Airway soluble CSF1R predicts progression in patients with idiopathic pulmonary fibrosis

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To the Editor:

Idiopathic pulmonary fibrosis (IPF) is a chronic debilitating lung disease, characterised by progressive deposition of excessive extracellular matrix in the lung parenchyma [1]. Though rare, IPF has a median untreated survival of 3 years from diagnosis, making it more deadly than many cancers [2, 3]. Two antifibrotic therapies, pirfenidone and nintedanib, have been shown to effectively slow IPF progression, but neither stop or reverse fibrogenesis [3]. Thus, there is an urgent unmet clinical need to develop more effective treatments for IPF and identify biomarkers.

Colony-stimulating factor (CSF)1, also known as macrophage-colony stimulating factor, is a glycosylated 40–70 kDa homodimer, and is a key regulator of the mononuclear phagocyte system [4]. CSF1 mediates its effects *via* binding the CSF1 receptor (CSF1R), a tyrosine kinase receptor [5]. CSF1R may also be activated by interleukin (IL)-34, a structurally unrelated ligand; CSF1 and IL-34 have distinct tissue expression and sequence homology, and have many nonredundant roles [6]. The CSF1–CSF1R pathway has emerged as a therapeutic target in IPF. Serum [7] and bronchoalveolar lavage (BAL) [8] of IPF patients have been reported to have higher concentrations of CSF1 compared to healthy controls. Mice deficient in CSF1 are protected from bleomycin-induced lung fibrosis in comparison to controls [8, 9]. Furthermore, pharmacological blockade of CSF1R ameliorates asbestos- [9] and radiation- [10] induced fibrosis in mice. In addition, increased CSF1 levels have been reported in lung lavage samples of radiation-treated human subjects and CSF1 activity has been implicated in mechanisms underlying radiation-induced fibrosis [10]. Under some macrophage-activating conditions, for example *via* activation of protein kinase C, or Toll-like receptor 4 [11], CSF1R is cleaved at the transmembrane region generating a soluble (s)CSF1R. However, the role of the CSF1–CSF1R axis in IPF is not clearly understood. We aimed to determine whether plasma and BAL levels of CSF1, sCSF1R and IL-34 are associated with IPF when compared to control subjects, and if these may serve as biomarkers of IPF mortality.

This was a retrospective study. Experimental protocols received ethical approval (10/HO720/12), and all subjects gave written informed consent. A diagnosis of IPF was made through multidisciplinary team discussion following the latest international guidelines [12]. Healthy volunteers had no history of lung disease or infection in the previous 6 months. Subject clinical characteristics are detailed in table 1. Pulmonary function testing was performed, clinical measurements were recorded and subjects underwent fibreoptic bronchoscopy with BAL *via* the oropharyngeal route according to a standard operating procedure [13]. Briefly, bronchoscopy of the right middle lobe was performed under a light sedation with midazolam in combination with local anaesthesia with lidocaine. Four 60-mL aliquots of warmed sterile saline were instilled in the right middle lung lobe and aspirated by syringe, and lavage aliquots were collected after each instillation was pooled for each patient. Volume and BAL appearance were recorded for all samples. BAL samples were processed on the day of sample collection. Whole BAL was strained through a 70-mm sterile strainer and subsequently centrifuged (700×*g*, 5 min, 4°C) and BAL supernatant carefully removed. BAL supernatant was divided into 1-mL aliquots, snap-frozen and sored at −80°C until analysis. Stored BAL and plasma were thawed and processed at Imperial College London (London, UK). Matrix metalloproteinase (MMP)-1, MMP-7, surfactant protein (SP)-D and Chitinase 3-like 1 (CHI3L1/YKL-40) concentrations were determined using a Luminex magnetic bead-based custom multiplex assay (R&D Systems) according to the manufacturer's protocol. Single-plex assays were performed for CSF1 (Meso Scale Discovery), sCSF1R (Ray Bio; high-specificity sCSF1R kit) and IL-34 (R&D Systems Quantikine ELISA kits) according to the manufacturers' instructions.

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This study provides the first evidence for a role of airway sCSF1R in IPF https://bit.ly/3KTBrCA

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TABLE 1 Comparison of mean biomarker concentration between idiopathic pulmonary fibrosis (IPF) and controls

	Luminex			RBH	Plasma				BAL			
	Luminex	Single-plex	Controls		Controls		IPF	p-value	Controls		IPF	p-value
Subjects	50	40		50								
Age	71.5±7.0	69.2±8.2	48±16	71.6±7.0								
Male	31 (62)	29 (72.5)	5 (38)	31 (62)								
FVC % predicted	81.1±16.7	85.0±21.4		81.1±16.7								
D_{LCO} % predicted				47.5±10.8								
Progression				13 (26)								
24-month progression	15/43 (34.9)	14/32 (43.8)										
36-month death/transplant	9 (18.0)	9 (22.5)										
Death				7 (14)								
10% FVC decline				8 (29)								
Proteins with known IPF association												
MMP-1 pg·mL^{-1}					12	2.96±4.57	50	11.84±29.04	0.09	12	0.04±0.01	50	0.17±0.28	0.0039
MMP-7 pg·mL^{-1}					12	4.70±2.66	50	16.57±19.14	0.0006	12	89.9±175.1	50	1135.5±2039.7	0.0005
SP-D pg·mL^{-1}					12	1883±1350.28	50	13 917.19±11 138.19	<0.001	12	16 39772±2238 109	50	64 5313±1422 002	0.011
YKL-40 pg·mL^{-1}					12	128.64±187.07	50	2126.97±3881.38	0.0004	12	1171.7±1499.6	50	1470.9±3273.6	0.89
CSF1–CSF1R pathway														
sCSF1R ng·mL^{-1}					10	175.10±96.34	51	227.79±181.14	0.68	17	1.16±0.83	76	1.98±1.13	0.001
IL-34 pg·mL^{-1}					12	[#]	50	[#]	[#]	12	0.34±0.18	50	0.21±0.13	0.0013
CSF1 pg·mL^{-1}					10	[#]	51	[#]	0.01	12	18.94±17.15	66	42.65±48.42	0.04

Data are presented as n, mean±SD or n (%), unless otherwise stated. Data compared using nonparametric rank-sum test. RBH: Royal Brompton Hospital; BAL: bronchoalveolar lavage; FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; MMP: matrix metalloproteinase; SP: surfactant protein; YKL: chitinase 3-like 1; CSF: colony-stimulating factor; CSF1R: CSF1 receptor; sCSF1R: soluble CSF1R; IL: interleukin. [#]: excluded due to >20% missing data.

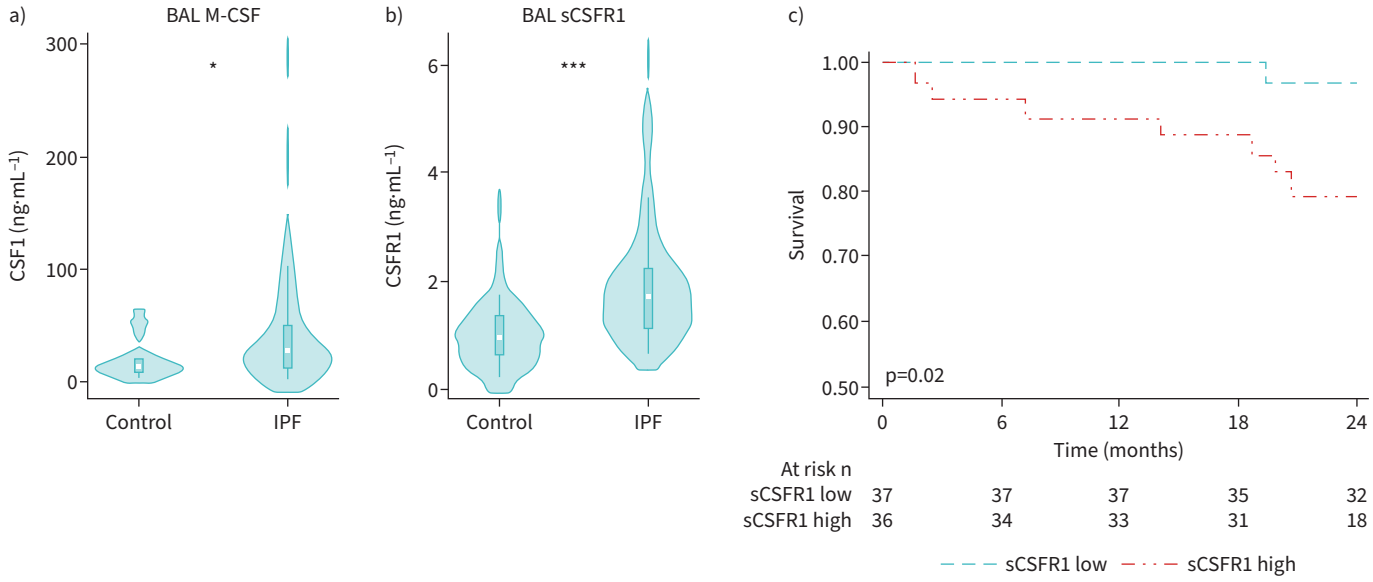

FIGURE 1 Concentrations of a) macrophage colony-stimulating factor (M-CSF) or b) soluble CSF1 receptor (sCSF1R) in the bronchoalveolar lavage (BAL) of patients with idiopathic pulmonary fibrosis (IPF; n=50) or healthy control subjects (n=17). c) Kaplan–Meier survival plot for subjects with IPF dichotomised above (high) and below (low) the median for sCSF1R levels in BAL. Individuals with an increased level of sCSF1R have worse survival than patients with a lower level of BAL-sCSF1R. *: p<0.05, ***: p<0.001.

Plasma/BAL protein concentrations are reported as mean±SD, compared using a rank-sum test due to skewed distribution and displayed visually using violin plots. The association between log-transformed protein concentration and all-cause mortality was assessed using univariable Cox proportional hazards regression and plotted using Kaplan–Meier analysis. Statistical analyses were performed using Stata (release 16; StataCorp, 2015) with statistical significance otherwise set at p<0.05.

This was a single-centre study. The mean age of the cohort was 71.5 years (62% male). CSF1 and IL-34 could not be detected in plasma for most subjects (table 1). Plasma sCSF1R was detectable in all subjects, but was similar between IPF patients and controls (control 175.10±96.34 ng·mL^{-1} *versus* IPF 227.79±181.14 ng·mL^{-1}; p=0.68). All three proteins could be detected in BAL, with higher concentration of CSF1 (control 18.94±17.15 pg·mL^{-1} *versus* IPF 42.65±48.42 pg·mL^{-1}; p=0.04) (table 1) and sCSF1R (control 1.16±0.83 ng·mL^{-1} *versus* IPF 1.98±1.13 ng·mL^{-1}; p=0.001) observed in patients with IPF compared to controls (table 1, figure 1a,b). Interestingly, sCSF1R levels in human BAL fluid was ∼100-fold lower than plasma levels in all subjects, whereas CSF1 levels were ∼10-fold higher in human BAL *versus* plasma. IL-34 levels were at or below the lower level of the assay performance, and these data should be interpreted with caution.

In outcome analysis, BAL sCSF1R concentration was associated with increased risk of death (hazard ratio 1.63, 95% CI 0.97–2.74; p=0.064), with those having sCSF1R concentration above the median having worse 3-year survival compared to those with concentration below the median (figure 1c; p=0.02). Among patients for whom progression data could be ascertained (n=58), progressors had higher mean BAL CSFR1 levels compared to nonprogressors (2.12±1.32 *versus* 1.75±0.94; p=0.28). There was no difference between progressors and nonprogressors when assessing BAL CSF1. No mortality association was observed for sCSF1R in plasma, nor any association observed for CSF1 or IL-34 in BAL.

To investigate whether CSF1–CSF1R axis findings mirrored those of previously identified biomarkers in IPF [14], we also compared IPF and control plasma and BAL concentration for MMP-1 and MMP-7, SP-D and Chitinase 3-like 1 (CHI3L1/YKL-40; table 1). In particular, MMP-7 [15], SP-D [16] and YKL-40 [17] have been shown to have value as prognostic biomarkers in IPF. Levels of plasma MMP-7 (control 4.70±2.66 pg·mL^{-1} *versus* IPF 16.57±19.14 pg·mL^{-1}; p=0.0006), but not plasma MMP-1 (control 2.96±4.45 pg·mL^{-1} *versus* IPF 11.84±29.04 pg·mL^{-1}; p=0.09) were significantly increased in IPF plasma compared to healthy controls. In the BAL, levels of both MMP-1 (control 0.04±0.01 pg·mL^{-1} *versus* IPF 0.17±0.28 pg·mL^{-1}; p=0.0039) and MMP-7 (control 89.9±175.1 pg·mL^{-1} *versus* IPF 1135.5±2039.7 pg·mL^{-1}; p=0.005) were significantly increased in IPF plasma compared to healthy controls. In our cohort, YKL-40

was significantly increased in plasma (control 128.64±187.07 pg·mL^{-1} *versus* IPF 2126.97±3881.38 pg·mL^{-1}; p=0.0004), but did not reach significance in BAL (control 1171±1499.6 pg·mL^{-1} *versus* IPF 1470.9±3273.6 pg·mL^{-1}; p=0.89) of IPF patients. In addition, we detected an increase in plasma SP-D (control 1883±1350.28 ng·mL^{-1} *versus* IPF 13 917.19±11 138.19 ng·mL^{-1}; p<0.001) and a significant decrease in BAL SP-D (control 1 639 772±2 238 109 pg·mL^{-1} *versus* IPF 645 313±1 422 002 pg·mL^{-1}; p=0.011) compared to controls.

There remains a pressing need for biomarkers in IPF. Herein, we describe, for the first time to our knowledge, sCSF1R levels in clinically relevant matrices of healthy *versus* diseased human subjects. Our data demonstrate that sCSF1R is present in greater abundance in the airways of subjects with IPF in comparison to healthy control subjects. Furthermore, baseline BAL sCSF1R was predictive of overall survival, even after accounting for baseline disease severity.

CSF1R blockade is an emerging therapeutic target for the treatment of fibrotic lung conditions. Release of sCSF1R can be observed following tumour necrosis factor-α-converting enzyme and γ-secretase action on macrophages and potentially *via* cleavage of the intracellular domain of CSF1R in epithelial cells [11]. Therefore, increased levels of sCSF1R may be due to enhance expression and/or cleavage of the receptor. Joshi *et al.* [9] identified that CSF1R is upregulated in human alveolar macrophage populations implicated in IPF pathogenesis. However, whether sCSF1R is a cause or consequence of the unique pulmonary milieu present in fibrotic lung tissue warrants further investigation. Our data would lend support to the role of the CSF1–CSF1R axis in IPF. Of note, our data indicate that total sCSF1R in the circulation is ∼0.2 µg·mL^{-1}; as sCSF1R probably binds antireceptor therapeutics, systemic exposures of these drugs may well need to exceed concentrations of this potential neutralising factor if they are to achieve optimal efficacy.

The current study has several strengths; for example, subjects were recruited before antifibrotic use, therefore biomarker values were not influenced by therapy. Furthermore, both local (BAL) and systemic (plasma) responses were analysed in our study. In terms of weaknesses, sample sizes were relatively small, which may have left some biomarker analyses underpowered. Although efforts were made to ensure consistency of measures (such as blinded sample randomisation), there were batch-by-batch variations; as the assay used to measure sCSF1R was a commercially available ELISA, further work will be required to validate specific assay methods. In addition, a further limitation was the lack of longitudinal sampling, which meant that a true representation of the relationship between cytokine levels and disease progression was not captured here, and a lack of validation in a second cohort.

To our knowledge, our study provides the first evidence for a role of airway sCSF1R in IPF. Additional research is needed to validate these findings and determine how sCSF1R levels can inform clinical decision-making in IPF. Thus, sCSF1R joins a growing number of biomarkers that hold promise for the assessment of patients with pulmonary fibrosis.

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