Biomarker signatures for progressive idiopathic pulmonary fibrosis

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Pathobiologically relevant circulatory biomarkers were found to be associated with IPF progression and mortality, and a statistical model incorporating these markers into a progression index score showed improved prognostication across all outcomes https://bit.ly/37L0oMl

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Abstract

Background Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease in which circulatory biomarkers have the potential for guiding management in clinical practice. We assessed the prognostic role of serum biomarkers in three independent IPF cohorts: Australian Idiopathic Pulmonary Fibrosis Registry (AIPFR), Trent Lung Fibrosis (TLF) and Prospective Observation of Fibrosis in the Lung Clinical Endpoints (PROFILE).

Methods In the AIPFR cohort, candidate proteins were assessed by ELISA as well as in an unbiased proteomic approach. LASSO (least absolute shrinkage and selection operator) regression was used to restrict the selection of markers that best accounted for the progressor phenotype at 1 year in the AIPFR cohort, and subsequently prospectively selected for replication in the validation TLF cohort and assessed retrospectively in the PROFILE cohort. Four significantly replicating biomarkers were aggregated into a progression index model based on tertiles of circulating concentrations.

Results 189 participants were included in the AIPFR cohort, 205 participants from the TLF cohort and 122 participants from the PROFILE cohort. Differential biomarker expression was observed by ELISA and replicated for osteopontin, matrix metallopeptidase-7, intercellular adhesion molecule-1 and periostin for those with a progressor phenotype at 1 year. Proteomic data did not replicate. The progression index in the AIPFR, TLF and PROFILE cohorts predicted risk of progression, mortality and progression-free survival. A statistical model incorporating the progression index demonstrated the capacity to distinguish disease progression at 12 months, which was increased beyond the clinical GAP (gender, age and physiology) score model alone in all cohorts, and significantly so within the incidence-based TLF and PROFILE cohorts.

Conclusion A panel of circulatory biomarkers can provide potentially valuable clinical assistance in the prognosis of IPF patients.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic disease of unknown aetiology, with a median survival of ∼2–5 years. While the overall prognosis is poor, the disease course and rate of progression are highly variable [1, 2]. The ability to stratify patients based on their predicted disease course has potential to inform management decisions, including the most appropriate time to commence antifibrotic treatment, potential transplant referral and planning end-of-life care. Identifying patients who are more likely to progress within a short time frame could also assist in stratifying clinical trial enrolment, allowing enrichment of trial populations with patients at greatest risk of decline.

There have been multiple studies in IPF aiming to identify reliable prognostic markers based on demographic data (age, gender and smoking status) [3, 4], clinical and physiological parameters (dyspnoea scores, and baseline and serial lung function) [5–7], as well as specific radiological features on high-resolution computed tomography (HRCT) scanning [8, 9]. While many of these parameters have been able to predict progressive disease either alone or in combination, detectable differences only occur after significant lung damage has occurred. Therefore, there is a need to identify molecules reflecting early underlying cellular and tissue damage; this led us to investigate blood biomarkers as predictors of disease progression and mortality. Many of these molecules have been associated with the pathogenesis of IPF as demonstrated by findings in bronchoalveolar lavage fluid and lung tissue studies [10–12]. Peripheral blood biomarkers are clinically more appealing than those obtained *via* other invasive procedures. Currently, there is a paucity of peripheral blood protein biomarkers that have been sufficiently replicated across different populations to be informative in clinical practice, even in conjunction with clinical/radiological parameters.

Using three international and well-characterised IPF populations, *i.e.* the Australian Idiopathic Pulmonary Fibrosis Registry (AIPFR) [13] as the primary cohort, and the Trent Lung Fibrosis (TLF) study [14] and the Prospective Observation of Fibrosis in the Lung Clinical Endpoints (PROFILE) cohort [11] for validation, we sought to assess biomarker profiles of patients with progressive *versus* stable disease, as markers indicating increased risk of progression at 1 year. We hypothesised that a panel of biomarkers could improve prediction of mortality and disease progression above current clinical scores. We used two methods of biomarker discovery: 1) a hypothesis-driven approach based on a selection of 12 molecules with known pathogenic roles in IPF, as well as 2) an unbiased proteomic approach to screen a large number of candidate biomarkers. After assessment of all these findings in the TLF validation cohort, we were able to formulate a progression index based on tertile levels of four biomarkers that sustained a signal which identified a population at risk of progression and then re-applied this index to test whether the addition of this progression index led to improved prognostic utility above that of the currently used clinical GAP (gender, age and physiology) score model.

Materials and methods

Study participants

The AIPFR is a national multicentre, prospective registry of IPF patients across Australia, collating comprehensive longitudinal data paired with a biobank of plasma and serum [13]. IPF diagnoses from physicians around Australia were centrally reviewed according to the 2011 American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association IPF guidelines for patients recruited to the AIPFR [15]. The TLF cohort recruited IPF patients from hospitals across England and Wales diagnosed by thoracic radiologists following HRCT review [14]. Blood samples from both the AIPFR and TLF cohorts were analysed at the same centre for both ELISA and proteomics (Harry Perkins Institute for Medical Research, Perth, Australia).

PROFILE is a multicentre, prospective cohort study of incident cases of IPF from secondary and tertiary centres in the UK, diagnosed by multidisciplinary discussion according to current diagnostic criteria (ClinicalTrials.gov: NCT01134822) [11]. The retrospective dataset generated from the PROFILE cohort was used as an additional replication cohort for the data generated by AIPFR and TLF. In the PROFILE cohort, the participants were missing biomarker values for POSTN (see supplementary table S1 for details of biomarkers), which impacted on subsequent score aggregation.

This study was approved by the Royal Perth Hospital Ethics Committee (HREC/2011-138) and the Sydney Local Health Network (HREC/15/RPAH/28). Ethical approval for the TLF study was granted by the Nottingham Research Ethics Committee (REC 09/H0403/59), while ethical approval for the PROFILE study was granted by the Royal Free Hospital Research Ethics Committee (REC 10/H0720/12) and PROFILE (Central England) Northampton Research Ethics Committee (REC 10/H0402/2). No patients were on pirfenidone or nintedanib at the time of blood collection.

Definitions

Our primary outcome of interest was progression status at 1 year, with date of consent being "time zero" across all three cohorts. In the AIPFR and TLF cohorts, disease stability was assessed in the 12 months following blood collection. "Progressive disease" was defined as a fall in forced vital capacity (FVC) ⩾10% and/or diffusing capacity of the lung for carbon monoxide (D_{LCO}) ⩾15% and/or death within 12 months from the time of blood collection, while "stable disease" was defined as the absence of progression over the same time frame. Classification and analysis of progression at 1 year was restricted to those with biomarker data and at least two lung function tests available.

Secondary outcomes included mortality and progression-free survival (PFS). Time to mortality was defined as the time from baseline (consent) to date of death. PFS was defined as the time to death or progression from consent.

Biomarker identification

ELISA analysis

12 biomarkers were selected on the basis of previous studies and measured by ELISA in serum using commercial kits according to the manufacturers' instructions. A list of all ELISA markers can be found in supplementary table S1.

Proteomic analysis

Plasma proteomics was performed in two stages as previously described [16, 17]. In brief, isobaric tags for relative and absolute quantitation (iTRAQ) and multiple reaction monitoring (MRM) were carried out to identify differential expression of circulating proteins in patients with stable and progressive IPF. First, iTRAQ was performed on pooled plasma samples from stable and progressive patients. Peptides that were differentially expressed were then quantified and validated using targeted mass spectrometry with MRM for all individual patient samples.

Protein identification and quantification were performed using ProteinPilot 4.5 beta software (AB Sciex, Macclesfield, UK) and spectra searched against the human SwissProt database as previously described [16]. A list of all proteomic metabolites can be found in supplementary table S1.

Statistical analyses

Identification of candidate prognostic biomarkers

The AIPFR cohort was used for initial exploratory analyses. The ELISA panel and proteomics were assessed in this cohort for candidate biomarkers predictive of clinical outcomes. Specifically, LASSO (least absolute shrinkage and selection operator) was used to shrink the selection to those that best account for this effect in AIPFR and key biomarkers associated with progression at 1 year were selected.

Univariable logistic regression and Cox regression analyses were performed for individual biomarkers to determine their relationship with progression status at 1 year, mortality and PFS outcomes. All univariable results are included in the supplementary material only. Multivariable adjustments for demographic and physiological parameters (age, gender, smoking status and baseline FVC) were also performed for each biomarker relative to each outcome. The false discovery rate (FDR) for all sets of analyses was controlled to 5% using Hochberg's method.

The ability of each biomarker to predict progression status was summarised using the area under the receiver operating characteristic curve (AUC) and 95% confidence intervals. Multivariable regression models were also estimated with all biomarkers (separately for ELISA and proteomics) using the LASSO estimation procedure to control for overfitting.

Development and validation of a progression index

The TLF cohort was selected as an independent cohort for the replication of all biomarker analyses obtained in the AIPFR cohort. A nonparametric Wilcoxon rank sum test was used to determine absolute differences in significant biomarkers between progressive and stable groups within this second cohort.

Associations were tested against progressor status as continuous data or as tertiles for ELISA panel concentration and mean peptide sequence protein ratio. Specifically, median values were compared between stable and progressors, and proteomic values were averaged across the contributing peptide sequences to estimate the concentration of protein. The biomarkers that proved significant were segregated into discrete tertiles based on circulating concentration levels and were aggregated into a progression index (0–8) from which they were assigned an index category (0–2, 3–5 or 6–8). The ability of each biomarker

to predict progression status using the tertiles was summarised using the AUC and 95% confidence intervals, and compared relative to the GAP score. Cox proportional hazards models were used to test associations with time to mortality and PFS, and proportional hazards assumptions were confirmed. A p-value of 0.05 defined the significance threshold. All analyses were performed using Stata version 16.0 SE (StataCorp, College Station, TX, USA).

All progression index models were adjusted for age, gender, baseline FVC and body mass index (BMI). Smoking data were not collected in the detail needed as part of the TLF study and therefore we did not include smoking in the multivariable models where comparisons were made across studies, in order to keep adjustments consistent. Unadjusted estimates of the progression index with disease progression, adjusted estimates as reported (FVC % pred, age, gender and BMI) and adjusted sensitivity estimates for D_{LCO} (D_{LCO}, age, gender and BMI) are included in the supplementary material only, to demonstrate comparable findings across TLF, AIPFR and pooled in multilevel analysis (supplementary figure S1).

Results

Patient demographics

Of the participants enrolled in the AIPFR at the time of this analysis, 189 had blood available for the investigation: 136 (72%) with stable disease and 53 (28%) with progressive disease. Demographic characteristics of this Australian cohort demonstrated predominantly males (n=136 (72%)), mean±SD age 69±8 years, FVC 82±19% predicted and D_{LCO} 49±15% predicted (table 1 and supplementary figure S2).

The TLF cohort comprised 211 IPF cases. Six individuals did not have serum available and so 205 individuals were included: 138 with stable disease (67%) and 67 with progressive disease (33%). Demographic characteristics demonstrated predominantly males (n=152 (74%)), mean±SD age 73±9 years, FVC 85±19% predicted and D_{LCO} 44±16% predicted (table 1 and supplementary figure S2).

The PROFILE cohort comprised 122 IPF cases with relevant biomarker data available: 68 with stable disease (56%) and 54 with progressive disease (44%). Demographic characteristics demonstrated

TABLE 1 Baseline demographics for the Australian Idiopathic Pulmonary Fibrosis Registry (AIPFR), Trent Lung Fibrosis (TLF) and Prospective Observation of Fibrosis in the Lung Clinical Endpoints (PROFILE) peripheral biomarker analysis

	Overall	Stable	Progressor	p-value
AIPFR	189	136	53	
Male	136 (72.0)	97 (71.3)	39 (73.6)	0.756
Age (years)	69.1±7.8	69.5±7.4	68.2±8.8	0.291
BMI (kg·m^{-2})	29.1±4.8	29.0±4.8	29.3±4.7	0.793
FVC (% pred)	82.3±19.1	82.8±19.7	80.8±17.2	0.576
D_{LCO} (% pred)	49.2±14.6	49.5±15.4	48.2±12.4	0.648
Ever-smoker	132 (69.8)	97 (71.3)	35 (66.0)	0.477
TLF	205	138	67	
Male	152 (74.2)	102 (73.9)	50 (74.6)	0.913
Age (years)	73.2±8.7	73.3±8.3	73.1±9.4	0.897
BMI (kg·m^{-2})	28.3±4.9	28.5±4.8	27.8±5.2	0.327
FVC (% pred)	84.7±18.7	87.0±17.8	80.1±19.7	0.017
D_{LCO} (% pred)	43.7±15.8	45.6±15.7	40.0±15.5	0.022
PROFILE	122	68	54	
Male	96 (78.7)	56 (82.4)	40 (74.1)	0.267
Age (years)	70.6±8.0	69.5±8.3	72.1±7.4	0.078
BMI (kg·m^{-2})	28.2±4.0	28.4±4.0	27.5±4.0	0.101
FVC (% pred)	81.2±19.1	83.4±19.1	78.4±19.0	0.164
D_{LCO} (% pred)	45.4±15.3	48.0±17.0	41.8±12.0	0.031
Ever-smoker	85 (69.7)	52 (76.5)	33 (61.1)	0.067

Data are presented as n, n (%) or mean±SD, unless otherwise stated. BMI: body mass index; FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide. The number of missing baseline FVC data across all cohorts is AIPFR 31 out of 189 (16.4%), TLF 16 out of 205 (7.8%) and PROFILE three out of 122 (2.5%); the number of missing baseline D_{LCO} data across all cohorts is AIPFR 46 out of 189 (24.3%), TLF 21 out of 205 (10.2%) and PROFILE eight out of 122 (6.6%).

predominantly males (n=96 (79%)), mean±SD age 71±8 years, FVC 81±19% predicted and D_{LCO} 45±15% predicted (table 1 and supplementary figure S2).

In the PROFILE cohort, five out of 122 had a record of antifibrotics (pirfenidone) at baseline (4.1%) and 14 out of 122 were prescribed during the course of 1 year follow-up (11.5%) with a median (interquartile range (IQR)) time to starting of 179 (132–201) days, with a further five prescribed after 1 year. Overall, for the 19 starting antifibrotics, the median (IQR) time to starting was 192 (161–367) days. There was no evidence of antifibrotics in concomitant medications in the AIPFR and TLF cohorts.

Identification of candidate prognostic biomarkers in the AIPFR cohort

To determine the association of biomarkers with clinical outcomes, empirical estimates of the AUC were made for each biomarker and those with AUC >0.6 were identified (supplementary table S2). Due to the large number of potential predictors, LASSO with 10-fold cross-validation was utilised to select the subset of variables that best predicted progression at 1 year. A total of 11 biomarkers were shrunk to zero <5 times, including six ELISA biomarkers (ENRAGE, ICAM1, OPN, POSTN, SPD and VCAM1) and five proteomic biomarkers (A2GL, APOE, GELS, PEDF and SAA4) (supplementary table S2).

For prediction of PFS, the biomarkers ICAM1 (p=0.007), OPN (p=0.0002) and SPD (p=0.0003) were associated with worse outcomes on multivariable Cox analysis, following Hochberg FDR adjustment (table 2), while an increase in mortality by multivariable Cox regression was observed with significant differences in OPN (p<0.0001) and POSTN (p=0.030).

TABLE 2 Multivariable analyses of the biomarkers and associations with progression-free survival and mortality in the Australian Idiopathic Pulmonary Fibrosis Registry cohort

Biomarker	Progression-free survival			Mortality		
	n	HR (95% CI)	p-value	n	HR (95% CI)	p-value
ELISA						
CCL18	161	1.009 (0.998–1.020)	0.1092	161	1.008 (0.995–1.022)	0.2211
CRP	160	1.008 (0.985–1.031)	0.4930	160	1.018 (0.992–1.045)	0.1675
CXCL13	161	1.000 (0.999–1.001)	0.9339	161	0.999 (0.997–1.001)	0.4612
ENRAGE	159	1.002 (1.000–1.005)	0.0651	159	1.003 (1.000–1.006)	0.0872
FBLN1	160	1.001 (0.997–1.005)	0.6048	160	0.999 (0.994–1.004)	0.7213
ICAM1	161	1.003 (1.001–1.005)	0.0068*	161	1.000 (0.997–1.003)	0.8926
MMP7	161	1.000 (1.000–1.000)	0.1799	161	1.000 (1.000–1.000)	0.2958
MUC1	40	1.019 (0.966–1.076)	0.4858	40	1.037 (0.980–1.098)	0.2068
OPN	161	1.028 (1.013–1.043)	0.0002*	161	1.033 (1.017–1.050)	<0.0001*
POSTN	159	1.011 (1.001–1.020)	0.0237	159	1.012 (1.001–1.024)	0.0303*
SPA	160	1.000 (0.996–1.004)	0.8470	160	1.003 (0.998–1.008)	0.2954
SPD	155	1.029 (1.013–1.045)	0.0003*	155	1.017 (0.997–1.038)	0.1029
VCAM1	160	1.000 (0.999–1.000)	0.6296	160	1.000 (0.999–1.000)	0.8854
Proteomics						
LUM	128	1.215 (0.746–1.979)	0.4331	128	1.003 (0.477–2.111)	0.9927
PGRP2	128	0.818 (0.298–2.242)	0.6956	128	0.343 (0.057–2.055)	0.2415
B2GPI	128	1.011 (0.813–1.257)	0.9228	128	0.624 (0.347–1.122)	0.1154
CO9	128	1.104 (0.729–1.671)	0.6395	128	0.817 (0.383–1.741)	0.6002
FN	128	0.330 (0.063–1.721)	0.1883	128	0.711 (0.109–4.649)	0.7217
ITIH1	128	1.020 (0.727–1.431)	0.9108	128	0.548 (0.217–1.387)	0.2046
A2GL	128	0.955 (0.716–1.275)	0.7564	128	0.751 (0.498–1.134)	0.1730
APOE	128	1.260 (0.817–1.941)	0.2957	128	1.099 (0.555–2.176)	0.7872
GELS	128	0.616 (0.190–1.998)	0.4200	128	0.205 (0.035–1.216)	0.0810
PEDF	128	0.818 (0.298–2.242)	0.6956	128	0.343 (0.057–2.055)	0.2415
AACT	128	1.336 (0.848–2.106)	0.2115	128	1.159 (0.522–2.573)	0.7176
APOCI	112	1.203 (0.276–5.241)	0.8058	112	1.603 (0.287–8.948)	0.5909
APOCII	124	0.672 (0.148–3.047)	0.6067	124	1.334 (0.274–6.485)	0.7208
APOCIII	117	1.151 (0.525–2.524)	0.7254	117	1.667 (0.807–3.444)	0.1676
SAA4	127	0.773 (0.316–1.895)	0.5739	127	0.558 (0.147–2.121)	0.3921

See supplementary table S1 for details of biomarkers. Multivariate adjusted hazard ratios (HRs) for all outcomes are reported for each of the ELISA biomarkers (adjusted for age, gender, ever-smoker and baseline forced vital capacity). *: p<0.05.

TABLE 3 Biomarker differences between progressor and stable groups in the Trent Lung Fibrosis cohort

Biomarker	Unit	Stable median (IQR)	Progressive median (IQR)	p-value
ELISA				
SPD	ng·mL^{-1}	37.32 (21.74–59.64)	41.64 (24.38–69.26)	0.1761
OPN	ng·mL^{-1}	22.37 (16.72–32.02)	27.69 (22.59–33.75)	0.0080*
MMP7	ng·mL^{-1}	2.72 (1.99–3.77)	3.28 (2.59–4.36)	0.0015*
ICAM1	ng·mL^{-1}	315.14 (226.78–453.99)	419.17 (320.96–640.63)	0.0001*
POSTN	ng·mL^{-1}	40.20 (30.54–60.75)	61.40 (39.13–80.67)	0.0001*
Proteomics[#]				
PGRP2	180 ratio	2.59 (2.04–3.40)	2.84 (2.08–3.70)	0.2964
CO9	180 ratio	8.08 (6.20–10.34)	8.26 (6.81–10.56)	0.6316
A2GL	180 ratio	5.89 (4.61–7.94)	6.04 (4.41–8.25)	0.7065
APOE	180 ratio	1.61 (1.21–2.05)	1.62 (1.17–2.16)	0.8526
GELS	180 ratio	1.48 (1.10–2.06)	1.58 (1.24–2.24)	0.1769
PEDF	180 ratio	2.24 (1.70–2.77)	2.37 (1.91–2.89)	0.3239
AACT	180 ratio	6.58 (5.07–9.39)	7.65 (5.19–9.87)	0.2716
SAA4	180 ratio	0.61 (0.46–0.87)	0.73 (0.44–1.01)	0.2104

See supplementary table S1 for details of biomarkers. IQR: interquartile range. [#]: proteomic unit is ratio of corrected unlabelled peak area/180 peak area. Nonparametric test. *: p<0.05.

TLF cohort

The TLF cohort was selected to validate biomarker analyses from the AIPFR cohort. Notably, in the TLF cohort, several of the ELISA biomarkers identified in the AIPFR cohort demonstrated a significant difference between the progressive and stable groups, including OPN (p=0.0080), MMP7 (p=0.0015), ICAM1 (p=0.0001) and POSTN (p=0.0001), but none of the proteomics markers (table 3).

We tested clinical outcomes using composite scores from the four replicating biomarkers confirmed as significant in the TLF cohort. The distributions of values for these four validated biomarkers were used to define tertiles (table 4 and figure 1), with assignment of an index value for low (0), medium (1) and high (2) tertiles. Estimates of the AUC were made for each biomarker tertile showing further discrimination of progressor status (supplementary table S4). Univariable analysis of tertile 3 relative to tertile 1 identified individual biomarkers (OPN, MMP7, ICAM1 and POSTN) as significantly associated with disease progression at 12 months, with OR 3.13 (95% CI 1.42–6.90) for OPN to OR 4.57 (95% CI 2.12–9.87) for POSTN (supplementary table S5).

PROFILE cohort

Analysis in the retrospective PROFILE dataset of the significant ELISA biomarkers (OPN, MMP7, ICAM1 and POSTN) showed significant differences in two of the ELISA biomarkers (MMP7 and ICAM1) between progressive and stable groups (p=0.033 and p=0.035, respectively) (supplementary table S6).

Development and validation of a progression index

These four candidate ELISA biomarkers (*i.e.* OPN (osteopontin), MMP7 (matrix metallopeptidase-7), ICAM1 (intercellular adhesion molecule-1) and POSTN (periostin)) were used to develop a clinically relevant progression index which was applied to all three cohorts. Thus, tertiles from the four biomarkers were aggregated into possible progression indexes from 0 to 8 based on the sum of tertiles, which were

TABLE 4 Tertile score aggregates across four significant biomarkers

	OPN (ng·mL^{-1})	MMP7 (ng·mL^{-1})	ICAM1 (ng·mL^{-1})	POSTN (ng·mL^{-1})
Tertile 1	<20.0	<2.46	<291.0	<36.0
Tertile 2	20.0–30.0	2.46–3.55	291.0–431.0	36.0–61.0
Tertile 3	>30.0	>3.55	>431.0	>61.0

The four most consistently across-centre significant ELISA markers, *i.e.* OPN, MMP7, ICAM1 and POSTN (supplementary table S1), were used in developing a progression score. Biomarker indexing into tertiles based on these biomarkers from the Trent Lung Fibrosis cohort.

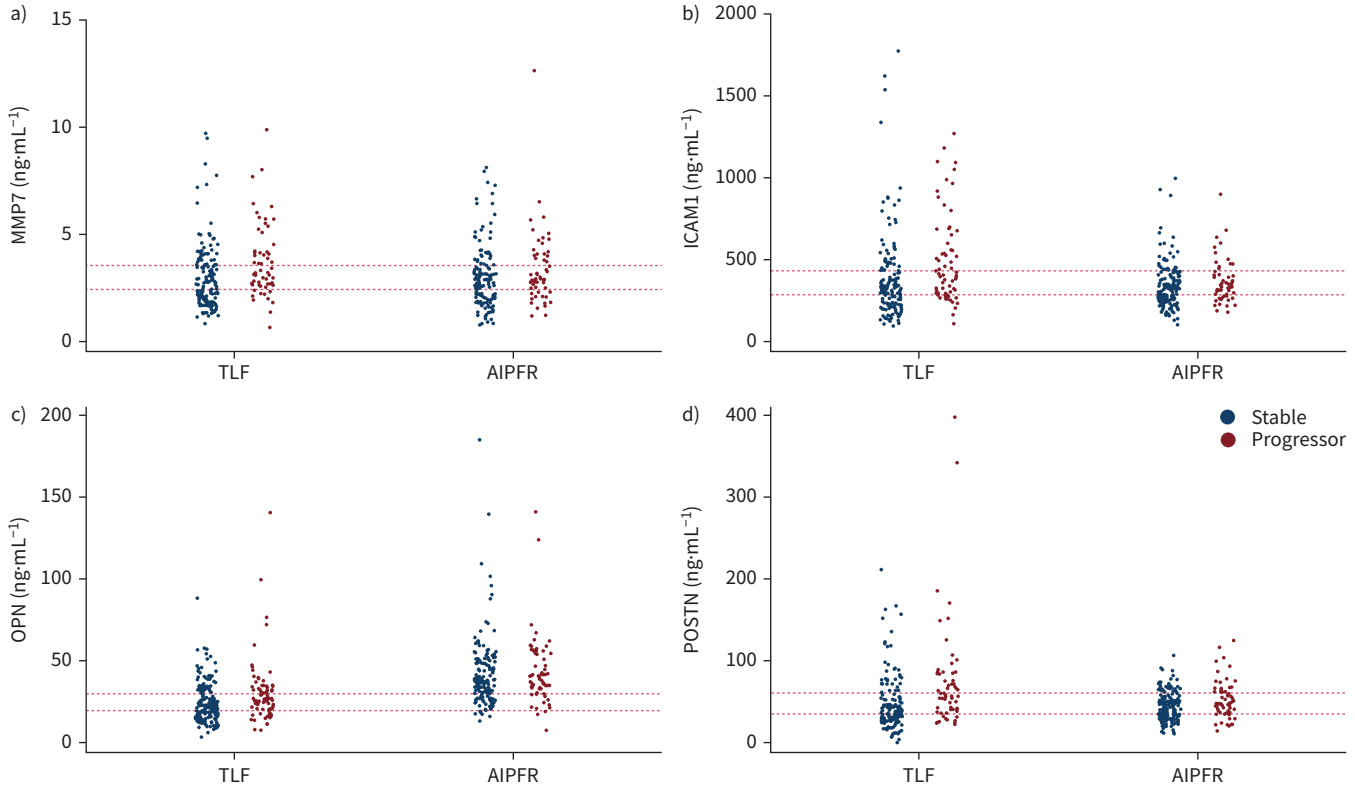

FIGURE 1 Scatter plots of significant biomarker values according to tertile score aggregates across the Trent Lung Fibrosis (TLF) and Australian Idiopathic Pulmonary Fibrosis Registry (AIPFR) cohorts. The four most consistently across-centre significant ELISA markers were used in developing a progression score: a) MMP7, b) ICAM1, c) OPN and d) POSTN (see supplementary table S1 for details of biomarkers). Tertile score aggregates compared between stable and progressor groups in both the AIPFR and TLF cohorts. The dashed lines indicate the TLF tertiles.

grouped into three clinically interpretable categories of sufficient sample size to test associations with clinical outcomes: 0–2 (26% of participants in TLF, 42% in AIPFR and 73% in PROFILE), 3–5 (49% in TLF, 53% in AIPFR and 27% in PROFILE) and 6–8 (25% in TLF, 5% in AIPFR and no participants in PROFILE) (supplementary table S7 and supplementary figure S3). In PROFILE, POSTN was not measured and was therefore missing for all participants, which unsurprisingly affected progression index frequencies when aggregated.

In the TLF cohort, progression index categories 3–5 and 6–8 were predictive of disease progression in adjusted analyses compared with people in the lowest score category (0–2) (OR 2.75, 95% CI 1.02–7.44; p=0.013 and OR 11.27, 95% CI 3.99–31.77; p<0.001, respectively). Relative to a score of 0–2, scores of 3–5 or 6–8 were associated with a 2.55-fold and 5.86-fold increase in the adjusted risk of mortality, respectively (hazard ratio (HR) 2.55, 95% CI 1.41–4.61; p=0.002 and HR 5.86, 95% CI 3.20–10.73; p<0.001, respectively). Significant association was also observed for scores of 6–8 regarding the outcome of PFS (HR 1.74, 95% CI 1.10–2.73; p=0.017) (table 5 and figure 2).

In the AIPFR cohort, a progression index score of 3–5 compared with 0–2 was predictive of disease progression in adjusted analyses (OR 2.30, 95% CI 1.04–5.09; p=0.04) and PFS (HR 1.61, 95% CI 1.07–2.42; p=0.023), and a progression index score of 6–8 was strongly predictive of PFS (HR 5.09, 95% CI 2.65–9.80; p<0.001) (table 5 and figure 2). Increasing progression index scores were not significantly associated with overall mortality in AIPFR, but trends were in the same direction but with wide confidence limits (score 3–5: HR 1.70, 95% CI 0.83–3.45; score 6–8: HR 2.92, 95% CI 0.37–22.91) (table 5 and figure 2).

In adjusted pooled analysis of TLF and AIPFR, progression index category 6–8 was significantly associated with a 54% increase in the odds of disease progression (OR 1.54, 95% CI 1.34–1.77; p<0.001), a 5.81-fold increase in the risk of mortality (HR 5.81, 95% CI 3.47–9.72; p<0.001) and a prediction of PFS (HR 2.29, 95% CI 1.60–3.27; p<0.001). Significant associations were also observed for progression

TABLE 5 Adjusted associations of the progression index score with outcome in the Trent Lung Fibrosis (TLF) and Australian Idiopathic Pulmonary Fibrosis Registry (AIPFR) cohorts

Progression index category	n	Progression at 12 months		Mortality		Progression-free survival	
		OR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
TLF							
0–2	53	1		1		1	
3–5	100	2.75 (1.02–7.44)	0.046*	2.55 (1.41–4.61)	0.002*	0.84 (0.54–1.30)	0.359
6–8	52	11.27 (3.99–31.77)	<0.001*	5.86 (3.20–10.73)	<0.001*	1.74 (1.10–2.73)	0.017*
AIPFR							
0–2	65	1		1		1	
3–5	81	2.30 (1.04–5.09)	0.040*	1.70 (0.83–3.45)	0.145	1.61 (1.07–2.42)	0.023*
6–8	7	2.98 (0.58–15.45)	0.193	2.92 (0.37–22.91)	0.309	5.09 (2.65–9.80)	<0.001*
Pooled data							
0–2	65	1		1		1	
3–5	81	1.17 (1.05–1.29)	0.003*	2.23 (1.42–3.51)	<0.001*	1.07 (0.80–1.43)	0.649
6–8	7	1.54 (1.34–1.77)	<0.001*	5.81 (3.47–9.72)	<0.001*	2.29 (1.60–3.27)	<0.001*

OR: odds ratio; HR: hazard ratio. Prediction of progression at 12 months, mortality and progression-free survival. All progression index models were adjusted for age, gender, baseline forced vital capacity % predicted and body mass index. Modelled with robust standard errors. *: p<0.05.

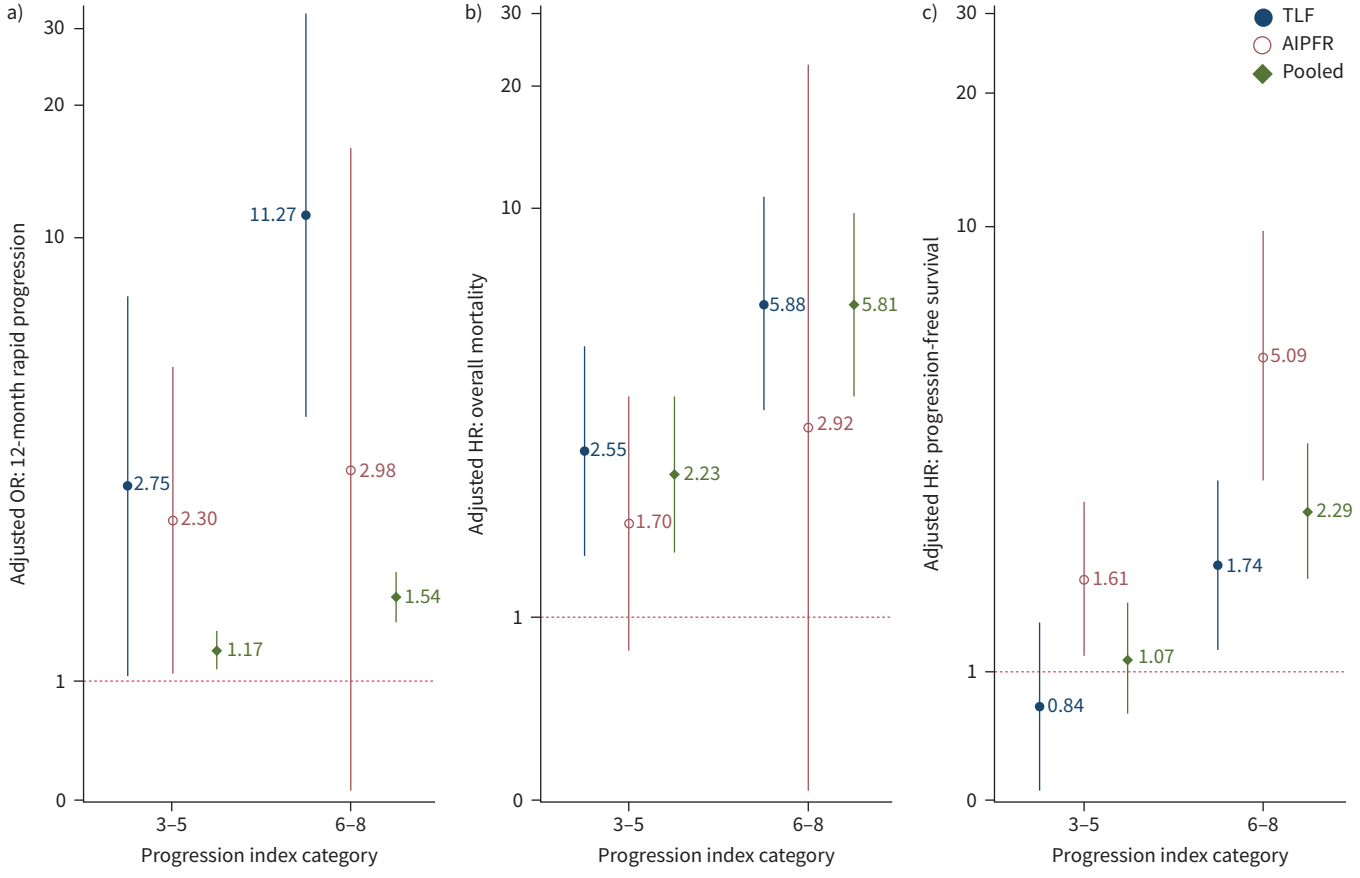

FIGURE 2 Adjusted associations of score category with outcomes comparing Trent Lung Fibrosis (TLF), Australian Idiopathic Pulmonary Fibrosis Registry (AIPFR) and pooled data: a) progression at 12 months, b) overall mortality and c) progression-free survival. Associations are presented as odds ratio (OR) or hazard ratio (HR) with 95% confidence intervals. The dashed line indicates the null effect. All progression index models were adjusted for age, gender, baseline forced vital capacity % predicted and body mass index.

TABLE 6 Area under the receiver operating characteristic curve (AUC) for progression at 12 months in all cohorts

Model	AUC for progression (95% CI)	p-value
TLF		
GAP	0.57 (0.49–0.66)	
Progression index	0.73 (0.66–0.80)	0.005*
GAP+progression index	0.71 (0.64–0.78)	<0.001*
AIPFR		
GAP	0.49 (0.38–0.59)	
Progression index	0.59 (0.48–0.69)	0.153
GAP+progression index	0.55 (0.45–0.66)	0.103
PROFILE		
GAP	0.55 (0.45–0.65)	
Progression index	0.62 (0.53–0.70)	0.281
GAP+progression index	0.60 (0.50–0.70)	0.049*

TLF: Trent Lung Fibrosis; AIPFR: Australian Idiopathic Pulmonary Fibrosis Registry; PROFILE: Prospective Observation of Fibrosis in the Lung Clinical Endpoints; GAP: gender, age and physiology. p-value for difference in AUC relative to the GAP score alone. *: p<0.05.

index category 3–5 in disease progression (OR 1.17, 95% CI 1.05–1.29; p=0.003) and mortality (HR 2.23, 95% CI 1.42–3.51; p<0.001) (table 5 and figure 2).

In the PROFILE cohort, the range of scores was limited by the missing biomarker (POSTN) data, but progression index category 3–5 was still predictive of mortality relative to category 0–2 (HR 2.23, 95% CI 1.02–4.85; p=0.043) (supplementary table S8). However, a significant association with disease progression was not observed in adjusted analyses (OR 2.06, 95% CI 0.59–7.24).

Addition of the progression index to the GAP model

In TLF, the GAP score and the progression index were used to calculate the AUC for the prediction models to determine the independent and combined outcome prediction power (table 6). The GAP score is used clinically to stage IPF and predict mortality. In the TLF cohort, the GAP score predicted disease progression at 12 months with AUC 0.57 (95% CI 0.49–0.66), while the progression index gave AUC 0.73 (95% CI 0.66–0.80). When combined with the GAP score, the progression index improved the AUC compared with the GAP score alone (AUC 0.71, 95% CI 0.64–0.78; p<0.001).

For the AIPFR cohort, the AUC for disease progression at 12 months using the GAP score alone was weak (AUC 0.49, 95% CI 0.38–0.59), while it improved using the progression index alone, although not significantly (AUC 0.59, 95% CI 0.48–0.69). The combined GAP score and progression index did not improve this (AUC 0.55, 95% CI 0.45–0.66) (table 6). In the PROFILE cohort, the GAP score offered an AUC for disease progression at 12 months of 0.55 (95% CI 0.45–0.65), while the progression index alone gave AUC 0.62 (95% CI 0.57–0.70). Combination of the progression index with the GAP score significantly improved prediction capacity above GAP alone (AUC 0.60, 95% CI 0.50–0.70; p=0.049) (table 6).

Discussion

In this multicentre study, we have shown differential biomarker expression in patients with progressive IPF compared with stable disease. In the AIPFR cohort, we derived a predictive model based on differences in expression of OPN, SPD, ICAM1 and MMP7. In the replication TLF cohort, the ELISA biomarkers OPN, MMP7, ICAM1 and POSTN showed significant differences between progressive and stable IPF. Using these four meaningful ELISA biomarkers, a progression index was generated according to tertile thresholds in TLF, which were associated with progression at 1 year, mortality and PFS. Furthermore, when combined in a statistical model with the GAP score this progression index improved the clinical predictive model for the identification of IPF progression in two of the cohorts tested. Notably, we were able to replicate the majority of our findings across three large, international, longitudinal and well-characterised IPF disease cohorts.

Our panel of predictive biomarkers has been previously implicated in the pathogenesis of IPF. Alveolar epithelial injury and impaired restitution are thought central to the development of this condition, and all these significant markers are known to be expressed by alveolar epithelial cells; their increased levels

emphasise ongoing alveolar epithelial cell damage in IPF [18, 19]. Indeed, OPN, MMP7, ICAM1 and POSTN have been previously associated with IPF progression, in line with our findings [20–27]. Furthermore, OPN and MMP7 overexpression may also reflect amplified fibroblast activity and increased extracellular matrix deposition in IPF lungs, while elevated ICAM1 expression may highlight the inflammatory and immune dysregulation of this condition [22, 28, 29].

Several lines of investigation have demonstrated that circulatory proteins are associated with patient outcome, increased mortality and disease severity [20–27]. Furthermore, studies have quantified clinically relevant circulating biomarkers in IPF to create a predictive performance index [22, 30, 31]. Richards *et al.* [22] defined a threshold of 203 and 4 $ng \cdot mL^{-1}$ for ICAM1 and MMP7, respectively, with plasma concentrations higher than these defining thresholds being associated with significantly lower median survival times in IPF.

Similar to our study, Ashley *et al.* [30] used unbiased proteomics to identify relevant biomarkers associated with disease progression in IPF. The group identified biomarker thresholds for six analytes involved in proteolysis, angiogenesis and immune function. They derived an index score that correlated with disease progression; however, limitations of the Ashley *et al.* [30] study are the small sample size and lack of a validation cohort.

A more recent study by Adegunsoye *et al.* [31] also defined circulating threshold concentrations of 47 and 3 $ng \cdot mL^{-1}$ for OPN and MMP7, respectively, which they found positively correlated with transplant-free survival. In addition, the group generated a clinical-molecular signature-risk score based on several biomarkers, age and FVC % pred, classifying IPF patients in “low-risk” and “high-risk” groups (the latter had worse transplant-free survival and increased mortality risk). Although there is merit to generating a single threshold value, we believe tertiles enable interpretable stratification, reflect the biomarker distribution more closely and minimise the impact of outlying values.

A limitation of our study was the application of the progression index to retrospective PROFILE study data, as there were insufficient data from some biomarkers to undertake a complete analysis of the progression index. Another limitation of the study was the inconsistency of the timing of pulmonary function test data in the AIPFR cohort, as these tests were collected for clinical follow-up rather than mandated at specific time-points as in a clinical trial. However, replication of the AIPFR data demonstrated generalisability and this real-world scenario translates into a possible strength. The reproducibility of OPN, MMP7, ICAM1 and POSTN, and the progression index, across international, multicentre cohorts that were prospectively (AIPFR and TLF) and retrospectively (PROFILE) analysed supports the robustness of our findings. A further limitation of the study was the lack of data for CA125 and CA19-9 in the initial discovery analysis of the AIPFR cohort, which therefore prevented the inclusion of these biomarkers in the progression index. It is likely that future biomarker studies will measure CA125 and CA19-9 in IPF, and it should be possible to build these into the current model. Indeed, as current research matures, advanced radiological image scoring, telomere length and related gene polymorphisms, as well as epithelial basal cell proteomics, can be incorporated into such prediction indices.

In conclusion, this study is in line with current literature and adds to rapidly evolving work that has demonstrated elevated circulating levels of OPN, MMP7, ICAM1 and POSTN in IPF. The progression index has provided us a new way of assessing IPF disease progression by using several well-known biomarkers in an index, with the scope to add and reassess the addition of new clinically relevant markers. Our reproducible findings across different sites and cohorts support additional validation in larger datasets, strengthening the potential prognostic value of these circulatory molecules and associated scores in clinical practice.

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