Plasma Surfactant Protein-D, Matrix Metalloproteinase-7, and Osteopontin Index Distinguishes Idiopathic Pulmonary Fibrosis from Other Idiopathic Interstitial Pneumonias

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Abstract

Rationale: Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal interstitial lung disease (ILD) characterized by abnormal extracellular matrix (ECM) remodeling. We hypothesized that ECM remodeling might result in a plasma profile of proteins specific for IPF that could distinguish patients with IPF from other idiopathic ILDs.

Objectives: To identify biomarkers that might assist in distinguishing IPF from non-IPF ILD.

Methods: We developed a panel of 35 ECM, ECM-related, and lung-specific analytes measured in plasma from 86 patients with IPF (derivation cohort) and in 63 patients with IPF (validation cohort). Comparison groups included patients with rheumatoid arthritis-associated ILD (RA-ILD; $n = 33$), patients with alternative idiopathic ILDs (a-ILD; $n = 41$), and healthy control subjects ($n = 127$). Univariable and multivariable logistic regression models identified biomarkers that differentiated patients with IPF from those with a-ILD. Both continuous and diagnostic threshold versions of

biomarkers were considered; thresholds were chosen to maximize summed diagnostic sensitivity and specificity in univariate receiver-operating characteristic curve analysis. A diagnostic score was created from the most promising analytes.

Measurements and Main Results: Plasma surfactant protein (SP)-D > 31 ng/ml, matrix metalloproteinase (MMP)-7 > 1.75 ng/ml, and osteopontin > 6 ng/ml each significantly distinguished patients with IPF from patients with a-ILD, both individually and in a combined index. The odds ratio for IPF when at least one analyte in the index exceeded the threshold was 4.4 (95% confidence interval, 2.0–9.7; $P = 0.0003$). When at least two analytes were elevated, the odds ratio for IPF increased to 5.0 (95% confidence interval, 2.2–11.5; $P = 0.0002$).

Conclusions: A biomarker index of SP-D, MMP-7, and osteopontin enhanced diagnostic accuracy in patients with IPF compared with those with non-IPF ILD. Our data suggest that this biomarker index may improve diagnostic confidence in IPF.

Keywords: fibrosis; biomarker; plasma

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At A Glance Commentary

Scientific Knowledge on the

Subject: Idiopathic pulmonary fibrosis (IPF) is the most common idiopathic interstitial pneumonia and carries the worst prognosis. With the recent U.S. Food and Drug Administration approval of new medications to treat IPF, making an accurate diagnosis is critical. Many patients cannot undergo surgical lung biopsy owing to respiratory compromise, and high-resolution computed tomography (HRCT) scans are not always diagnostic.

What This Study Adds to the

Field: Our study demonstrates that plasma levels of three proteins implicated in the pathogenesis of IPF (surfactant protein-D, matrix metalloproteinase-7, and osteopontin) can reliably distinguish patients with IPF compared with patients with other idiopathic interstitial lung diseases, irrespective of HRCT appearance, lung function, or smoking history. This biomarker panel may improve the diagnostic accuracy for patients with IPF.

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disorder of extracellular matrix (ECM) remodeling in the lung that leads to respiratory failure and ultimately death. A confident diagnosis of IPF may be made in patients in whom alternative causes of interstitial lung disease (ILD) are not identified and in whom a pattern of usual interstitial pneumonitis (UIP) is seen on high-resolution chest computed tomography (HRCT) or on surgical lung biopsy (1). Being able to confidently distinguish IPF from alternative idiopathic ILDs (a-ILD) is now of greater importance because of the recent approval in the United States of both pirfenidone and nintedanib for treatment of patients with IPF (2) and the documentation of the negative effects of combined immunosuppression in this population (3). However, many patients with IPF are unable to tolerate surgical lung biopsy due to respiratory compromise; in addition, the risk of subsequent complications [such as the development of an acute exacerbation of IPF (4)] may be unacceptably high. Moreover, interobserver

agreement among expert radiologists who evaluate HRCT is only low to moderate (5), suggesting that alternative markers to diagnose IPF are necessary.

The pathogenesis of IPF remains unknown, although abnormal ECM remodeling is believed to contribute to the relentless deposition of collagenous scar tissue in the lung (6, 7). To this end, we previously found substantial differences in ECM composition of normal versus IPF lungs (8), suggesting that fibrotic ECM may promote ongoing fibrogenesis. In addition, ECM stiffness, which is substantially increased in fibrotic lungs (8, 9), may also amplify a fibrotic response in a feed-forward loop (10, 11).

Because ECM or ECM-modifying proteins may be instrumental in IPF pathogenesis (12–16), we questioned whether such proteins could be used as biomarkers to distinguish IPF from a-ILD. Previous studies have identified ECM-modulating enzymes such as matrix metalloproteinase (MMP)-7 and MMP-1 as potential biomarkers for IPF (17), lending further credence to the possibility that ECM remodeling might be diagnostic and/or prognostic in IPF. More recently, and consistent with the previous study, our group showed that MMP-7, together with surfactant protein (SP)-D and endothelin-1 (among others), is elevated in the plasma of at-risk, first-degree relatives of patients with familial interstitial pneumonia (18). Notably, elevated plasma or serum SP-D levels have previously been associated with IPF severity (19, 20) but have not been shown to be specific for IPF.

To address whether ECM-related, and lung-specific proteins could be useful as biomarkers to distinguish IPF from a-ILDs, we measured a panel of 35 analytes in blood plasma from well-characterized IPF, a-ILD, rheumatoid arthritis-associated ILD (RA-ILD), and healthy control subjects. Putative biomarker analytes were curated from the literature as potentially being instrumental in the pathogenesis of IPF (Table 1).

Methods

Study Subjects

The derivation cohort of 86 patients with IPF consisted of blood plasma and data provided by the Lung Tissue Research Consortium (LTRC) supported by the NHLBI. The validation cohort of 63 patients with IPF and a separate group of 33 patients

Table 1. Biomarker Analytes Assayed in FibroPlex

Biomarker Analytes		
α -defensins	IGF-1	SP-D
BMP-7	IGFBP-3	Osteopontin
CCL-2	IGFBP-5	PICP
CCL-18	IL-13	PYD
Collagen IV	IL-13R α 2	Syndecan-1
Collagen VI	Laminin-332	Tenascin-C
CTGF	MIP-1 α	TIMP-1
Desmosine	MMP-1	TIMP-2
EDA cFn	MMP-2	TIMP-3
Endothelin-1	MMP-7	TIMP-4
FasL	MMP-8	TSP-1
ICTP	MMP-9	

Definition of abbreviations: BMP-7 = bone morphogenetic protein-7; CCL = C-C chemokine ligand; CTGF = connective tissue growth factor; EDA cFn = extra domain A-containing cellular fibronectin; FasL = Fas ligand; ICTP = type I collagen telopeptide; IGF = insulin-like growth factor; IGFBP = IGF binding protein; MIP = macrophage inflammatory protein; MMP = matrix metalloproteinase; PICP = procollagen I C-terminal peptide; PYD = pyridinoline; SP = surfactant protein; TIMP = tissue inhibitor of metalloproteinases; TSP = thrombospondin.

with RA-ILD were obtained through the Pulmonary Clinics as part of a separate study at Brigham and Women's Hospital (Boston, MA); all research subjects provided informed consent, and institutional review board approval was granted by the Partners Human Research Committee at the Brigham and Women's Hospital (Protocol # 2012P000840). The IPF groups were compared with patients with RA-ILD and a-ILDs (n = 41). The a-ILD cohort, recruited through LTRC, included patients with nonspecific interstitial pneumonitis (NSIP; n = 14), uncharacterized fibrosis (n = 15), cryptogenic organizing pneumonia (n = 6), respiratory bronchiolitis-ILD (n = 4), and desquamative interstitial pneumonitis (n = 2). All final a-ILD diagnoses were provided by a multidisciplinary diagnostic review that was completed in a standardized and validated approach by the LTRC investigative group before biomarker analysis and separate from the present study. Healthy control subjects identified by LTRC (n = 127) were also analyzed. Exemption for use of LTRC samples was provided by the University of Michigan Institutional Review Board because it involved the collection or study of existing data and biologic specimens by the

Table 2. Demographic Data of the Derivation Cohort

	IPF	a-ILD	Healthy Control Subjects	P Value*	P Value†
N	86	41	127		
Age, yr (mean ± SD)	63 ± 8.8	58 ± 14.0	64 ± 10.4	0.02	0.6
Male, n (%)	62 (72)	22 (54)	57 (45)	0.04	<0.0001
Ever smoked, n (%)	60 (72.3)	21 (56.8)	74 (65.5)	0.09	0.3
FVC, % predicted (mean ± SD)	62 ± 16	65 ± 22	97 ± 15	0.45	<0.0001
FEV ₁ , % predicted (mean ± SD)	68 ± 16	67 ± 21	97 ± 15	0.81	<0.0001
DL _{CO} , % predicted (mean ± SD)	44 ± 18	56 ± 20	86 ± 18	0.002	<0.0001

Definition of abbreviations: a-ILD = alternative interstitial lung disease; DL_{CO} = diffusing capacity of the lung for carbon monoxide; IPF = idiopathic pulmonary fibrosis.

*P value represents the comparison between the IPF and a-ILD cohort.

†P value represents the comparison between the IPF and control cohort.

investigator in such a manner that subjects could not be identified directly or through identifiers linked to the subjects. In the derivation IPF cohort, 49 patients (57%) underwent surgical lung biopsy; the remainder had UIP confirmed in the explant during transplantation or in a lobectomy specimen. In the validation IPF group, 55 (80%) patients underwent surgical lung biopsy with the remainder confirmed by computed tomographic scan and/or clinical data. Demographics of the derivation and validation cohorts are shown in Tables 2 and 3, respectively.

Plasma Samples

Frozen plasma samples in the derivation cohort were obtained from the LTRC, thawed once, and analyzed for biomarkers as described in the following. Per the LTRC protocol, plasma samples were collected in lithium heparin-coated tubes. Plasma samples in the validation group were collected and analyzed as described in the following.

Biomarker Measurements

Biomarker analytes (FibroPlex version 2) as outlined in Table 1 were produced as described previously (21). Briefly, analytes were divided into five individual multiplexed assays [to maintain interanalyte cross reactivity at <10% (22)] and were performed simultaneously on a high-speed pipetting robot (Tecan Systems, Inc., San Jose, CA). The lower limit of detection and intra-assay coefficient of variation for the 35 analytes has been published previously (21, 22).

Statistical Analyses

All analyses were performed using SAS software (version 9.4, SAS Institute, Inc., Cary, NC) or the R software package, version 3.2.0 (The R Foundation for

Statistical Computing, Vienna, Austria). Statistical significance was defined for P values <0.05. The biomarker screening strategy is depicted in Figure 1. The relationship between patient diagnosis and individual biomarkers initially was explored using adjusted logistic regression. Primary predictors in these models were measurability levels of each individual

biomarker (1 if out-of-range low, 2 if measurable, and 3 if out-of-range high) and measured biomarker levels when in range. Demographic (age, sex, race, and smoking status) and functional (baseline FVC) data were used to accommodate potential confounding factors. Biomarkers were screened out in this first stage if the statistical signal based on adjusted logistic

Figure 1. Flow diagram depicting the screening analytic approach used to identify 3 biomarkers for consideration in diagnostic index. AUC = area under the ROC curve; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; MMP = matrix metalloproteinase; OOR = out of range; ROC = receiver-operating characteristic; SP = surfactant protein.

regression at the 0.3 significance level was related to either the measured biomarker level or being out of range (low or high) was not met.

Receiver-operating characteristic (ROC) curves (23) were constructed to evaluate sensitivity and specificity of each remaining biomarker. Threshold values of biomarkers were determined by maximizing the sensitivity and specificity of the biomarker to discriminate IPF from a-ILD (24). A binary threshold variable was created, defining each biomarker measurement as being above or below the threshold value. Biomarkers lower than the lower limit of detection (out-of-range low) were considered below the threshold. Similarly, biomarkers above the upper limit of quantitation (out-of-range high) were treated as above the threshold. Based on the binary threshold variable, the area under the curve (AUC) from unadjusted ROC analysis was calculated; biomarkers with $AUC < 0.6$ were removed from further consideration. Subsequently, for each individual biomarker, binary threshold variables to predict IPF were analyzed using both unadjusted and adjusted logistic regression analyses, with adjustment variables including sex, age, smoking status, and FVC (% predicted). This screening stage for biomarkers required $P < 0.05$ in both adjusted and unadjusted analyses, leaving three biomarkers under consideration. The distribution of the three biomarkers that survived the screening process are summarized by diagnostic group via boxplots and kernel density plots assuming Gaussian kernel and default smoothing options as described by Deng and Wickham (Figure 2) (25).

Different combinations of significant biomarkers were explored with two goals in mind: (1) to produce a score that contributed to diagnostic accuracy beyond the aforementioned adjustment variables that are available in standard clinical practice; and (2) simplicity of use in the constrained time of a clinical encounter. For goal (1), we built a score by incorporating individual biomarkers into a multivariable model according to magnitude of odds ratios (ORs) and ROC curve AUCs that separated IPF from a-ILD in adjusted analyses. For goal (2), biomarkers had to pass a series of screening steps, including statistical significance following both univariable and multivariable adjustments as outlined in the Statistical Analysis section.

Figure 2. Distribution of significant analytes among diagnostic groups. ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; MMP = matrix metalloproteinase; OOR = out of range; SP = surfactant protein.

As part of a sensitivity analysis, we also considered alternative scoring systems based on maximizing the adjusted AUC for a particular number of included biomarkers. All potential diagnostic indexes were

calculated by adding +1 for each biomarker exceeding its respective threshold. Thus, the range of each index is from zero to the total number of biomarkers included in the score, with higher values increasing the

odds of IPF. Cochran-Armitage trend tests (26, 27) were used to detect significant linear associations between increasing index scores and IPF diagnosis versus either the patients with a-ILD or the healthy control subjects. For biomarkers with out-of-range values, instead of means and two-sample *t* tests, descriptive tables and figures report Yuen's symmetric trimmed means with corresponding 2-sample test statistics, because these allowed for out-of-range values to be removed from calculations without altering the median of the biomarker distribution (28). As noted earlier, this did not affect how thresholds or scores based on these were calculated.

The value of our recommended diagnostic index was assessed separately in our derivation and validation cohorts via univariable and multivariable logistic models and the area under the ROC curve.

Results

Measurement of FibroPlex Biomarkers in the Derivation Cohort

In our derivation cohort, 254 plasma samples were assayed for the 35 biomarkers included in FibroPlex (Table 1). Demographics of the derivation and validation cohorts are shown in Tables 2 and 3, respectively. Compared with patients with a-ILD, patients with IPF were, on average, older (63 yr vs. 58 yr; $P = 0.02$), more likely to be men (72.1% vs. 53.7%; $P = 0.04$), and had a lower mean diffusing capacity of the lung for carbon monoxide (DL_{CO}) (44% vs. 56%; $P = 0.002$). There were no significant differences between the derivation IPF and validation IPF groups (data not shown). For the RA-ILD group, we noted that patients were, on average, significantly older than patients with a-ILD in the derivation cohort ($P < 0.05$).

Figure 1 shows a flow diagram depicting the screening analytical approach used to ultimately target three biomarkers (SP-D, MMP-7, osteopontin) for inclusion in our IPF diagnostic index versus the a-ILD diagnostic index. Twelve biomarkers passed an initial screening that required statistical signal at the $P \leq 0.3$ significance level for either the continuous biomarker level (collagen IV, collagen VI, extra domain A-containing cellular fibronectin, type I collagen telopeptide, laminin, osteopontin, procollagen I C-terminal peptide, and SP-D) or the ordered

Table 3. Demographic Data of the Validation Cohort

	IPF	RA-ILD	P Value*
N	63	33	
Age, yr (mean $\pm$ SD)	65.4 $\pm$ 9.1	65.5 $\pm$ 11.3	0.96
Male, n (%)	41 (63.1)	23 (69.7)	0.52
Ever smoked, n (%)	43 (66.2)	21 (63.6)	0.80
FVC, % predicted (mean $\pm$ SD)	63.3 $\pm$ 17.7	74.3 $\pm$ 23.7	0.013
FEV ₁ , % predicted (mean $\pm$ SD)	68.4 $\pm$ 18.8	73.5 $\pm$ 26.2	0.35
DL_{CO} , % predicted (mean $\pm$ SD)	42.3 $\pm$ 18.1	48.5 $\pm$ 19.4	0.15

Definition of abbreviations: DL_{CO} = diffusing capacity of the lung for carbon monoxide; IPF = idiopathic pulmonary fibrosis; RA-ILD = rheumatoid arthritis-associated interstitial lung disease.

**P* value represents the comparison between the IPF and RA-ILD cohort.

measurability levels (bone morphogenetic protein-7, endothelin-1, MMP-7, pyridinoline). Table E1A in the online supplement displays unadjusted logistic regression results for diagnosing IPF versus non-IPF ILD for each of the 12 biomarkers that passed the initial screening based on the continuous biomarkers levels (when measurable) and a three-level measurability index of the biomarker (1 = out-of-range low, 2 = measurable, and 3 = out-of-range high). Table E1B in the online supplement shows the same logistic regression analyses of those biomarkers excluded after the initial screen. For each of the included 12 biomarkers, Table E2 in the online supplement shows thresholds that maximize the summed sensitivity plus specificity (24) for distinguishing between IPF and a-ILD, as well as the corresponding sensitivity, specificity, and AUC curve ROC results when using a binary indicator of exceeding a biomarker threshold in an unadjusted ROC analysis. In the final stage of screening, only three biomarkers (SP-D, MMP-7, and osteopontin) yielded an AUC > 0.6 and significantly enhanced the odds of an IPF diagnosis in both unadjusted and adjusted analyses, with ORs ranging from 3.1 to 4.7 (Table 4).

The Final Biomarker Index Includes Plasma SP-D, MMP-7, and Osteopontin

Table 5 provides the statistical significance of the diagnostic scores when the biomarkers were included one by one in the order of decreasing adjusted ORs and AUCs for separating patients with IPF and patients with a-ILD. For each row in this table, the score ranges from 0 (all biomarkers below threshold) to the number of biomarkers used in the score for that row (all biomarkers above threshold). The combination of SP-D,

MMP-7, and osteopontin gave an unadjusted ROC AUC = 0.709 and an adjusted ROC AUC = 0.766.

Boxplot distribution and frequency analysis of the three selected biomarkers are shown in Figure 2 (also see Figure E1 in the online supplement for biomarker distribution in the control group). SP-D, MMP-7, and osteopontin demonstrated higher symmetrically trimmed means in the IPF group compared with the a-ILD group. Tables E3A and E3B in the online supplement show the differences in symmetrically trimmed means for all 35 biomarkers in the IPF group compared with the a-ILD group.

Frequency analysis demonstrated that a plurality of patients in the IPF group had plasma levels of SP-D and MMP-7 above the identified threshold values as opposed to the patients with a-ILD, which improved sensitivity of the score. Conversely, nearly all patients with a-ILD had osteopontin levels below the threshold, which improved the specificity of the score (Figure 2). Shown differently, we plotted Yuen's symmetric trimmed mean values (28) for SP-D, MMP-7, and osteopontin for patients with IPF, patients with a-ILD, and the control subjects on *x*-, *y*-, and *z*-axes (Figure 3).

The percentage of patients with IPF (vs. patients with a-ILD) significantly increased with increasing score [$P = 0.0001$ by pairwise Cochran-Armitage trend testing (26, 27)] (Figure 4A; see also Table E4A in the online supplement). As might be expected, this trend remained significant when comparing the percentage of patients with IPF versus healthy control subjects ($P < 0.0001$). Evaluated in a different way, 71 of 86 patients with IPF (82.6%) demonstrated at least 1 biomarker above the threshold, compared with the those with a-ILD (22 of 41 patients, 54%;

Table 4. Specificity, Sensitivity, and Threshold Values of Three Biomarkers with Unadjusted Area under the Receiver-Operating Characteristic Curve >0.6, Ordered by Decreasing Adjusted Odds Ratio

Biomarker	Spec	Sens	Threshold	Adjusted*				Unadjusted			
				OR	95% CI	P Value	AUC	OR	95% CI	P Value	AUC
OPN, ng/ml	0.91	0.35	6	3.8	1.0–14.3	0.045	0.743	4.7	1.5–14.5	0.007	0.620
SP-D, ng/ml	0.65	0.7	31	3.1	1.2–7.8	0.016	0.747	3.8	1.0–8.3	0.0008	0.660
MMP-7, ng/ml	0.63	0.71	1.75	3.1	1.2–7.7	0.015	0.771	3.9	1.7–8.7	0.0009	0.662

Definition of abbreviations: AUC = area under the receiver-operating characteristic curve; CI = confidence interval; MMP-7 = matrix metalloproteinase-7; OPN = osteopontin; OR = odds ratio; Sens = sensitivity; SP-D = surfactant protein-D; Spec = specificity.

*Adjustment variables include sex, age, smoking status, and FVC (% predicted).

$P = 0.0006$) and the control subjects (58 of 127 subjects, 45.6%; $P < 0.0001$) (Figure 4B).

An Index Score of Elevated Plasma SP-D, MMP-7, and Osteopontin Levels Increases the Odds of an IPF Diagnosis

Based on the preceding findings, we next evaluated the usefulness of the panel of SP-D, MMP-7, and osteopontin in discriminating IPF from a-ILD diagnoses. As shown in Table 6, various delineations of the biomarker index score provided significantly enhanced odds of an IPF diagnosis, with and without adjustment for confounders. The adjusted odds of an IPF diagnosis was 1.9 times higher for each 1-point increase in the index score (e.g., from a score of 1 to 2 or 0 to 1) ($P = 0.005$). Similarly, the unadjusted odds of an IPF diagnosis was 2.1 times higher for each 1-point increase in the index score ($P = 0.0001$). Thus, our data indicated that the index score significantly and substantially enhanced the odds of diagnosing IPF in a patient with ILD when plasma SP-D, MMP-7, and osteopontin all exceeded a threshold level.

Stratifying each individual diagnosis within the a-ILD cohort by index score

revealed consistently lower scores for the unclassified fibrosis group and more evenly spread indexes within the NSIP group (see Table E4B in the online supplement). For the NSIP group, this might indicate varying degrees of fibrosis captured in our cohort. In addition, we investigated individual-level demographics from Tables 2 and 3 for healthy control subjects with an index score of 3 (false positives), patients with a-ILD with a score = 3, and patients with IPF with an index score of 0 (false-negatives) (see Tables E5A to E5C in the online supplement). Interestingly, patients with IPF with an index score of 0 demonstrated significantly better lung function compared with those with an index score > 0, with increased FVC and DL_{CO} (FVC: 69% vs. 60%, respectively; $P = 0.03$; DL_{CO} : 53% vs. 41%, respectively; $P = 0.03$). In contrast, healthy control subjects with an index = 3 showed no significant difference in FVC or DL_{CO} compared with healthy control subjects with an index < 3 (data not shown). Healthy control subjects with an index score = 3 were significantly older than those with an index score < 3 (72 yr vs. 64 yr; $P = 0.03$), whereas the age of patients with IPF with a score = 0 was no different

than those with a score > 0 (data not shown). Taken together, these data indicate that in healthy control subjects, our biomarker index might be influenced by subject age. However, our analyses when adjusted for age and other confounders continued to independently predict the IPF diagnosis.

Validation of the Three-Analyte Biomarker Panel

To ensure our biomarker panel reliably distinguished patients with IPF from those with a-ILDs, we also determined levels of SP-D, MMP-7, and osteopontin in plasma samples from a validation cohort of patients with IPF and an additional ILD group that typically manifests the UIP pattern, which is RA-ILD. As shown in Table 7, the adjusted and unadjusted odds of an IPF diagnosis were significantly increased when the biomarker index was used to discriminate between either healthy control subjects or patients with a-ILD in the derivation cohort. This result was replicated in the validation cohort (OR for IPF vs. a-ILD when at least one analyte in the index exceeded the threshold was 10.1; 95% confidence interval, 3.8–26.4; $P < 0.0001$). When at least two analytes were elevated, the OR for IPF was 4.4 (95% confidence interval, 1.8–10.4; $P = 0.0009$). We found that our biomarker profile did not distinguish either derivation or validation patients with IPF from patients with RA-ILD, whose ILD often manifests as a UIP pattern.

Table 5. Statistical Significance of Diagnostic Scores When Biomarkers Are Included One by One, according to Magnitude of Odds Ratios from Table 4

Biomarkers Used to Construct the Index	Adjusted*				Unadjusted			
	OR	95% CI	P Value	AUC	OR	95% CI	P Value	AUC
OPN	3.8	1.0–14.3	0.045	0.743	4.7	1.5–14.5	0.007	0.620
OPN, SP-D	2.5	1.3–4.8	0.008	0.756	2.9	1.6–5.1	0.0003	0.698
OPN, SP-D, MMP-7	1.9	1.2–2.8	0.005	0.766	2.1	1.4–3.0	0.0001	0.709

Definition of abbreviations: AUC = area under receiver-operating characteristic curve; CI = confidence interval; MMP-7 = matrix metalloproteinase-7; OPN = osteopontin; OR = odds ratio for index comprised of markers listed in each row; SP-D = surfactant protein-D.

AUCs are shown based on separating idiopathic pulmonary fibrosis from alternative interstitial lung disease in adjusted and unadjusted analyses.

*Adjustment variables include sex, age, smoking status, and FVC (% predicted).

Discussion

Confirming a diagnosis of IPF is of critical importance when patients present with ILD. The recent approval of two therapeutic agents for patients with IPF and documentation of harm from combined immunosuppression necessitates making an accurate diagnosis.

Figure 3. Trimmed mean values of surfactant protein (SP)-D, matrix metalloproteinase (MMP)-7, and osteopontin plotted using three-dimensional coordinates. ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis.

Unfortunately, many patients are unable to tolerate surgical lung biopsy, and HRCT scans do not always show diagnostic features (1). We demonstrated that results of a three-analyte panel of plasma SP-D, MMP-7, and osteopontin might markedly enhance diagnostic accuracy when trying to distinguish IPF from alternative idiopathic interstitial pneumonias, but not RA-ILD.

The pathogenesis of IPF remains elusive; however, we and others have postulated that abnormal remodeling of the ECM plays a significant role in the development and/or progression of the disease (7, 8, 11, 12, 29). Thus, we created FibroPlex analytes based on experimental data in human IPF samples that demonstrated a functional role for the

ECM and ECM-related proteins in cellular biology believed to be critical in fibrogenesis and lung-specific markers. We recently showed that some of these markers, including SP-D and MMP-7, significantly differ between healthy control subjects and first-degree relatives of patients with familial interstitial pneumonia (18), which suggests that at-risk individuals develop identifiable abnormalities in these proteins before the onset of clinically relevant disease. Therefore, in addition to acting as biomarkers of disease, our data, and that of others (14, 17, 30), strongly implies a pathogenic role for these proteins in IPF and warrants further study.

Although MMP-7 and MMP-1 levels have previously been identified as possible

biomarkers to distinguish patients with IPF from healthy control subjects and others with chronic lung diseases, clinical mimics such as NSIP and other idiopathic fibrotic disorders were not evaluated (17). Thus, it is not necessarily surprising that our data did not recapitulate the discriminatory nature of MMP-1, whereas MMP-7 levels were confirmed. It is plausible that MMP-1 levels do not differ significantly between patients with IPF and a-ILD, which is similar to what was observed in our study. This interesting finding perhaps provides a hint into the commonalities and differences in the pathogenesis of a-ILD and IPF, and would be a topic of interest for future investigation.

Most of the patients in the IPF derivation cohort in this study (57%) had an unclear diagnosis that required surgical lung biopsy to confirm. This implies that other diagnostic modalities (such as HRCT) were nondiagnostic for IPF. Clinically, this group is the ideally targeted cohort in which diagnostic biomarkers should be used, thereby potentially obviating the need for surgical biopsy. Our present data suggest that, even without adjusting for HRCT scores, our three-analyte panel, when elevated above the threshold values, increased the odds of an IPF diagnosis. Not only is this finding important for clinical care of patients, but it can easily be used in the clinical research environment as an additional screening tool to confirm the diagnosis of IPF in potential study subjects.

Osteopontin is a matricellular protein produced by macrophages that induces migration, proliferation, and adhesion of

Figure 4. (A) Distribution of diagnoses among the biomarker index score levels. Patients with idiopathic pulmonary fibrosis (IPF) were most of the patients with a score of 2 or higher. Cochran-Armitage trend testing demonstrates that, compared with alternative interstitial lung disease (a-ILD) and healthy control groups, the IPF diagnosis increases significantly as the index score increases. (B) Distribution of scores within diagnostic groups. Most of the patients with IPF demonstrated an index score of 2 or greater.

Table 6. Odds Ratios (95% Confidence Intervals) of an Idiopathic Pulmonary Fibrosis Diagnosis Using the Index Score

Comparison	Adjusted*				Unadjusted			
	OR	95% CI	P Value	AUC	OR	95% CI	P Value	AUC
All biomarkers high vs. all others	3.1	0.8–11.6	0.09	0.735	3.4	1.1–10.5	0.04	0.585
At least 2 high vs. all others	4.0	1.5–10.2	0.004	0.781	5.0	2.2–11.5	0.0002	0.686
At least 1 high vs. none high	3.3	1.3–8.6	0.014	0.753	4.4	2.0–9.7	0.0003	0.671
Each analyte above threshold	1.9	1.2–2.8	0.005	0.766	2.1	1.4–3.0	0.0001	0.709

Definition of abbreviations: AUC = area under the receiver-operating characteristic curve; CI = confidence interval; OR = odds ratio comparing 0 to 3 point index score delineations in each row.

For each 1-point increase in index score, odds of an idiopathic pulmonary fibrosis diagnosis increases by a factor of 1.9 (adjusted for confounders) or 2.1 (unadjusted).

*Adjustment variables include sex, age, smoking status, and FVC percent predicted.

fibroblasts (31, 32) and has been implicated in the pathogenesis of other fibrotic disorders (33–36). Osteopontin has previously been implicated in human IPF (14). It is largely up-regulated in IPF compared with normal lungs, and interestingly, it co-localizes with MMP-7 in IPF (14). To our knowledge osteopontin has not previously been shown to discriminate IPF from a-ILDs. However, osteopontin has been proposed to be a biomarker in other fibrotic disorders (37, 38); it has been shown to predict the degree of hepatic fibrosis in patients with hepatitis C viral infection (38). Thus, it is plausible that osteopontin is a viable biomarker in IPF.

Dynamic changes in collagen degradation products, presumably as a surrogate marker for ECM remodeling, have recently been shown to predict progression in IPF, with a rate of change that predicts survival (39). Although our panel contained markers of both collagen I production (type I collagen telopeptide and procollagen I C-terminal peptide) and collagen breakdown

(pyridinoline), we did not find that these markers provided any statistically significant discriminatory reliability between the patients with IPF and a-ILD in our cohort. This may reflect the “final common pathway” of ECM turnover in fibrotic lung disease, making these markers nonspecific for diagnostic purposes. Clearly, our biomarker panel needs to be assessed in prediction of disease progression as well, and those studies are ongoing.

The three-analyte panel of SP-D, MMP-7, and osteopontin enhanced the odds of an IPF diagnosis when each biomarker exceeded its threshold value (Table 5) in both adjusted and unadjusted analyses. We chose to use a simple three-point scale that would be easy to adopt in clinical practice, and it showed value after adjusting for other clinically available information such as age. We believe this is less cumbersome to apply in practice than a formula that requires input of multiple variables that may or may not be available to the clinician at the point of care.

However, it remains to be seen whether a

combinatorial model that includes our panel plus other predictors of an IPF diagnosis [e.g., age > 70 yr (9) or HRCT scores (40)] will enhance diagnostic accuracy.

Diagnostic accuracy of our biomarker panel to distinguish IPF from other idiopathic interstitial pneumonias was validated in a second cohort of patients with IPF, although we noted no ability to discriminate between IPF and RA-ILD; clinically, this distinction might be less difficult because RA-ILD is more easily distinguished by the presence of serological markers such as rheumatoid factor, cyclic citrullinated peptide levels, and others (41), as well as a compatible HRCT. There are a number of potential explanations for why our panel was unable to discriminate IPF from RA-ILD. First, the RA-ILD group was older than the a-ILD group within the derivation cohort; as noted previously, it was possible that age played some role in biomarker levels or diagnostic ability, although when adjusted for age or other confounding variables, we were still able to distinguish IPF from idiopathic interstitial

Table 7. Odds Ratio for IPF versus Alternative Diagnoses Shown in Columns for Each Unit Increase in Index Score (Derivation and Validation IPF Cohorts)

	Adjusted*			Unadjusted		
	Healthy Control Subjects	a-ILD	RA-ILD	Healthy Control Subjects	a-ILD	RA-ILD
Derivation IPF						
OR	1.9	1.9	1.0	2.7	2.1	1.1
95% CI	1.1–3.3	1.2–2.8	0.6–1.5	2.0–3.6	1.4–3.0	0.7–1.6
P value	0.015	0.005	0.82	<0.0001	0.0001	0.65
Validation IPF						
OR	2.7	2.3	0.8	3.2	2.5	1.0
95% CI	1.5–4.7	1.3–3.9	0.5–1.5	2.2–4.6	1.6–4.0	0.6–1.8
P value	0.0009	0.004	0.56	<0.0001	0.0001	0.89

Definition of abbreviations: a-ILD = alternative interstitial lung disease; CI = confidence interval; IPF = idiopathic pulmonary fibrosis; OR = odds ratio comparing 0 to 3 point index score delineations in each row; RA-ILD = rheumatoid arthritis-associated interstitial lung disease.

*Adjustment variables include sex, age, smoking status, and FVC (% predicted).

pneumonias but not RA-ILD. Second, we noted that patients with RA-ILD typically displayed a UIP pattern on biopsy, which might be indistinguishable from UIP seen in IPF. Thus, our biomarker panel might be more capable of distinguishing UIP from

non-UIP patterns in idiopathic interstitial pneumonias.

In conclusion, we identified a three-analyte panel of peripheral plasma biomarkers that enhanced the odds of an IPF diagnosis in patients with ILD. These

findings have potential implications as a diagnostic option for patients who are unable or unwilling to undergo surgical lung biopsy. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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