 (29%) | 1 (6%) | 0 | 2 (40%) | 22 (20%) |
| Avoid starting immunosuppressants | 5 (17%) | 6 (19%) | 1 (6%) | 0 | 2 (40%) | 14 (13%) |
| Decrease dose of immunosuppressants | 4 (14%) | 2 (6%) | 0 | 0 | 0 | 6 (6%) |
| Stop current immunosuppressants | 1 (3%) | 1 (3%) | 0 | 0 | 0 | 2 (2%) |
| Start immunosuppressants | 5 (17%) | 3 (10%) | 1 (6%) | 0 | 0 | 9 (8%) |
| Start antifibrotics | 3 (10%) | 2 (6%) | 0 | 2 (4%) | 1 (20%) | 8 (7%) |
| Any change | 15 (52%) | 14 (45%) | 2 (13%) | 2 (7%) | 2 (40%) | 35 (32%) |

cHP = chronic hypersensitivity pneumonitis; CTD-ILD = connective tissue disease-related interstitial lung disease; IPF = idiopathic pulmonary fibrosis; UILD = unclassifiable interstitial lung disease.
[a]Immunosuppressants: mycophenolate, azathioprine, or prednisone. Antifibrotics: nintedanib or pirfenidone.





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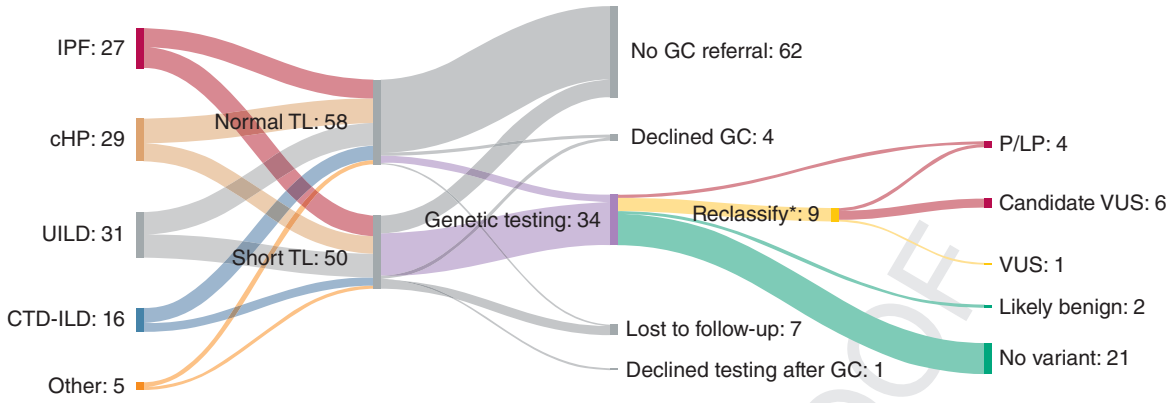


Figure 3 – *Genetic counseling referral and clinical genetic testing after telomere length testing. Sankey plot demonstrates the proportion of patients from each interstitial lung disease diagnosis identified to have short vs normal telomeres. Subsequent referral to a genetic counselor and follow-up status after telomere length testing is demonstrated, including results of genetic testing. A candidate diagnostic finding indicates variants of uncertain significance with a telomere length below the 1st percentile. cHP = chronic hypersensitivity pneumonitis; CTD-ILD = connective tissue disease-related interstitial lung disease; GC = genetic counselor; IPF = idiopathic pulmonary fibrosis; P/LP = pathogenic/likely pathogenic; TL = telomere length; UILD = unclassifiable interstitial lung disease; VUS = variant of uncertain significance. *Reclassification of variants accounting for telomere length as supporting evidence of pathogenicity.*

($\kappa = 0.59$) and FlowFISH TL in granulocytes < 1st percentile ($\kappa = 0.56$) (e-Fig 10).

## Discussion

To our knowledge, this is one of the first studies to systematically evaluate the implementation of TL testing in the clinical treatment of patients with fibrotic ILD. Given the emerging role of TL for aiding genetic diagnosis,[17,21] personalizing prognosis,[2,27] and risk-stratifying immunosuppressant therapy[8,14,15] in fibrotic ILD, we sought to understand the clinical impact of this test in a real-world setting. We found a high prevalence of short TL, defined as < 10th age-adjusted percentile by FlowFISH, in nearly one-half of patients undergoing testing. TL testing directly impacted the pharmacologic treatment of 35 of 108 patients (32%) and led to actionable findings by genetic testing for 10 of 34 patients (29%).

In our cohort of patients selected for TL testing, the overall prevalence of short TL < 10th percentile for either lymphocytes or granulocytes was 46%. This was similarly high for patients with IPF (52%) and patients without IPF (44%) and higher than in prior retrospective studies that used qPCR-based percentiles.[14,15] The high prevalence of short TL in this cohort is likely due to enrichment for patients with FPF or a personal or family history of extrapulmonary manifestations of short telomere phenotypes. We found that the number of short telomere features, but not any single feature, was associated with short TL. Prior reports have described variable penetrance of individual extrapulmonary manifestations of short TL[29] even among carriers of *TERT* and *TERC* mutations.[30,31] As nearly one-half of patients with ILD with FlowFISH TL < 10th percentile did not have any short telomere features, noninherited etiologies such as smoking,[32] chronic infection,[33] or inflammation[34] may contribute to their telomere shortening.

In our study, changes in immunosuppression medical therapy occurred predominantly in patients with cHP and UILD, diagnoses for which no consensus first-line therapy exists.[20,35] Most often, the identification of short TL led to cessation, reduction, or avoidance of immunosuppression, consistent with retrospective studies describing a harmful pharmacogenetic interaction between short TL and immunosuppression in patients with cHP[8,15] and UILD.[15] Patients with short TL may have an intrinsic immunodeficiency that is unmasked by exogenous immunosuppressants,[36] leading practitioners to consider alternative options such as antifibrotic therapy. However, for patients with CTD-ILD with short TL, practitioners often judged that the benefits of immunosuppression outweigh the risks. Further studies are needed to identify specific subsets of patients with ILD for whom identification of short TL may shift clinical equipoise toward avoiding immunosuppression and favoring antifibrotics as first-line therapy.

Consistent with prior reports,[37] our study identified telomere gene mutations in patients across multiple ILD diagnoses and highlights additional benefits of TL testing by reclassifying these variants from VUS to pathogenic or likely pathogenic. Curation of individual





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variants into ACMG pathogenicity classes[22-24] is often limited by lack of supporting experimental or phenotypic evidence. Patient-derived evidence of telomere shortening offers preliminary functional evidence in favor of pathogenicity (PP4 criteria) in telomere-related gene variants.[4,5] In the absence of rigorous testing in experimental models (PS3 criteria), which provides strong evidence of pathogenicity,[24] wider reporting of variants to curation databases such as ClinVar[26] can also help reclassify VUS if found in multiple affected unrelated individuals (PS4 criteria). Accurate reporting of ACMG pathogenicity class is especially important because cascade testing of family members is recommended for pathogenic or likely pathogenic variants but not for VUS. Given the high diagnostic rate of actionable mutations in our study, genetic counseling and testing should ideally accompany a finding of FlowFISH TL $<$ 1st percentile. Additional efforts are needed to expand the availability of genetic counseling and to understand patient barriers to testing so that the full benefit of TL testing can be realized. Until then, referral to specialized centers with genetic counseling expertise may be appropriate.

Our study identified similarly strong correlations between various measures of TL, whether between cell populations by FlowFISH (granulocytes vs lymphocytes; $R = 0.77$), or between different assays (qPCR vs FlowFISH; $R =$ 0.76-0.77). While the 10th age-adjusted percentile cutoff for both assays showed comparable sensitivity and specificity for associations with short telomere features or actionable genetic findings, we find that absolute cutoffs are not equivalent between the two assays. In this study, we defined short TL as being less than the 10th percentile based on multiple studies encompassing thousands of patients with ILD that have identified this cutoff for both qPCR and FlowFISH assays to be associated with risk of pulmonary fibrosis,[6,38,39] extrapulmonary consequences,[29,40,41] adverse outcomes,[7,10,15,29,38,42,43] or harm from immunosuppression.[14,15] As with any continuous assay, the association between risk and TL exists along a spectrum, with more adverse events associated with the shortest TL.[2,7,11,13,27,44-46] Similarly, dyskeratosis congenita is a rare syndromic disorder of extreme telomere shortening for which lymphocyte TL $<$ 1st percentile has a sensitivity of 97% and a specificity of 91% for differentiating patients from their unaffected relatives.[25] Further studies will be needed to identify clinically relevant cutoffs for TL, recognizing that cutoffs may depend on the type of measurement and the type of cell measured.

In our cohort, we identified a skew in the racial/ethnic makeup toward more non-Hispanic White patients found to have short TL. Discrepancies in TL by race/ethnicity have been previously described in both healthy[28] and ILD populations.[27] We identified fewer cases of short TL among patients with ILD of Black, Asian, and Hispanic racial/ethnic groups, consistent with prior studies.[28,47] This observation may be derived from differences in genetic variation,[42] epigenetic inheritance,[48] as well as medical[33,49] and social stressors,[50] or to the control populations used to validate FlowFISH nomograms. Additional studies are needed to determine if race- and ethnicity-specific reference panels could improve resolution for identifying critically short TL in these populations.

Our study has several limitations. First, our study was retrospective and there may be inconsistencies in provider assessment or patient recall of short telomere features. Second, because our study was a single-center experience and we did not perform TL testing on all patients, clinical associations with short TL should be independently validated. Third, the FlowFISH assay differs from the qPCR assay for TL, from which most clinical outcome data in ILD are derived. Applying the same age-adjusted 10th percentile cutoff for these two tests may yield differences in ability to distinguish patients with ILD at increased risk for worse outcomes. Fourth, only FlowFISH measurements from one clinical laboratory were used; these were not compared with other CLIA-certified laboratories. Despite these limitations, we demonstrate the feasibility of our approach to TL testing with a high detection rate of short TL in an identifiable subset of patients with ILD in a real-world setting. Although our findings demonstrate short-term changes in clinical management resulting from TL testing, continued follow-up of this cohort will determine how TL measurement impacts long-term clinical outcomes. Future studies, including randomized clinical trials, are needed to quantify the clinical benefit of TL testing on relevant outcomes like mortality and lung function decline.

In summary, we found that clinical TL testing in ILD is feasible, actionable, and impactful for clinical management. The real-world prevalence of short telomeres is high in patients with fibrotic ILD with a personal or family history suggestive of a short telomere syndrome. TL testing not only impacted pharmacologic treatment of non-IPF patients, but also led to upclassification of telomere gene VUS to likely pathogenic mutations or to candidate diagnostic





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variants. Thus, TL testing led to actionable results for patients with fibrotic ILD and their family members.

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