Ultrastructural alterations in the bronchial mucosa of patients with early interstitial lung disease associated with systemic sclerosis

Pulmonary fibrosis represents the most common disease related cause of death in patients with systemic sclerosis (SSc) (1).
The pathobiological hallmark of SSc is tissue fibrosis. In interstitial lung diseases (ILD), we have learned to recognise and understand this process by means of radiology and light microscopy. We hypothesized that SSc-ILD may cause altered deposition of extracellular matrix also in radiologically normal parts of the lung. We have therefore explored the sub-microscopic architecture of extracellular matrix in patients with early SSc-ILD and control subjects.
Three consecutive patients with new-onset SSc-ILD were invited for endoscopic bronchial biopsy before being subject to immunosuppressive therapy (2). Five healthy non-smokers served as controls. Bronchial mucosal biopsy was taken from lobar and segmental carinae of the right lung and analysed with transmission electron microscopy (TEM) (Supplementary methods) (3-5). Patients had a median disease duration of 15 months and mean age of 59 years, while control subjects had a mean age of 41 years (Supplementary Table S1).
SSc subject 1 suffered from mild ILD that did not progress. SSc subject 2 suffered from moderate ILD that halted after treatment with cyclophosphamide. SSc subject 3 suffered from progressive pulmonary fibrosis, causing terminal respiratory failure with autopsy-proven extensive, ubiquitous pulmonary fibrosis (6).
Using TEM we were able to localise the basal lamina of the subepithelial basement membrane in biopsies from all subjects (Fig. 1A). In every participant, at least 300 collagen fibrils were measured. SSc subject 1 exhibited similar mean (SD) diameter of collagen fibrils as control subjects; 31.2 (3.3) *vs*. 31.5 (4.3) nm. In contrast, both SSc subject 2 and 3 exhibited altered, wider collagen fibrils with the presence of large diameter fibrils not seen in the control group. Mean (SD) diameter of collagen fibrils in both subject 2 and subject 3 were larger than in controls; 37.9 (5.3) and 34.1 (4.1) *vs*. 31.5 (4.3) nm, $p<0.001$ for both comparisons (Fig. 1B-C).
To our knowledge, this is the first report of altered extracellular matrix composition in the airways of early SSc. Previous similar studies have mainly included patients with established SSc-ILD and analyses made post-mortem in reference to light microscopical features of fibrosis (7). In our study, we investigated patients with newly diagnosed SSc using TEM.
Saglani *et al*. analysed collagen fibril di-

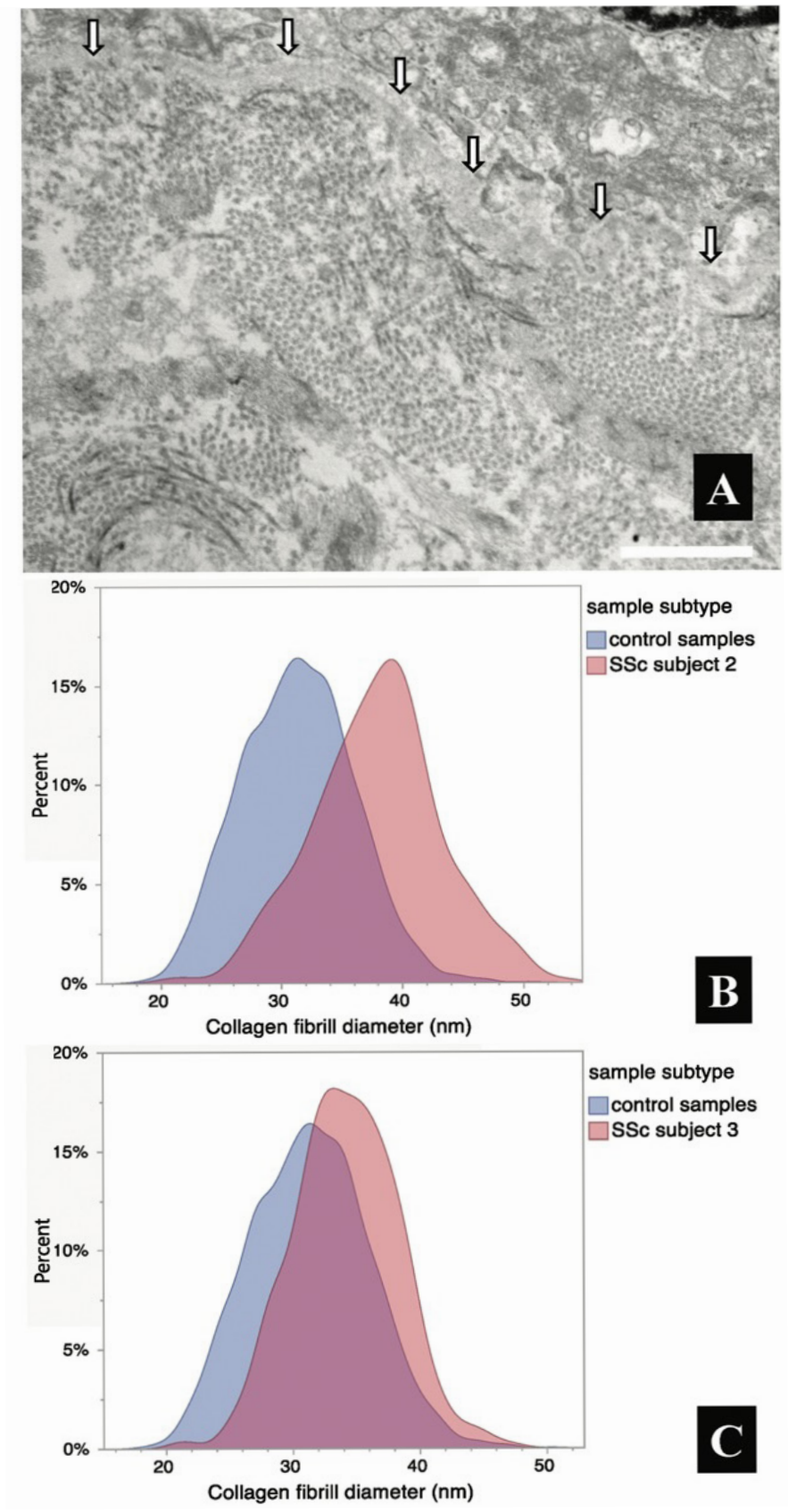

Fig. 1. A. Transmission electron micrograph of bronchial biopsy. The laminar layer (arrows) between the epithelial layer and the extracellular matrix below can be identified. Notice that collagen fibres are only present inferior to the basal lamina. Scalebar represents 1000 nm.
B-C. The distribution of collagen fibril diameter indicated that SSc subject 2 and 3 had wider collagen fibrils compared to control subjects.

ameter in the bronchial mucosa of patients with cryptogenic lung fibrosis and healthy controls (8). In agreement with our results, they also reported wider fibrils in patients with lung fibrosis compared to controls, results also numerically in line with those we report.

Collagen fibril diameter varies in different tissues and disease states. It is dependent on number of factors including the presence of small leucine rich repeat proteins (*e.g.*, decorin and fibromodulin) and cross-linking of collagen molecules by lysyl oxidase enzymes during the assembly of collagen fibrils. The expression of above-mentioned proteins has been shown to be altered in SSc related fibrosis including SSc-ILD (9, 10).

Unfortunately, we were unable to obtain sufficient material to complement our study with light microscopy including immunohistochemistry. Also, control subjects were on average older than participants with SSc. However, SSc subject 2 exhibited wider collagen fibrils than both control A and control C, participants that were older than SSc subject 2. We suggest our results to be interpreted as a hypothesis generating contribution to the field.

In conclusion, we report extracellular alterations on the ultrastructural level in the central airways of patients with early SSc-ILD. We suggest that further exploration of pulmonary remodelling in SSc-ILD also include sub-microscopic analyses of the extracellular matrix.

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