AGORA
RESEARCH LETTER

Telomere length in patients with unclassifiable interstitial lung disease: a cohort study

To the Editor:

Up to 15% of patients with chronic interstitial lung disease (cILD) will remain clinically unclassifiable (*i.e.* unclassifiable ILD, uILD) despite thorough clinical evaluation and multidisciplinary team discussion (MDT) [1, 2]. This diagnostic uncertainty translates into uncertainty in expected prognosis and initial treatment approach (*e.g.* immunosuppression *versus* anti-fibrotic medications) for patients with uILD, and it often precludes enrolment into clinical trials. Peripheral blood telomere length (TL) is a genomic biomarker that has been associated with prognosis and harm from immunosuppression in IPF [3, 4]. TL has recently been associated with idiopathic pulmonary fibrosis (IPF)-like morphologic features (*i.e.* features of usual interstitial pneumonia, UIP) and reduced survival in other forms of cILD [5–7]. Whether TL demonstrates similar associations in patients with uILD is unknown, but if so, its clinical measurement could reduce diagnostic and therapeutic uncertainty by determining which patients with uILD will have an IPF-like course. The aim of this study was to determine whether TL is associated with clinical features and outcomes in a cohort of patients with uILD.

This is an observational cohort study of 219 patients with uILD drawn from the University of California San Francisco ILD longitudinal clinical database and biorepository enrolled between February 2004 and September 2017. Baseline clinical information and blood samples were collected at the time of enrolment. All diagnoses were made by in-person MDT, aided by available clinical guidelines [8, 9]. When a confident MDT diagnosis could not be made, cases were labelled as "unclassifiable" in the database along with a differential diagnosis where the first-choice diagnosis was considered the most likely diagnosis by the MDT. 689 patients with a confident diagnosis of IPF during the study period served as a comparison for transplant-free survival.

TL was measured from genomic DNA isolated from whole blood using quantitative PCR as previously described [10]. DNA sequencing was not performed to evaluate for telomere-related variants as part of this study. High-resolution computed tomography (HRCT) images were reviewed by expert chest radiologists and scored for UIP pattern [8], semi-quantitative extent of fibrosis, and presence or absence of individual morphologic features [5].

Bivariate associations between TL and clinical and radiographic features were made using the t-test (for binary variables), ANOVA (for multi-category variables), or Pearson's correlation (for continuous variables). Logistic regression models were used to evaluate the association of TL with radiographic variables, adjusted for age. The primary outcome was transplant-free survival, defined as the time from enrolment to either death or lung transplantation. Kaplan–Meier plots were constructed to visualise transplant-free survival by quartile of TL in patients with uILD along with patients with IPF. The log-rank test was used to compare these groups. An adjusted Cox proportional hazards model was constructed to examine the association of TL with transplant-free survival accounting for potential confounders including age, sex, baseline pulmonary function, family history of ILD, race/ethnicity (non-Hispanic white *versus* other), first choice diagnosis, and mucin 5B (MUC5B) single nucleotide polymorphism rs35705950 (any minor allele *versus* wildtype). A second multivariable Cox model was constructed to compare transplant-free survival of IPF to uILD patients grouped by quartile of TL, adjusting for age, sex and baseline pulmonary function.

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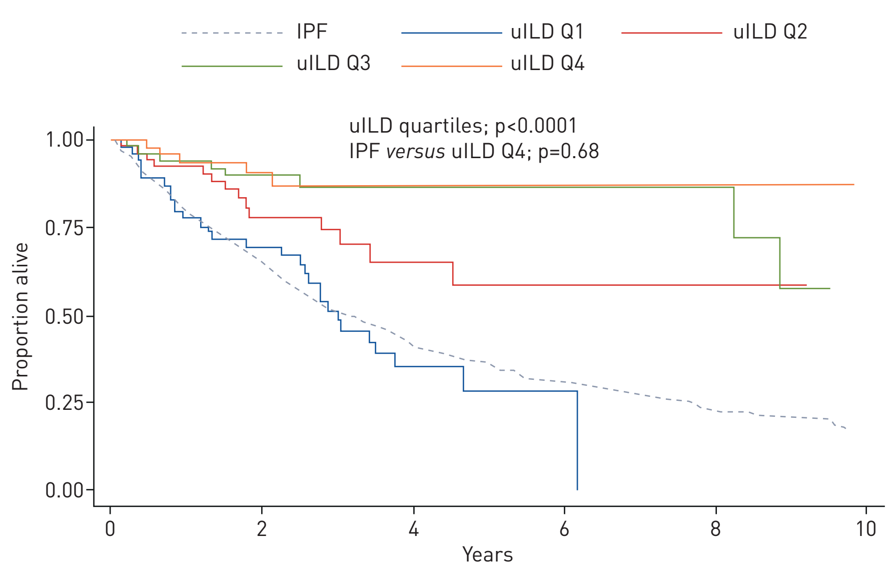
Of 247 patients identified with uILD and DNA samples, 28 were excluded for poor quality DNA, leaving a final cohort of 219 with TL measurement. The mean±SD TL in the cohort was 5773±524 base pairs (bp). TL was associated with age at diagnosis (r=−0.22, p=0.0012) but not sex, race/ethnicity, family history of ILD, smoking history or baseline pulmonary function. Average TL was associated with first choice diagnosis (from shortest to longest: smoking related-ILD; nonspecific interstitial pneumonia; IPF; hypersensitivity pneumonitis; connective tissue disease-associated ILD; other diagnoses; p=0.0075). While there was not a dose-dependent reduction in TL by MUC5B genotype (p=0.13), TL was shorter in patients with one or more MUC5B minor alleles (5698±535 bp) compared to homozygous wild-type (5839 ±508 bp) (p=0.046). In logistic regression models adjusted for age, TL was significantly associated with moderate to severe fibrosis (OR per 100-bp decrease 1.083, 95% CI 1.018–1.153; p=0.012) and traction bronchiectasis (OR per 100-bp decrease 1.163, 95% CI 1.062–1.273; p=0.001) on HRCT. TL was not associated with HRCT UIP pattern (p=0.28). Only 63/219 (28%) of patients underwent surgical lung biopsy, and having surgical lung biopsy was not associated with TL (p=0.61). The subset of patients with surgical lung biopsy was too small to formally compare histopathologic associations with TL.

There was a total of 58 deaths (n=56) or lung transplants (n=2) during a median follow-up of 2.3 years. TL was associated with transplant-free survival on unadjusted analysis (HR per 100-bp decrease 1.121, 95% CI 1.074–1.172, p<0.00001), and after adjustment for potential confounders (HR per 100-bp decrease 1.130, 95% CI 1.067–1.197; p=0.00003). Unclassifiable patients with TL in the lowest quartile had transplant-free survival nearly identical to that of patients with IPF (HR 1.119, 95% CI 0.766–1.636; p=0.56) (figure 1).

This large single-centre cohort study found that shorter TL in patients with uILD is associated with more severe radiographic fibrosis, traction bronchiectasis, and shorter transplant-free survival. This study adds to the growing evidence base demonstrating that TL is an independent and consistent prognostic biomarker across subtypes of cILD including IPF, chronic hypersensitivity pneumonitis, connective tissue disease-associated ILD and, now, unclassifiable ILD [5–7].

These findings suggest that TL may be a useful biomarker in the management of patients with uILD where the initial treatment strategy remains unclear. In the current clinical classification system of cILDs there is a dichotomy of initial treatment approach: anti-fibrotic medications for IPF and immune suppression for most other cILDs. In light of data suggesting harm from immune suppression in IPF patients with short telomeres [3], and recent RCTs showing benefit of anti-fibrotics in progressive non-IPF



Number at risk

	0	2	4	6	8	10
IPF	689	365	170	89	43	20
uILD Q1: 3849–5428 bp	54	31	9	1	0	0
uILD Q2: 5429–5802 bp	55	27	13	3	1	0
uILD Q3: 5803–6113 bp	53	32	14	7	6	2
uILD Q4: 6114–6898 bp	52	29	15	13	12	7

FIGURE 1 Transplant-free survival by quartile of peripheral blood telomere length in patients with unclassifiable interstitial lung disease compared to patients with idiopathic pulmonary fibrosis. IPF: idiopathic pulmonary fibrosis; uILD: unclassifiable interstitial lung disease; Q: quartile.

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fibrotic ILDs [11], we propose that TL measurement could be useful in selecting the initial treatment approach for patients with uILD. For example, short TL in an uILD patient might indicate upfront anti-fibrotic therapy as the most appropriate treatment approach, rather than a trial of immune suppression (which could be harmful) or watchful waiting for disease progression (which is very likely). On the other hand, for uILD patients with longer telomeres, the usual of approach of treatment selection based on the leading clinical diagnosis and/or clinical course may be most appropriate. However, further studies are needed to define the role of TL as a predictive (of treatment response) biomarker in uILD.

Strengths of this study include its relatively large size of well-characterised uILD patients and prospective data collection. Its major limitation is the lack of an independent replication cohort as we were unable to identify a separate, sizable cohort of well-characterised uILD patients with high-quality DNA samples. Other limitations include the lack of adequate data to determine differential responses to (and harms from) treatments and use of a non-clinical grade TL test with well-validated age-based thresholds that could be translated directly to the clinical setting. Additional studies are needed to replicate these results, and prospective studies should be designed to test the role of TL measurement in predicting response to and harms from available treatments for cILDs.

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