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ORIGINAL ARTICLE

Fibroblast activation protein and disease severity, progression, and survival in idiopathic pulmonary fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is characterized by progressive fibrosis in the lungs. Activated fibroblasts play a central role in fibrogenesis and express fibroblast activation protein α. A truncated, soluble form (sFAP) can be measured in blood and is a potential novel biomarker of disease activity. The aim was to study the association between sFAP and clinical, radiological, and histopathological measures of disease severity, progression, and survival in a prospective, multicentre, real-world cohort of patients with IPF. Patients with IPF were recruited from the tertiary interstitial lung disease centres in Denmark and followed for up to 3 years. Baseline serum levels of sFAP were measured by ELISA in patients with IPF and compared to healthy controls. Pulmonary function tests, 6-minute walk test and quality of life measures were performed at baseline and during follow-up. The study included 149 patients with IPF. Median sFAP in IPF was 49.6 ng/mL (IQR: 43.1–61.6 ng/mL) and in healthy controls 73.8 ng/mL (IQR: 62.1–92.0 ng/mL). Continuous sFAP was not associated with disease severity, progression or survival ($p > 0.05$). After dichotomization of sFAP below or above mean sFAP + 2 SD for healthy controls, higher levels of sFAP were associated with lower FVC % predicted during follow-up ($p < 0.01$). Higher than normal serum levels of sFAP were associated with longitudinal changes in FVC % predicted, but sFAP did not show clear associations with other baseline or longitudinal parameters. As such, sFAP has limited use as a biomarker of disease progression or survival in patients with IPF.

KEYWORDS
fibroblast activation protein, idiopathic pulmonary fibrosis, prognosis

1 | INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is an irreversible progressive scarring interstitial lung disease (ILD) characterized by self-sustained, progressing formation of fibrosis in the lungs. Data support the clinical experience that not all patients with IPF will develop a progressive phenotype from the time of diagnosis, and that some patients may remain stable for years. In a recent retrospective study from Canada, 59% of patients with IPF (66% on antifibrotic therapy) fulfilled commonly accepted criteria for a progressive fibrosing phenotype (during 24 months: ≥10% relative decline in FVC, 5%–10% relative decline in FVC and worsening of symptoms, 5%–10% relative decline in FVC and worsening of fibrosis on computed tomography of the lungs, or worsening of symptoms and fibrosis on computed tomography[1]), but 41% remained stable.[2] This emphasizes the urgent need of biomarkers to help identify not only patients with IPF but also patients with other forms of fibrosing interstitial lung diseases with a progressive phenotype. The advantages are obvious with respect to, for example, reduced latency in diagnostics with early onset of treatment initiation with antifibrotic therapies and early referral to lung transplant evaluation.

The pathological process in IPF is driven by a combination of several factors, for example, environmental exposure, genetic predisposition, behavioural, epigenetic and immunological triggers as well as increasing age, resulting in recurrent alveolar epithelial damage leading to dysregulation of epithelial repair mechanisms. A complex signalling pathway between epithelial, immune, mesenchymal and endothelial cells result in an inflammatory and fibrotic response that recruits fibroblasts and activates myofibroblasts to deposit collagen and other proteins in the extracellular matrix.[3] In IPF, activated fibroblasts and myofibroblast are often aggregated in areas close to injured alveolar epithelial cells, termed fibroblast foci, and are pathognomonic histological manifestations of lung damage.

Previously studied biomarkers related to the extracellular matrix include types I, III and VI collagen turnover. These biomarkers measure formation and degradation of collagens central to fibrosis in IPF and were associated with disease progression and survival.[4–6] As fibroblast play an important role in extracellular matrix secretion and remodelling, other biomarkers of fibroblast activity could be of interest. Activated fibroblasts in healing wounds express fibroblast activation protein α (FAP), which is a transmembrane glycoprotein with dipeptidyl peptidase and endopeptidase activities. Overexpression of FAP has been shown in fibroblast foci and fibrotic areas of the lung in histopathological samples, bronchoalveolar lavage (BAL) fluid, and on positron emission tomography (PET) scans.[7–10] Treatment with a FAP inhibitor in a murine model had a protective effect against bleomycin-induced fibrosis.[11] As FAP can be measured in a truncated, soluble form in blood,[12] we hypothesized that soluble FAP (sFAP) serum levels had potential as a new biomarker of disease activity in IPF. The aim of this study was to study the association between serum levels of sFAP and clinical, radiological and histopathological measures of disease severity and progression as well as survival in a prospective, multicentre, real-world cohort of patients with IPF.

2 | MATERIALS AND METHODS

2.1 | Study subjects

Patients with IPF were recruited from August 2016 to March 2018 at the three tertiary ILD centres in Denmark (Aarhus, Odense and Copenhagen). Incident and prevalent patients aged >18 years with IPF based on international guidelines were included.[13,14] Exclusion criteria were inability or unwillingness to adhere to the study as reported in studies based on the same cohort on patient related outcome measures and comorbidities in IPF.[15–19] Patients were followed for up to 3 years. Blood samples from healthy controls were analysed to obtain reference values for sFAP.

2.2 | Study measures

Patients attended study visits at baseline, 6, 12, 24 and 36 months. A high-resolution computed tomography (HRCT) scan was performed at baseline. Radiological analysis divided findings into usual interstitial pneumonia (UIP), possible UIP, or inconsistent with UIP. Histopathological samples were divided into UIP, probable UIP, possible UIP or not UIP, according to guidelines.[13] All diagnoses were the result of a multidisciplinary team discussion.[13] At each visit, pulmonary function tests (i.e. forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide (DLCO) performed in accordance with international guidelines[20,21]), 6-minute walk test distance (6MWD) and patient reported outcome measures (IPF-specific version of St. George's Respiratory Questionnaire (SGRQ-I),[15,17,22] King's Brief Interstitial Lung Disease questionnaire (K-BILD)[16,17,23] and University of California San Diego Shortness of Breath questionnaire (SOBQ)[24]) were completed, and blood samples were collected. The gender, age and physiology (GAP) index was calculated to assess mortality risk.[25] Disease progression was defined

as an absolute decline in FVC ≥5% or DLCO ≥10% after 12 months. Survival was registered up to 3 years after baseline. Progression-free survival was defined as a composite outcome of disease progression or death.

2.3 | FAP ELISA

Baseline serum levels of sFAP were measured by enzyme-linked immunosorbent assay (ELISA) according to the instructions from the manufacturer (RayBiotech, ELH-FAP). The assay was tested for binding of heterophilic antibodies and spike recovery prior to running the samples.[26] Mouse and bovine IgG for blocking samples were purchased from Jackson ImmunoResearch (catalogue numbers 015-000-003 and 001-000-003). Wash buffer was prepared with PBS pH 7.4 with 0.05% Tween-20. Samples were diluted 1:50.

2.4 | Statistics

Continuous data are presented as means with standard deviations (SD) or medians with interquartile range (IQR) after testing for normality using quantile–quantile plots (QQ-plots) and categorical data as frequencies. Categorical data were analysed using Chi-squared test, and continuous data were analysed using univariate or multivariate linear regression adjusted for antifibrotic treatment or Wilcoxon rank-sum test as appropriate. Repeated measurements were analysed using mixed-effects models with random intercept, and cluster effect for centre (using the 'Clustered Sandwich Estimator') was applied to the model to account for the possible within centre correlation. Survival estimates were analysed by the Kaplan–Meier method and Cox regression analyses adjusted for GAP index and antifibrotic treatment. Data were initially analysed for sFAP as a continuous variable and consequently dichotomized at below or above mean sFAP +2 SD for healthy controls to identify patients with higher-than-normal sFAP levels. Patient reported outcome measures with more than 15% missing items or missing domain or total scores were not included in the analyses. Imputation was not performed. Data were analysed using STATA, version 14.2 (StataCorp, College Station, Texas).

3 | RESULTS

3.1 | Patient characteristics

A total of 149 patients with IPF were included in the study (Table 1). The cohort comprised a majority of males with a history of smoking, preserved FVC and moderately reduced DLCO, and more than half of the patients received antifibrotic treatment. Progression after 12 months was observed in 49 patients (33%). The median sFAP in patients with IPF was 49.6 ng/mL (IQR: 43.1–61.6 ng/mL), whereas the median for healthy controls was 47.5 ng/mL (IQR: 43.1–50.0 ng/mL). No significant difference in sFAP was observed between patients with IPF and healthy controls ($p = 0.35$, Figure S1 in Additional file 1).

TABLE 1 Baseline characteristics.

Clinical characteristics	IPF patients	Healthy controls
Total cohort, *n*	149	17
Male/female, *n* (%)	121/28 (81%/19%)	14/3 (82%/18%)
Age, years (SD)	72.9 (6.3)	56.7 (11.1)
Smoking status		
Never, *n* (%)	40 (27%)	
Former, *n* (%)	100 (67%)	
Current, *n* (%)	9 (6%)	
FVC, % predicted (SD)	87.0 (23.1)	
DLCO, % predicted (SD)	48.5 (14.1)	
6MWD, m (SD)	451.7 (113.3)	
Long-term oxygen therapy (%)	19 (13%)	
Antifibrotic treatment, *n* (%)	84 (56%)	
Pirfenidone, *n* (%)	51 (34%)	
Nintedanib, *n* (%)	33 (22%)	

Note: Values are presented as *n* (%) or mean with standard deviation (SD).[15–19]

Abbreviations: 6MWD, 6-minute walk test distance; DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity.

3.2 | sFAP continuous

At baseline, no significant associations between continuous measures of sFAP and gender, smoking status, GAP index, HRCT pattern, histopathological pattern, disease duration or antifibrotic treatment were observed (all $p > 0.05$). Likewise, no association was found between sFAP and age, FVC, DLCO, 6MWD, K-BILD, SGRQ-I or SOBQ at baseline in univariate or multivariate analyses or with survival (Table 2). sFAP was neither associated with disease progression after 12 months in the entire cohort nor in a subgroup analysis of treatment-naïve patients at baseline, who initiated treatment within 6 months after inclusion.

TABLE 2 Association between sFAP and other outcomes.

	sFAP		Mean sFAP +2 SD for healthy controls			
			Below $n = 126$		Above $n = 23$	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
Age, years	−0.02 (−0.06–0.02)	−0.02 (−0.07–0.02)	Ref.	Ref.	−1.55 (−4.36–1.25)	−1.65 (−4.43–1.14)
FVC, % predicted	0.03 (−0.14–0.19)	0.02 (−0.14–0.18)	Ref.	Ref.	−2.63 (−7.52–2.26)	−2.76 (−7.64–2.11)
DLCO, % predicted	0.06 (−0.04–0.16)	0.06 (−0.04–0.16)	Ref.	Ref.	0.96 (−2.02–3.95)	0.91 (−2.07–3.89)
6MWD, m	0.18 (−0.62–0.99)	0.24 (−0.56–1.03)	Ref.	Ref.	23.9 (−2.4–50.3)	25.1 (−1.0–51.2)
K-BILD	−0.04 (−0.12–0.05)	−0.03 (−0.12–0.05)	Ref.	Ref.	−1.32 (−3.97–1.33)	−1.35 (−4.00–1.31)
SGRQ-I	0.04 (−0.12–0.19)	0.04 (−0.12–0.20)	Ref.	Ref.	0.42 (−4.42–5.26)	0.51 (−4.32–5.34)
SOBQ	−0.08 (−0.26–0.10)	−0.08 (−0.26–0.10)	Ref.	Ref.	−0.93 (−6.39–4.53)	−0.84 (−6.30–4.61)
Survival	0.996 (0.984–1.001)	0.999 (0.988–1.012)	Ref.	Ref.	0.94 (0.50–1.79)	1.31 (0.68–2.53)

Note: Differences in pulmonary function, exercise capacity and patient reported outcome measures at baseline and survival associated with sFAP as a continuous variable and dichotomized below or above mean sFAP +2 SD for healthy controls. Data are presented as slopes, differences or hazard ratios with 95% confidence intervals. Multivariate analyses were adjusted for antifibrotic treatment, and survival analyses were adjusted for antifibrotic treatment and GAP index.

Abbreviations: 6MWD, 6-minute walk test distance; DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; GAP, gender, age, physiology.; K-BILD, King's Brief Interstitial Lung Disease questionnaire; SD, standard deviation; sFAP, soluble fibroblast activation protein alpha; SGRQ-I, IPF-specific version of St. George's Respiratory Questionnaire; SOBQ, University of California, San Diego Shortness of Breath Questionnaire.

3.3 | sFAP dichotomized

After dichotomizing sFAP below or above mean sFAP +2 SD for healthy controls, similar results at baseline were observed for gender, smoking status, GAP index, HRCT pattern, histopathological pattern, antifibrotic treatment, age, FVC, DLCO, 6MWD, K-BILD, SGRQ-I and SOBQ (Table 2). However, FVC, DLCO, 6MWD and K-BILD scores developed differently in the two groups ($p < 0.001$ for interaction, Figure 1). In patients with higher sFAP levels at baseline, FVC was consistently lower at all follow-up visits, whereas DLCO in two and 6MWD in four of the visits were higher. K-BILD levels were neither consistently higher nor lower. No differences were found for SGRQ-I or SOBQ in relation to dichotomized sFAP values. No association with disease progression was observed in the total cohort or in the subgroup of incident patients initiating antifibrotic treatment within 6 months of baseline ($p > 0.05$). Survival (HR 0.94 [95% CI: 0.50–1.79]) and progression-free survival (HR 1.02 [95% CI: 0.62–1.66]) were similar in the two groups (Figure 2, Figure S2 in Additional file 1).

4 | DISCUSSION/CONCLUSION

Activated fibroblasts and myofibroblasts play a central role in the pathogenesis of IPF with overexpression of FAP in these cells in fibrotic areas of the lungs in patients with IPF. This is the first study to assess the association between the soluble form of FAP measured in serum and clinical, radiological and histopathological measures of disease severity and progression in a prospective, multicentre, real-world cohort of patients with IPF. Continuous levels of sFAP were similar in patients with IPF and healthy controls and were not associated with disease severity at baseline, disease progression or survival during follow-up of up to 36 months. When compared with healthy controls, IPF patients with sFAP levels above the upper normal values had lower FVC % predicted during follow-up, but no significant differences in other outcomes.

FAP has been found predominantly in fibroblast foci in lung biopsies from patients with IPF.[27] However, the same study showed a large variation in the expression of FAP in IPF lung tissue ranging from low levels comparable to healthy controls up to higher levels than seen in patients with silicosis. This indicates that there is a large heterogeneity in the number of activated fibroblasts in patients with IPF. This could partly explain the variation in measured sFAP and why no associations between baseline characteristics and sFAP levels were observed in our study. As such, it would be interesting to compare sFAP to FAP expression in histological samples from fibrotic lung tissue. It has been shown that disease progression in IPF does not follow a straight trajectory, and cross-sectional analyses at baseline does not necessarily reflect current activity in the disease.[28] Our study showed an association between higher sFAP and a larger decline in FVC % predicted during follow-up, but no significant differences in other outcomes. As

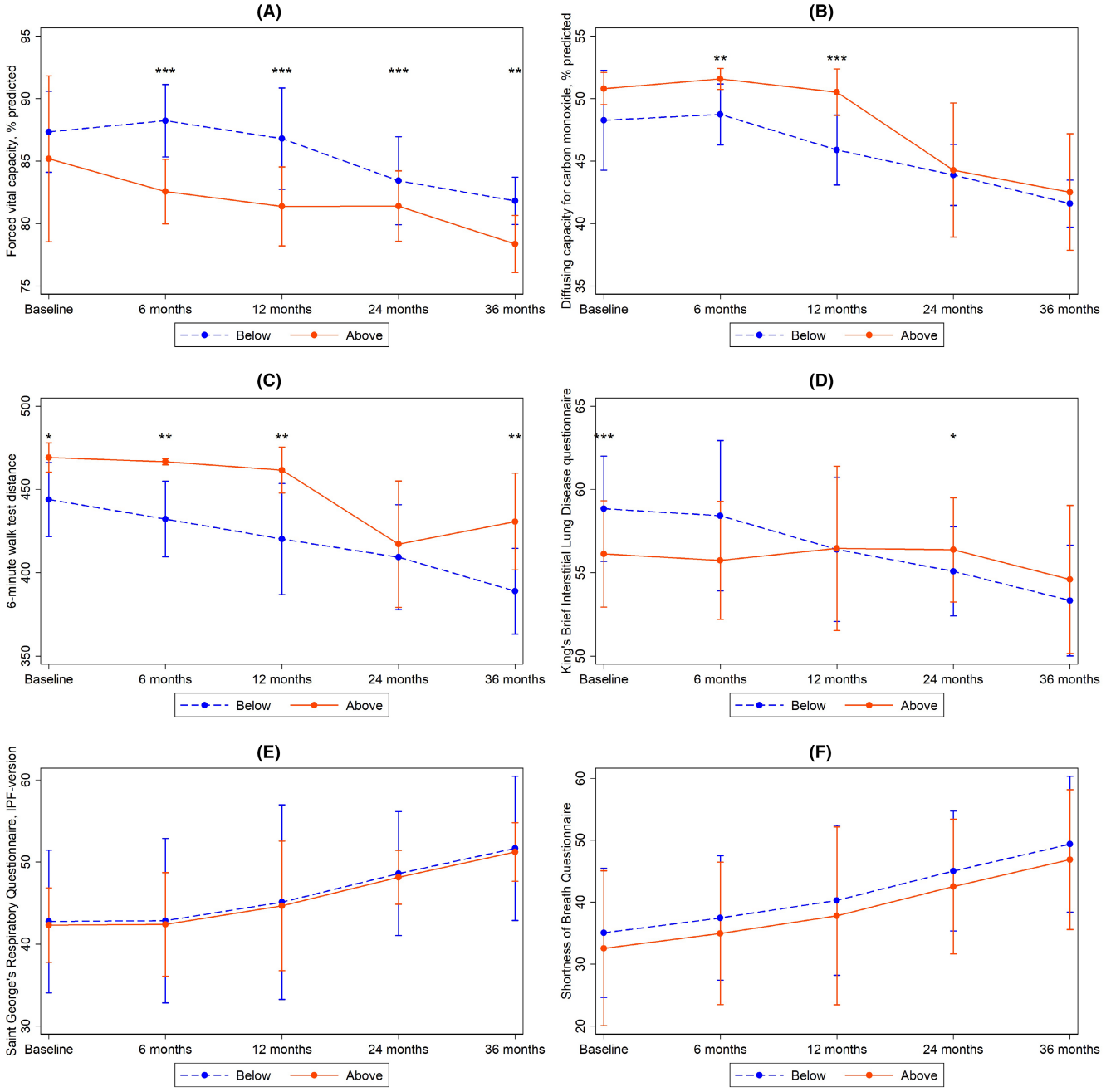

FIGURE 1 sFAP dichotomized below or above mean sFAP +2 SD for healthy controls related to (A) Forced vital capacity, % predicted, (B) Diffusing capacity for carbon monoxide, % predicted, (C) 6-minute walk test, (D) King's Brief Interstitial Lung Disease questionnaire, (E) Saint George's Respiratory Questionnaire, IPF-specific version, (F) Shortness of Breath Questionnaire. Data were analysed using mixed-effects models with random intercept and cluster effect for centre. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

such, sFAP has a limited use as a biomarker of disease progression or survival. The significant association with FVC may be caused by FVC being a more sensitive marker of disease progression compared to DLCO, as DLCO has a larger measurement variation than FVC.[29] Hence, smaller changes in FVC can be detected.

FAP measured in BAL fluid of IPF patients was higher than in controls and associated with disease progression.[10] A PET study using FAP inhibitor as tracer also showed a correlation between total standardized uptake value (SUVtotal) and decline in lung function during follow-up.[27] Other PET studies using murine models with bleomycin induced pulmonary fibrosis have shown similar results.[9,10] This supports FAP as a biomarker applicable for predicting disease progression whether measured in blood or BAL fluid or used as inhibiting tracer in PET scans.

Other blood biomarkers related to fibrosis have associations with disease progression and survival in IPF.

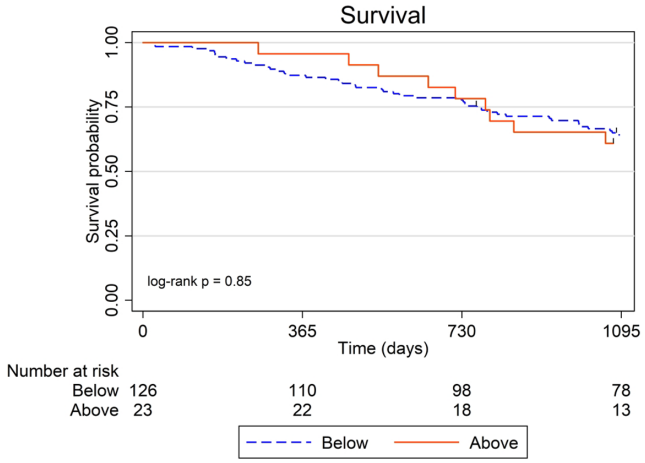

FIGURE 2 Survival in patients dichotomized below or above mean sFAP +2 SD for healthy controls measured at baseline. Data were analysed using the Kaplan–Meier estimator and log-rank test.

Imbalance in remodelling of the extracellular matrix involving formation and degradation of different types of collagens is a central mechanism in IPF disease progression. Recent studies have shown that cross-sectional and longitudinal measures of type I, III and VI collagen turnover are related to disease progression and mortality.[4–6,30,31] A combination of sFAP and other biomarkers, for example, collagen turnover measures, could be another approach to a prognostic tool. Similarly, longitudinal measurements of sFAP could potentially show associations with disease progression and mortality, as increasing levels of sFAP could indicate increasing disease activity. Future studies should investigate this hypothesis, as early identification of progression in patients with IPF and other types of ILD is important for choosing the best treatment for individual patients. Furthermore, it could be interesting to measure sFAP in patients with interstitial lung abnormalities to examine whether patients progressing to ILD have higher levels of sFAP. Knowledge of future progression should lead to early initiation of antifibrotic treatment, pulmonary rehabilitation, advance care planning and early palliation.[32] On the other hand, patients with little risk of progression might benefit from a watchful waiting approach to avoid side effects from antifibrotics while still receiving other treatment and care options.

A strength of this study is the nationwide, prospective, multicentre, real-world setting including patients with IPF from all tertiary ILD centres in Denmark without the strict in- and exclusion criteria of clinical trials. This allows for a broader and more generalizable sample of the entire IPF population. Also, the IPF diagnosis was based on multidisciplinary discussions, further increasing the reliability of the diagnosis. A limitation was the cross-sectional analysis of sFAP, which does not capture changes in serum levels of sFAP. Also, healthy controls were younger than patients with IPF. This may affect the measured levels of sFAP in the two groups.

Higher than normal serum levels of sFAP were associated with longitudinal changes in FVC % predicted, but sFAP did not show clear associations with other baseline or longitudinal parameters. As such, sFAP has limited use as a biomarker of disease progression or survival in patients with IPF.

AUTHOR CONTRIBUTIONS

TSP and EB initiated the project. TSP, EB, TWK and BWD designed the project. Data were collected by TSP, SBS, JRD, NH and EB. Laboratory analyses were performed by TWK, MPH and SL. Data analyses and initial draft of the manuscript were performed by TSP. Interpretation of the data and critical revision of the manuscript were conducted by all authors. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

TP has received research funding from Boehringer Ingelheim and Galapagos and lecture fees from Boehringer Ingelheim. JRD has received lecture fees from Boehringer Ingelheim and received financial support to attend ERS Congress from Boehringer Ingelheim. SBS has received lecture fees from Boehringer Ingelheim, Roche and Novartis, and received financial support to attend scientific meetings and the ERS Congress from Boehringer Ingelheim. BWD has been part of an advisory board for Boehringer Ingelheim and AstraZeneca, received lecture fees from Eli Lilly, and received research funding from Gilead and the Danish Rheumatoid Association. EB has received lecture fees from Boehringer Ingelheim. TWK is co-founder of a company making aptamer-based drugs to

treat rheumatoid arthritis and holds 20% of total shares and has received lecture fees from Abbvie. NH, MPH and SL have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data sets collected and analysed during the current study are not publicly available due to information that could compromise research participants' privacy but are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Written informed consent was obtained from all participants before inclusion in the study. Before initiation, the study was registered at clinicaltrials.org (NCT02818712) and approved by the Central Denmark Region Committee on Health Research Ethics (case no. 1-10-72-87-16, approved 20 June 2016).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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