The prognostic role of matrix metalloproteinase-7 in scleroderma-associated interstitial lung disease

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To the Editor:

Systemic sclerosis (SSc) has the highest mortality rate amongst rheumatologic diseases [1]. It is characterised by endothelial dysfunction resulting in a small vessel vasculopathy leading to inflammation and fibrosis of skin and internal organs, including the lungs [1]. Pulmonary manifestations of SSc (pulmonary hypertension and interstitial lung disease (ILD)) account for the majority of deaths in these patients, and ILD alone accounts for a third of the mortality observed in SSc patients [2].

While ILD is a common manifestation of SSc, not every patient with SSc-ILD will have clinically significant ILD and prognosis remains a challenge. Mortality risk prediction algorithms using clinical and radiological variables have been developed in SSc-ILD, though these are often not used in clinical practice [3–5].

Disease-relevant biomarkers could refine understanding of prognosis among patients with SSc-ILD. Candidate biomarkers include matrix metalloproteinase-7 (MMP7), chemokine (C-C motif) ligand 18 (CCL18) and chemokine (C-X-C motif) ligand 13 (CXCL13). These biomarkers are prognostic in a similar fibrosing interstitial lung disease, idiopathic pulmonary fibrosis (IPF) [6–9]. In this study, we investigate these candidate biomarkers in SSc-ILD and their association with baseline lung function and prognosis.

Subjects with SSc-ILD were identified retrospectively from the University of California San Francisco (UCSF) ILD biorepository. Subjects were included in this study if they had a rheumatologist-confirmed diagnosis of scleroderma, evidence of ILD on high-resolution computed tomography (HRCT) scan or lung biopsy, and consented to the parent registry with baseline serum samples available. Clinical data (*e.g.* demographics, pulmonary function, comorbidities and smoking history) and serum samples were collected prospectively as part of a longitudinal registry, and approval was obtained from the local institutional review board.

Explanted lungs from patients with SSc-ILD and IPF were obtained at UCSF at the time of transplant. Tissues were either immediately frozen in liquid nitrogen or fixed in formaldehyde. Unused donor controls were processed similarly. Lung tissue sections were immunostained for MMP7 using a polyclonal goat antibody against human MMP7 (R&D Systems). RNA isolated from homogenised lung tissue underwent quantitative PCR for MMP7.

Commercially available ELISA assays for MMP7, CXCL13 and CCL18 were used. All biomarkers were measured in triplicate and the average value used for analysis.

The bivariate association between biomarkers and lung function were measured using Spearman correlations, t-tests and Wilcoxon rank-sum tests, as appropriate. We also assessed the adjusted association between log-transformed biomarkers and outcomes using multivariable regression methods to account for confounding. Continuous outcomes of forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide (D_{LCO}) % predicted were analysed *via* linear regression adjusting for age, sex, pulmonary hypertension and ever-smoking status, within which each effect of interest was tested *via* a Wald t-test. A significantly nonzero effect in these models suggests the biomarker had an association with baseline lung function even after controlling for these confounders. The outcome of transplant-free survival was assessed *via* Cox regression models adjusted for measures of disease severity (*e.g.* GAP and SADL

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Matrix metalloproteinase-7 level is associated with worse baseline pulmonary function and worse transplant-free survival in scleroderma interstitial lung disease https://bit.ly/3zZhja7

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scores, separately) [3] to assess the extent to which each biomarker had prognostic relevance after adjusting for disease severity. Based on exploratory modelling using generalised additive models, we categorised MMP7 into three categories (low, medium and high). For the transplant-free survival outcome, this categorical variable was tested *via* a log-rank test. A minor amount of data were missing (3.2%); however, 22.6% of subjects were missing at least one variable, so bivariate and multivariable results were pooled after multiple imputation (m=25) using chained random forests [10]. Group differences between tissue samples were assessed *via* Wilcoxon rank sum tests.

The study cohort included 115 SSc-ILD subjects with a median follow-up time of 4.34 years. The subjects were predominantly female (83.2%) with a mean age of 57.5 years and a history of smoking in 46.5% of individuals. The average baseline lung function among this cohort was 77.1% predicted FVC and 57.0% predicted D_{LCO}. Echocardiographic findings of pulmonary hypertension were present in 24.3%. The mean±SD extent of fibrosis scored on HRCT scans in this cohort was 14.9±14.6%. 24 subjects (21%) were on mycophenolate and no subjects were on antifibrotic therapy at the time of study enrolment.

For subjects with SSc-ILD, higher levels of serum MMP7 were associated with lower FVC % predicted (Spearman r=−0.423; p<0.001) and lower D_{LCO} % predicted (Spearman r=−0.516; p<0.001) (figure 1a). These associations remained significant (p<0.001) after adjusting for potential confounders including age, pulmonary hypertension, smoking status and sex. In the adjusted models, for every doubling of the value of MMP7, we observed a 6.64 unit decrease in FVC % predicted (95% CI −9.17, −4.11), and a 7.00 unit decrease in D_{LCO} % predicted (95% CI −10.09, −3.92). There was no association between CXCL13 or CCL18 level and baseline FVC % predicted or D_{LCO} % predicted in this cohort.

During the follow-up period, 20 subjects with SSc-ILD died or underwent lung transplantation. MMP7 level was associated with death or lung transplant among SSc-ILD subjects (hazard ratio (HR) 2.05, 95% CI 1.23–3.42, per doubling; p=0.009). Based on exploratory modelling using generalised additive models, we categorised MMP7 into three categories (low, medium and high: MMP7 <2000 pg·mL^{-1}, MMP7 2000–4000 pg·mL^{-1} and MMP7 >4000 pg·mL^{-1}), which corresponded well to disease severity (p<0.001) (figure 1b). The association between MMP7 and transplant-free survival remained significant when adjusting for the SADL score (HR 1.62, 95% CI 1.05–2.66; p=0.03) and the GAP score (HR 1.66, 95% CI 1.05–2.83; p=0.03). There was no association between CXCL13 or CCL18 level and transplant-free survival in this cohort.

To assess the biological plausibility of MMP7 in SSc-ILD, using immunohistochemistry, we found MMP7 expressed in SSc-ILD lung tissue, primarily in the alveolar type 2 cells and bronchiolar epithelium (figure 1e). This was similar in appearance to those with IPF (figure 1d). Further, MMP7 RNA levels were higher in SSc-ILD lung lysates compared to controls (p<0.001) and similar to IPF lung lysates (figure 1c).

In this study we found an association between serum MMP7 levels and baseline lung function in SSc-ILD (FVC % predicted and D_{LCO} % predicted). MMP7 levels were also predictive of death or lung transplant in this population. Like IPF tissue, expression of MMP7 is found in SSc-ILD lung epithelial cells and is over-expressed compared to controls.

Previous studies have shown that serum levels of MMP7 are associated with the presence of ILD among patients with SSc and that serum levels of MMP7 are significantly higher in SSc-ILD than normal controls [11, 12]. MMP7 has biological plausibility as a predictive biomarker for SSc-ILD. In IPF, MMP7 has been suggested to promote epithelial to mesenchymal transition, increase profibrotic mediators and reduce antifibrotic mediators [13]. Further, in IPF, MMP7 has been correlated with worse pulmonary physiology (FVC % predicted and D_{LCO} % predicted) and worse transplant-free survival [14, 15]. While the potential role of MMP7 in pulmonary fibrosis is not specific to SSc, it does highlight the potential shared pathogenesis across different aetiologies of pulmonary fibrosis.

Past studies investigating the association between biomarkers and baseline lung function and survival in SSc-ILD have been inconsistent, particularly in relation to to CCL18 [16–22]. While our study did not show an association between baseline CCL18 and CXCL13 and SSc-ILD outcomes, given the differences in study design, disease severity, treatment and sample size in our study compared to others, further data are needed to better understand the association between biomarkers and outcomes in SSc-ILD.

There are limitations to the interpretation of our findings, primarily due to the lack of a validation cohort.

This study demonstrates an association between baseline MMP7 and baseline lung function and survival in a single cohort of subjects with SSc-ILD. While MMP7 has been shown to be prognostic in IPF, the use

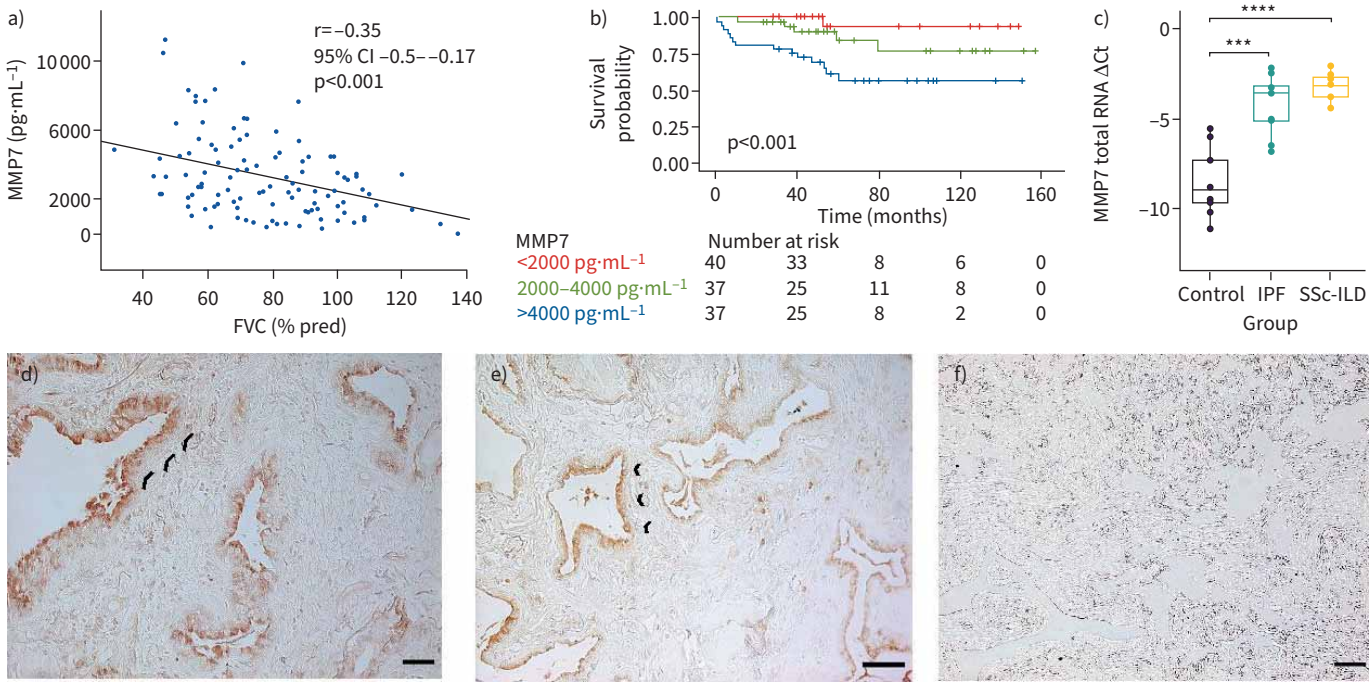

FIGURE 1 a) Plot of serum matrix metalloproteinase-7 (MMP7) levels on the *y*-axis and forced vital capacity (FVC) % predicted on the *x*-axis. There is a linear relationship between higher MMP7 levels and lower FVC % predicted (p<0.001). b) Kaplan–Meier survival curves based on a tertile distribution of MMP7 (tertile 1: MMP7 <2000 pg·mL^{-1}; tertile 2: MMP7 2000–4000 pg·mL^{-1} and tertile 3: MMP7 >4000 pg·mL^{-1}) (p<0.001). The *y*-axis here represents survival probability (from 1.0 to 0.0), while the *x*-axis represents time from blood draw date for this study (0 to 160 months). c) Relative MMP7 expression in control, idiopathic pulmonary fibrosis (IPF), and scleroderma-associated interstitial lung disease (SSc-ILD) total RNA. Average threshold cycle (Ct) values of the target gene (MMP7) were normalised to the Ct values of the reference gene (β-actin). Group differences were assessed *via* pairwise Wilcoxon rank sum test. ***: p=0.001; ****: p<0.001. This cohort (n=27) included samples from control subjects (n=9), and subjects with IPF (n=9) and SSc-ILD (n=9), matched for sex and age. d) Immunohistochemical staining of MMP7 taken from explanted lung from patient with IPF. MMP7 staining is denoted by arrowheads pointing out the brown immunoreactive staining which is noted in the alveolar and bronchiolar epithelium. e) Immunohistochemical staining of MMP7 in an explanted lung taken from a patient with SSc-ILD. As in (d), the scleroderma lung slide shows MMP7 staining, denoted by the arrowheads indicating brown immunoreactive staining, in the alveolar and bronchiolar epithelium. f) Negative control image of immunohistochemical staining of MMP7 in an explanted lung taken from a patient with SSc-ILD. Unlike (d and e), this slide was stained only with the secondary antibody (no MMP7 primary stain) which rules out non-specific or false positive staining from the secondary antibody alone. d–f) Scale bars represent 50 µm.

of MMP7 has yet to gain widespread clinical use. In contrast to IPF, SSc-ILD mortality is more heterogeneous, disease progression is not as predictable, and decisions regarding timing and initiation of pharmacological treatment are less straightforward. Future studies should further explore the utility of biomarkers like MMP7 in SSc-ILD, including validation of these findings in another cohort.

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