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Original Article

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Basic science

Transforming growth factor-beta is increased in sputum from individuals with rheumatoid arthritis-associated pulmonary fibrosis

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Abstract

Background: Interstitial lung disease (ILD) develops in 5–10% of patients with RA and contributes significantly to morbidity and mortality, particularly in those with a fibrotic phenotype. Yet, biomarkers to reliably identify RA patients with underlying pulmonary fibrosis are inadequate. Herein, we used sputum to identify lung-based biomarkers that distinguish RA patients with underlying pulmonary fibrosis and may better inform underlying pathogenesis in RA-ILD.

Methods: We included 37 RA patients with pulmonary fibrosis (RA-PF) and 30 RA patients without ILD (RA-no-ILD). Induced sputum and serum were tested for TGF-β levels by immunoassay. DNA was extracted to determine presence of the *MUC5B* ILD-risk allele ('T'). High-resolution CT (HRCT) and pulmonary function tests (PFTs) were completed within 3 months of sputum collection and quantified to determine lung disease severity.

Results: Sputum TGF-β was significantly elevated in individuals with RA-PF compared with RA-no-ILD ($P < 0.001$) and correlated with more fibrosis on HRCT ($P = 0.005$) and lower forced vital capacity ($P = 0.006$) and diffusion capacity of carbon monoxide ($P = 0.044$) on PFTs. Within RA-PF patients, sputum TGF-β was higher in those with the *MUC5B* ILD-risk genotype (GT/TT) ($P = 0.038$). There were no differences in serum levels of TGF-β between groups.

Conclusion: We demonstrate that sputum levels of TGF-β are significantly elevated in individuals with RA-PF, correlate with lung disease severity, and are elevated in those with the *MUC5B* ILD-risk polymorphism. These findings could identify novel approaches to ILD screening in RA and potential targeted therapeutic strategies for RA-ILD.

Keywords: RA, interstitial lung disease, pulmonary fibrosis, biomarkers.

Rheumatology key messages

- Enhanced biomarkers are needed to better understand the pathogenesis of rheumatoid arthritis-associated pulmonary fibrosis (RA-PF).
- Sputum transforming growth factor-beta (TGF-β) is increased in RA-PF compared to RA patients without lung disease.
- Sputum TGF-β levels are associated with decreased pulmonary physiology and increased lung fibrosis.

Introduction

Rheumatoid arthritis-associated interstitial lung disease (RA-ILD) is a severe and potentially life-threatening extra-articular manifestation of RA, which occurs in ~5–10% of RA patients [1]. RA-ILD most commonly develops in a fibrotic pattern termed usual interstitial pneumonia (UIP), and it is notably the fibrotic UIP subtype of RA-ILD that is associated with the worst outcomes and highest mortality [2]. In addition, ~30% of individuals with RA have evidence of pulmonary fibrosis (PF) on high-resolution CT (HRCT) imaging in the absence of

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symptoms or abnormal spirometry [3]. Regardless of whether a patient with RA is diagnosed with RA-UIP or found to have evidence of fibrosis on HRCT, most patients with RA-PF have a progressive lung disease that contributes significantly to morbidity and mortality [4–7], highlighting the importance to more effectively identify patients with RA-PF, better understand RA-PF pathogenesis and ultimately improve patient outcomes.

Transforming growth factor-beta 1 (TGF-β) is a multifunctional cytokine that has been implicated in various biological processes and importantly has been shown to have a central role in the development and progression of fibrosis within various organs, including the lung [8, 9]. TGF-β is thought to exert fibrotic effects in the lung through a variety of mechanisms including fibroblast activation to myofibroblasts, mesenchymal cell transition of alveolar epithelial cells and deposition of extracellular matrix proteins [10–12]. In RA-ILD, serum levels of TGF-β have been shown to be elevated, although levels were also increased in RA patients without lung fibrosis [13]. Data using lung-based biomarkers in RA-PF are limited but could be a more informative reflection of the local lung microenvironment that is more directly related to the pathogenesis of lung disease. Notably, induced sputum sampling, in comparison to bronchoalveolar lavage fluid, offers a non-invasive approach to sampling the biologic environment of the respiratory tract that could better inform the relevant pathogenic pathways.

Induced sputum also samples the distal airway of the lung, which has become of increasing interest in the pathogenesis of RA-PF given that one of the strongest risk factors for developing RA-PF is the presence of a polymorphism (rs35705950) within the promoter region of the *MUC5B* gene [14]. *MUC5B* encodes for the mucin protein Mucin 5B which is the predominant mucin found in the distal airways of the lung. The presence of the mutant allele T is thought to result in enhanced mucin production and results in up to a roughly 6-fold increased risk of developing ILD in individuals with RA [14]. Yet, the mechanisms driving lung fibrosis in RA, particularly in the presence of the *MUC5B* risk allele, remain poorly understood.

In this study, we measured lung levels of TGF-β using induced sputum from individuals with RA with and without PF and determined associations with lung disease severity.

Methods

Study subjects

Participants were recruited from both rheumatology and pulmonary clinics at National Jewish Health and the University of Colorado. We identified 37 individuals with RA who had evidence of pulmonary fibrosis (RA-PF) on high-resolution computed tomography (HRCT). The RA-PF group included RA patients with clinically diagnosed ILD who exhibited a radiographic pattern of UIP ($n = 18$) and RA patients with no clinical diagnosis of ILD but evidence of pulmonary fibrosis on HRCT that was identified through participation in a lung HRCT screening study ($n = 19$) [3]. Pulmonary fibrosis on HRCT was defined as the presence of irregular reticulation, traction bronchiectasis and/or honeycombing and was confirmed and agreed upon by two thoracic radiologists. Patients with other forms of ILD (i.e. nonspecific interstitial pneumonia, organizing pneumonia) or patients in the lung HRCT screening study who exclusively had non-UIP radiographic findings (i.e. ground glass opacities, centrilobular nodules) in the absence of pulmonary fibrosis on HRCT were not included as the focus of the study was on lung fibrosis. As a comparator group, we included 30 individuals with RA and no ILD (RA-no-ILD). These RA-no-ILD patients had no clinical diagnosis of ILD as well as no evidence of pulmonary fibrosis or other parenchymal abnormalities on HRCT. Medical chart review was performed to obtain RA clinical characteristics, including RF positivity, anti-CCP positivity and RA disease duration.

Sputum collection

Induced sputum was collected using established protocols [15, 16]. Briefly, sputum samples were collected over 15 min using hypertonic nebulized saline. The cell differential of the sample was determined by cytocentrifugation. Samples were considered to be of appropriate sputum quality if the cell differential contained <80% squamous epithelial cells. Collected sputum was diluted with phosphate-buffered saline (PBS) and mechanically homogenized using syringe-based protocols. Following centrifugation, the cell-free supernatant was removed from the cell pellet, and both supernatant and pellet were stored at −80°C.

Sputum cytokines/chemokines

The sputum supernatant was used to quantify lung levels of TGF-β using the Meso Scale Discovery (MSD) TGF-β1 antibody set (catalog # B20XW-3). The supernatant was also used to quantify additional cytokines and chemokines in the lung using the MSD V-PLEX Human Cytokine 30-Plex Kit (catalog # K15054D), which included quantification of the following: eotaxin, eotaxin-3, granulocyte-macrophage colony stimulating factor (GM-CSF), IFN gamma (IFN-γ), IL 1-alpha (IL-1α), IL 1-beta (IL-1β), IL 2 (IL-2), IL 4 (IL-4), IL 5 (IL-5), IL 6 (IL-6), IL 7 (IL-7), IL 8 (IL-8 HA), IL 10 (IL-10), IL 12/IL 23 heterodimer (IL-12/IL-23p40), IL 12 (IL-12p70), IL 13 (IL-13), IL 15 (IL-15), IL 16 (IL-16), IL 17 (IL-17A), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), monocyte chemoattractant protein 4 (MCP-4), macrophage-derived chemokine (MDC), macrophage inflammatory protein-1 alpha (MIP-1α), macrophage inflammatory protein-1 beta (MIP-1β), TNF alpha (TNF-α), VEGF-A. Of note, three analytes on the original V-PLEX assay were removed from the analysis due to failure of quality control measures (TARC), lack of expression within any sample (TNF-β) and redundancy (IL-8 was removed while IL-8HA, which measures higher range levels of IL-8, remained).

MUC5B genotyping

In samples with an adequate sample available, DNA was extracted from peripheral blood buffy coat or sputum cell pellet using QIAamp DNA Mini Kit and tested for presence of the ILD-risk *MUC5B* promoter allele using the assay (ThermoFisher, catalog # C___1582254_20) per the manufacturer's protocol. Each reaction analyzed 10–20 ng of genomic DNA. TaqMan assays were run on Thermo ABI 7500 system, and the results were analyzed with the accompanying Real-Time PCR software.

Lung disease severity

Lung disease severity was assessed using pulmonary function tests (PFT), which included percent predicted forced vital

capacity (% FVC) and percent predicted diffusing capacity of the lungs for carbon monoxide (% DLCO), as well as quantitative fibrosis scores. In 36 RA-PF and 27 RA-no-ILD participants, PFTs were collected within 3 months of the sputum collection, either through routine clinical care or during a dedicated research visit. HRCT scans from 29 RA-PF subjects were of adequate quality such that quantitative fibrosis could be calculated using a data-driven textural analysis (DTA) score, as described previously [17]. This approach applies machine learning to detect and quantify lung fibrosis (including reticular abnormalities, honeycombing and traction bronchiectasis) on HRCT images and expresses an extent score as the percentage of total lung volume involved.

Serum biomarkers

In a subset of participants with a sufficient serum sample collected at the same time point as sputum ($n = 21$ RA-PF and $n = 25$ RA-no-ILD), cytokine profiling was completed using the assays described above for sputum testing (TGF-β and multiplex MSD panel).

Statistical analysis

Participant characteristics were compared using χ^2 tests and independent samples *t*-tests. Cytokine/chemokine levels were log-transformed given their highly right-skewed distributions and compared between groups with independent samples *t*-tests. Simple unadjusted logistic regression models were also used to test the association between cytokines and odds of RA-PF. Multivariable logistic regression models were used to model these associations after adjustment for age and sex, which were baseline characteristics that significantly differed between groups ($P < 0.05$), as well as smoking status, which has a well-established association with the development of ILD in RA and potential impact on local cytokine production. For sputum cytokines/chemokines found to be significantly associated with RA-PF in these linear regression models, we further evaluated their relationship with RA-PF while mitigating any potential influence of collinearity using partial least squares discriminant analysis (PLS-DA) and determining variable importance in projection (VIP) scores. Spearman's correlation coefficient was calculated to determine the relationship between sputum cytokine levels and lung disease severity as measured by % FVC, % DLCO and DTA fibrosis score. The natural log of DTA fibrosis was used in visualizations of these correlations for ease of interpretation. In addition, a receiver operating characteristic (ROC) curve was completed using the true positive rate compared with the false-positive rate at various sputum TGF-β level thresholds and the area under the curve was identified to characterize the general diagnostic utility (sensitivity and specificity) of the test.

Study approval

All study procedures were approved by the Colorado Institutional Review Board and in compliance with the Helsinki Declaration. Written informed consent was obtained from all subjects.

Results

Participant characteristics

Participant demographics and clinical characteristics are included in Table 1. Individuals with RA-PF were significantly older (64.5 vs. 50.4 years, $P < 0.001$) and less often female (57% vs. 90%, $P = 0.003$) compared with individuals with RA-no-ILD. There was a non-significant trend towards more former/current smokers in the RA-PF group. The mean fibrosis score across all individuals with RA-PF was 7.8%. The mean fibrosis score was less in those with radiographic fibrosis compared with those with clinically significant ILD (1.47% vs. 18.15%). While there is no established DTA score parameter that represents clinically significant or radiographically significant ILD, prior studies in patients with idiopathic pulmonary fibrosis (IPF) report that a change of >3.4 represents a clinically significant progression of ILD [17]. Immunosuppression therapy at the time of sample collection is included in Supplementary Table S1, available at *Rheumatology* online. There were significantly more participants with RA-PF on rituximab compared with participants with RA-no-ILD (24% vs. 0%, $P = 0.002$). There were no other significant differences in medications between groups.

Table 1. Demographics and clinical characteristics

	RA-no-ILD	RA-PF	*P*-value[a]
n	30	37	
Age	50.4 (15.1)	64.5 (10.8)	<0.001
Female	27 (90%)	21 (57%)	0.003
Smoking status			
Former/current smokers	10 (33%)	19 (51%)	0.215
Pack-years	14.4 (15.5)	24.2 (21.9)	0.240
RA-associated autoantibodies			
RF positive	23 (77%)	30 (81%)	0.766
Anti-CCP positive	23 (77%)	27 (73%)	0.784
RA disease duration (years)	8.3 (8.5)	10.2 (10.5)	0.413
Lung disease parameters[b]			
% pred FVC	102.4 (14.4)	88.3 (18.0)	0.001
% pred DLCO	94.2 (20.1)	76.9 (18.6)	<0.001
% DTA fibrosis	N/A	7.8 (11.6)	N/A
Radiographic fibrosis	N/A	19 (51%)	N/A
RA-ILD (UIP)	N/A	18 (49%)	N/A
MUC5B genotype[c]			
GG	19 (83%)	24 (69%)	0.359
GT/TT	4 (17%)	11 (31%)	
Sputum TGF-beta levels (pg/ml)[d]	6.84 (1.02)	7.86 (1.12)	<0.001

All values are displayed as mean (S.D.) or *n* (%).
[a] *P*-value based on independent samples *t*-test or χ^2 where appropriate.
[b] %FVC and %DLCO unavailable for three RA-no-ILD and one RA-PF.
[c] MUC5B genotype was unavailable for 7 RA-no-ILD and 2 RA-PF.
[d] Raw levels were natural log-transformed due to right-skewed distribution.
DLCO: diffusing capacity of the lungs for carbon monoxide; DTA: data-driven textural analysis; FVC: forced vital capacity; RA-no-ILD: RA patients without Interstitial Lung Disease; RA-PF: RA patients with pulmonary fibrosis; UIP: usual interstitial pneumonia; GG: homozygous for *MUC5B* wild type polymorphism G; GT/TT: heterozygous or homozygous for *MUC5B* at risk polymorphism T.

Sputum TGF-β is increased in individuals with RA-PF

Mean sputum TGF-β levels were significantly higher in individuals with RA-PF compared with those with RA-no-ILD (7.86 ± 1.12 vs. 6.84 ± 1.02, $P < 0.001$) (Fig. 1A). There were no significant differences in sputum TGF-β levels amongst different immunosuppressive therapies or anti-fibrotics ($P > 0.05$). In a simple unadjusted logistic regression model, sputum TGF-β was significantly associated with PF in RA such that the odds of an individual having RA-PF were increased 2.58-fold for every one unit increase in the natural log of sputum TGF-β (95% CI [1.43, 4.65], $P = 0.002$). After adjusting

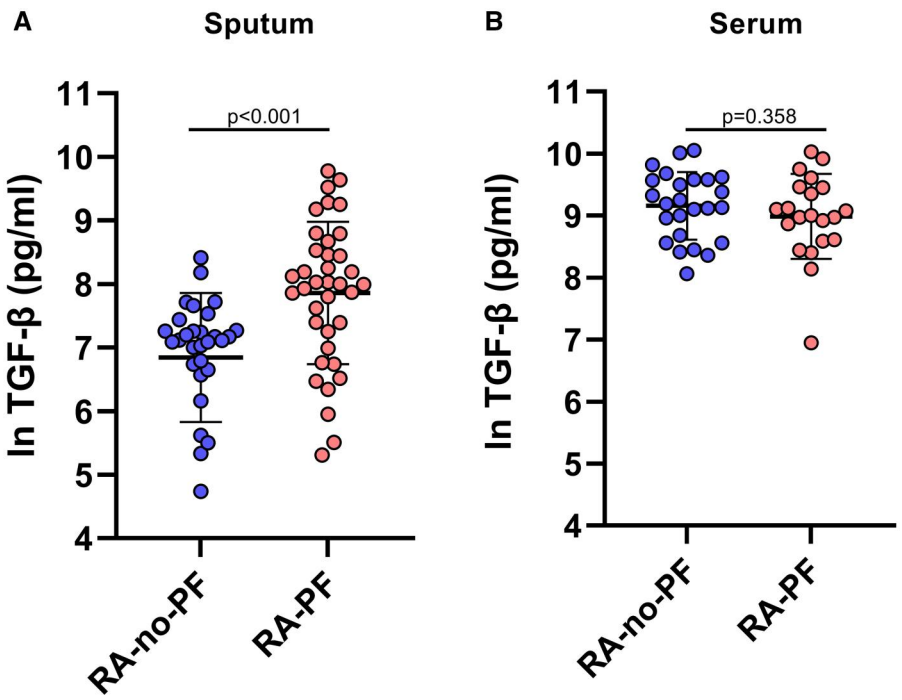

Figure 1. Relationship of natural log-transformed levels of TGF-β in sputum (A) and serum (B) between RA with pulmonary fibrosis compared with RA without ILD (RA-no-interstitial lung disease). Values are plotted as mean (S.D.). *P*-value determined by independent samples *t*-test

for age, sex, and smoking status, sputum TGF-β levels remained significantly associated with RA-PF such that the odds of an individual having RA-PF were increased 2.02-fold for every one unit increase in the natural log of sputum TGF-β (95% CI: [1.03, 3.97], $P = 0.041$). Additionally, using an ROC curve analysis examining the potential utility of sputum TGF-β as a screening tool for differentiating individuals with RA-PF from individuals with RA-no-ILD, the area under the curve (AUC) was 0.76 with a sensitivity of 73% and specificity of 77% at a log adjusted sputum TGF-β level of 7.33.

A subgroup analysis was performed comparing the portion of individuals with RA-PF who did not have a clinical diagnosis of ILD ($n = 19$) and individuals with RA-no-ILD, with the rationale to investigate the potential role of sputum TGF-β as an early biomarker for fibrosis. In this subgroup, sputum TGF-β remained significantly associated with RA-PF such that the odds of an RA individual having early fibrosis were increased 2.67-fold for every one unit increase in the natural log of sputum TGF-β (95% CI [1.19, 5.98], $P = 0.017$). In models adjusting for age, sex, and smoking status in this subgroup, the odds of an individual having RA-PF were increased 2.47-fold for every one unit increase in the natural log of sputum TGF-β (95% CI: [0.993, 6.16], $P = 0.052$).

Sputum TGF-β is associated with lung disease severity and fibrosis

There were significant inverse correlations between sputum TGF-β with % FVC ($r = -0.344$, $P = 0.006$) (Fig. 2A) and % DLCO ($r = -0.257$, $P = 0.044$) (Fig. 2B). In individuals with RA-PF, sputum TGF-β levels had a significant correlation with DTA fibrosis score ($r = 0.508$, $P = 0.005$) (Fig. 2C).

Sputum TGF-β and *MUC5B* genotype

As expected, the *MUC5B* promoter variant (T) was observed at a higher frequency in individuals with RA-PF compared with those with RA-no-ILD (31% vs. 17%, $P = 0.149$) (Table 1), although this was not statistically significant. Within individuals with RA-PF, there was no difference in sex between those with and without the ILD-risk *MUC5B* polymorphism (GT/TT) ($P > 0.05$), although those with GT/TT were ~7 years older ($P = 0.051$). Within individuals with RA-PF, sputum TGF-β levels were significantly higher in those with the ILD-risk polymorphism ($P = 0.038$) (Fig. 3A), although this relationship did not remain significant in models adjusted for age ($P = 0.153$).

Sputum cytokine profile in RA-PF

Using a multiplex cytokine panel, the relationships between log-transformed sputum levels of 27 different cytokines/chemokines and ILD status were analyzed with logistic regression models. In simple unadjusted models, several cytokines were significantly positively associated with increased odds of RA-PF, including MCP-1, IL-2, IL-5, IL-16 and TNF-α (Supplementary Table S2, available at *Rheumatology* online), even after adjustment for multiple comparisons (Bonferroni adjusted *P*-value < 0.002). After adjusting for age, sex and smoking status, MCP-1, IL-2, IL-5 and TNF-α remained positively associated with increased odds of RA-PF. These associations also persisted for MCP-1, IL-2 and IL-5 in the subclinical RA-PF subgroup (Supplementary Table S3, available at *Rheumatology* online). Additionally, IL-15, IL-16 and IL-1β trended towards an association with disease status, though did not meet the cutoff criteria for Bonferroni correction or reach *P*-value <0.05 in the adjusted models. When including sputum TGF-β levels in the model along with age, sex and smoking, IL-5 remained significantly associated with RA-PF (OR = 1.40, 95% CI [1.02, 1.92], $P = 0.035$), whereas MCP-1 (OR = 2.75, 95% CI [0.99, 7.66], $P = 0.053$), IL-2 (OR = 2.08, 95% CI [0.73, 5.94], $P = 0.169$) and TNF-α (OR = 1.36, 95% CI [0.0.65, 2.82], $P = 0.412$) were no longer significantly associated. Using PLS-DA plots

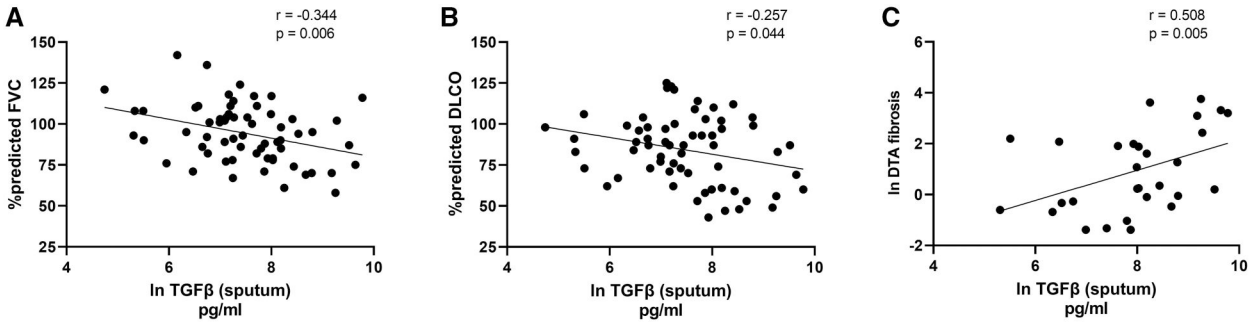

Figure 2. Correlation of natural log-transformed levels of TGF-β in sputum with % predicted forced vital capacity (A), % predicted diffusion capacity of carbon monoxide (B), and log-transformed data-driven textural analysis quantitative fibrosis score (C). (A) and (B) include available data from all subjects (RA-nointerstitial lung disease and RA- pulmonary fibrosis) while (C) includes only RA-pulmonary fibrosis subjects. Correlation coefficient (r) and P-value determined by Spearman correlation

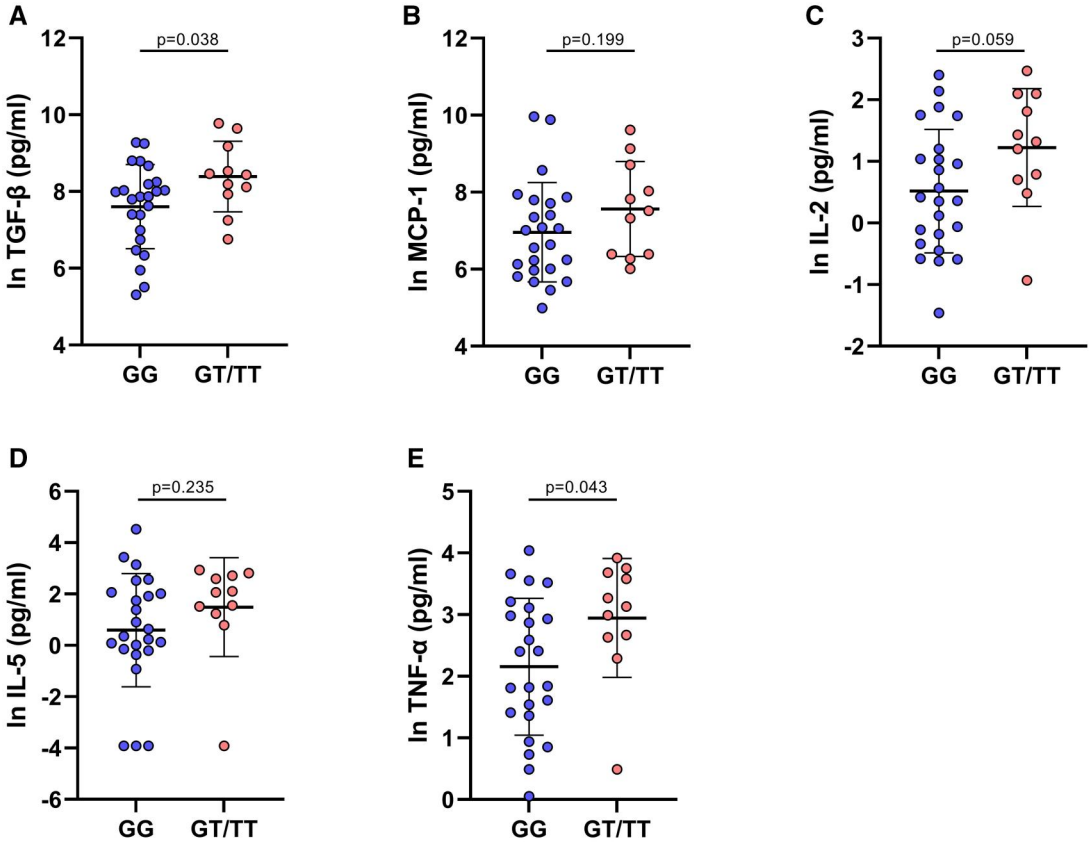

Figure 3. Sputum levels (log-transformed by natural logarithm) in RA-pulmonary fibrosis of TGF-β (A), monocyte chemoattractant protein 1 (B), IL-2 (C), IL-5 (D), and TNF-α (E) based on the presence (GT/TT) or absence (GG) of the ILD-risk MUC5B polymorphism. Values are plotted as mean (S.D.). P-value determined by independent samples t-test

that included TGF-β, MCP-1, IL-2, IL-5 and TNF-α, we found moderate separation between RA-PF and RA-no-ILD groups, with the highest drivers of this variation being IL-5 followed by MCP-1 and TGF-β (Supplementary Fig. S1, available at *Rheumatology* online).

Similar to sputum TGF-β, sputum MCP-1, IL-2 and IL-5 demonstrated a significant inverse correlation with %FVC (MCP-1: $r=-0.292$, $P=0.021$; IL-2: $r=-0.250$, $P=0.048$; IL-5: $r=-0.352$, $P=0.005$) and % DLCO (MCP-1: $r=-0.323$, $P=0.011$; IL-2: $r=-0.282$, $P=0.026$; IL-5: $r=-0.363$, $P=0.004$) as well as a significant correlation with DTA fibrosis scores (MCP-1: $r=0.519$, $P=0.005$; IL-2: $r=0.446$, $P=0.016$; IL-5: $r=0.578$, $P=0.001$) within individuals with RA-PF. Sputum TNF-α did not significantly correlate with %FVC ($r=-0.215$, $P=0.090$) or %DLCO ($r=-0.200$, $P=0.119$), but did show a significant correlation with DTA fibrosis scores ($r=0.479$, $P=0.009$) within individuals with RA-PF. Lastly, sputum TNF-α levels were also increased in RA-PF patients with the ILD-risk *MUC5B* allele ($P=0.043$), whereas sputum levels of MCP-1 ($P=0.199$), IL-5 ($P=0.235$) and IL-2 ($P=0.059$) were not significantly different by *MUC5B* risk status (Fig. 3B–E).

Serum TGF-β levels and cytokine profile in RA-PF

Serum levels of TGF-β were not significantly increased in RA-PF compared with RA-no-ILD individuals (8.99 ± 0.68 vs.

9.16 ± 0.54, $P = 0.358$) (Fig. 1B). There were also no significant associations between the other 27 cytokines/chemokines and odds of RA-PF in simple unadjusted logistic regression models after a correction for multiple comparisons (Supplementary Table S4, available at *Rheumatology* online), nor in multivariable models after adjustment for age, sex and smoking status with the exception of IL-2 (OR = 0.38, 95% CI [0.12, 0.82], $P = 0.045$) and eotaxin (OR = 5.56, 95% CI [1.01, 30.72], $P = 0.049$).

Discussion

While studies have established mechanisms by which TGF-β contributes to lung fibrosis, few have evaluated levels of TGF-β in RA-ILD or RA-PF. Lung levels of TGF-β are reported to be elevated in patients with IPF [18, 19], but this is the first study to report elevated sputum levels of TGF-β in RA-associated PF that correlated with lung disease severity and fibrosis, suggesting that these elevated levels of TGF-β in the lung may directly contribute to RA-PF pathogenesis.

Several clinical risk factors have been established for PF in patients with RA, including age, sex, history of smoking and the presence of ILD-at risk *MUC5B* genotype. However, there are limited biomarkers that can differentiate RA patients with and without PF. Identification of individuals with RA-PF can be challenging because current screening options include HRCT which can be limited by cost and radiation exposure. There is an unmet need for safe, non-invasive, easily repeatable biomarkers that can aid in screening for RA-PF. Such a biomarker would be of great clinical utility as the field moves toward earlier identification of RA-PF and as more treatment options become available for progressive PF. Our study used induced sputum to measure TGF-β levels, which is notable because induced sputum is a safe and non-invasive approach to measure the biology of the lung, in contrast to approaches such as bronchoalveolar lavage fluid collection. It is also notable that we did not see associations with peripheral blood TGF-β levels and RA-PF, suggesting a direct measure from the lung may be more specific for lung disease. It has been reported that TGF-β levels are increased in synovial fluid in patients with RA [20]. As such, peripheral blood TGF-β levels may pick up TGF-β elevations at non-pulmonary sites. Lastly, our findings showing an association of sputum TGF-β levels even in the subset of RA-PF with radiographic fibrosis support the notion that elevated sputum TGF-β can be identified early in the disease process.

TGF-β is a well-established inducer of lung fibrosis through direct effects including myofibroblast differentiation [21]. However, TGF-β could also have indirect influences on RA-PF. For example, TGF-β is a neutrophil chemoattractant, and given that RA neutrophils are more prone to neutrophil extracellular trap (NET) formation, and NETosis is suggested to contribute to other forms of PF, the association of TGF-β and NETosis in the lung in RA needs further study [22]. TGF-β is also involved in T cell homeostasis, which may be more specifically relevant in the pathogenesis of RA-PF than in other forms of PF. We also found higher sputum TGF-β levels in RA-PF patients with the *MUC5B* ILD-risk genotype. While in our cohort this relationship was not significant after adjusting for age, it would be of interest in future studies to explore the potential relationship between the MUC5B ILD-risk genotype and increased lung production of the pro-fibrotic cytokine TGF-β. Moreover, while the focus of this work was on differential cytokine/chemokine production in the lung in RA-PF, it will be important in future studies to understand the relationship between local cytokine/chemokine production and other biomarker proteins that have been associated with the presence and severity of lung fibrosis, such as Krebs von den Lungen-6 (KL-6), which is a glycoprotein expressed on alveolar epithelial cells and increased in ILD in association with alveolar epithelial cell injury [23, 24], and matrix metalloproteinases (MMPs), which are enzymes that degrade extracellular matrix components and can contribute to tissue remodeling in ILD [25].

In this study, we also evaluated the relationship between a range of cytokines and RA-PF. Several sputum cytokine levels were found to be increased in RA-PF even after adjusting for age, sex and smoking status, including MCP-1, IL-2, IL-5 and TNFα. These signalling molecules have been previously implicated in IPF and RA-ILD, although prior studies have primarily evaluated only serum levels [26–30]. It is of note that IL-5 remained associated with RA-PF after adjusting for TGF-β levels and was the strongest driver of variation between RA-PF and RA-no-ILD in PLS-DA plots. Thus, additional studies are needed to understand if these cytokines are increased in a shared pathway, parallel pathways or are mediators of the increased TGF-β production in RA-PF.

Our study has several limitations. Participants were evaluated in cross-section and longitudinal studies are needed to establish the relationship between these sputum cytokine levels and the progression of PF over time. Furthermore, the individuals with RA-PF included those with known prior diagnosis of UIP as well as individuals with newly discovered pulmonary fibrosis based on screening imaging. This was done in order to include a range of severity of PF; however, there may be inherent differences between these groups and in the underlying genetic risk, pathophysiology and progression which will need to be explored in future studies. We also focused on fibrotic lung disease, and additional studies are needed to understand if TGF-β or different cytokines are elevated in the sputum in non-UIP forms of RA-ILD.

In conclusion, we found that sputum-derived TGF-β levels were significantly elevated in patients with RA and pulmonary fibrosis, correlated with more severe lung disease, were elevated in those with the ILD-risk *MUC5B* polymorphism, and were associated with higher odds of RA and pulmonary fibrosis even after adjustment for age and sex. These results support future studies to better understand the relationship between cytokine generation within the lung, *MUC5B* genotypes and the associated mechanisms driving pulmonary fibrosis in RA.

Supplementary material

Supplementary material is available at *Rheumatology* online.

Data availability

Data are available upon reasonable request to the corresponding author but will not be shared publicly.

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