PLASMA PROTEOME ANALYSIS REVEALS SHARED AND UNIQUE BIOMARKERS OF ILD SUBTYPES

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BACKGROUND

Robust diagnostic and prognostic biomarkers of interstitial lung disease (ILD) are urgently needed to facilitate early patient diagnosis and treatment decisions. Recently, the Olink proteomic discovery platform was used to screen up to 3000 proteins in ILD patients, identifying dozens of circulating biomarkers predictive of ILD progression or mortality at 12 months.[1,2] Here we utilized, for the first time, the 5400 protein panel from Olink to investigate circulating plasma biomarkers of ILD in patients with idiopathic pulmonary fibrosis (IPF), rheumatoid arthritis-associated ILD (RA-ILD) and scleroderma-associated ILD (SSc-ILD). RA patients with or without radiologist-identified subclinical RA-ILD were also included to identify potential early biomarkers of ILD.

METHODS

Randomized plasma samples collected from patients with IPF (n=30), SSc-ILD (n=24), RA-ILD (n=30), RA (n=30), and subclinical RA-ILD[3] (n=32), along with those from healthy donors (n=24) were run on the Olink Explore HT 5400 panel. Comparative analyses between groups were performed using uncorrected t-tests for previously validated biomarkers[1,2] and multiple comparison adjusted t-tests for discovery.

RESULTS

Comparative analysis of plasma from three ILD subtypes (IPF, RA-ILD and SSc-ILD) and healthy subjects identified 30 previously described prognostic biomarkers of ILD progression that were significantly dysregulated across all ILD subtypes, including PRSS8, OCLN, PLAUR, AGER, AREG, FASLG, WFDC2 and KRT19.

PRSS8 and OCLN were also found to be significantly upregulated in patients with subclinical RA-ILD relative to patients with RA alone, providing potential early diagnostic plasma biomarkers of ILD.

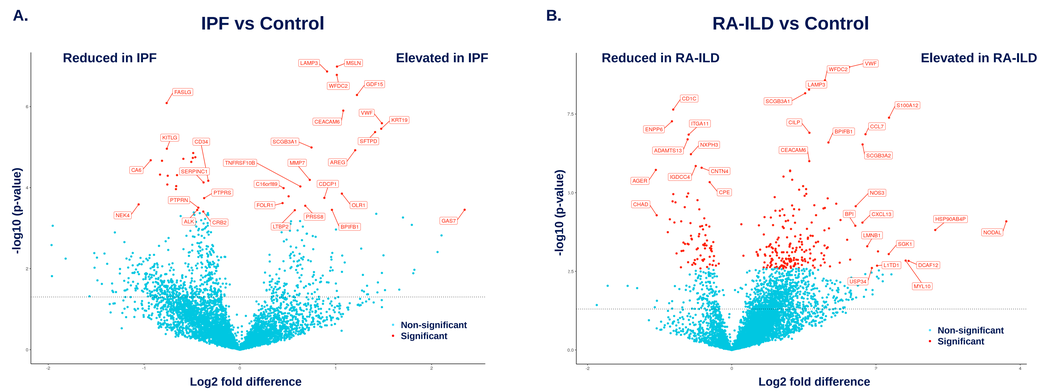
Plasma concentrations of ITGB6, the β6 subunit of the integrin heterodimer αVβ6 expressed by injured lung epithelium, were elevated in patients with IPF, SSc-ILD, and RA-ILD relative to healthy subjects.

Multi-comparison analysis of the full 5400 protein panel also identified novel putative biomarkers of ILD consistent across multiple ILD subtypes (e.g. C16orf89, CRB2, IL23R, and ALK) and of subclinical ILD in patients with RA, including AKT2, IGFBP3, and C5.

Table 1: Overview of ILD and healthy plasma donor demographics

Groups	IPF	RA-ILD	SSc-ILD	RA	Subclinical RA-ILD	Healthy Donors
Source	UCSF	UCSF	UCSF	Univ Colorado	Univ Colorado	UCSF
# Donors	30	30	24	30	32	24
Mean age (min,max)	63 (42, 71)	68 (55, 86)	55 (34, 73)	64 (42, 83)	64 (46, 83)	61 (52, 71)
% Female	77	70	79	75	75	75

Figure 1: Proteomic screening (Olink Explore HT 5400 panel) was used to compare plasma from patients with interstitial lung disease and healthy donors



Volcano plots presenting log2 fold change of plasma proteins between **(A)** patients with IPF and healthy donors and **(B)** patients with RA-ILD and healthy donors. Red dots indicative of significance (q<0.05) following t-test adjustment for multiple comparisons. IPF: idiopathic pulmonary fibrosis, RA-ILD: rheumatoid arthritis-associated interstitial lung disease

30 plasma proteins previously shown to be prognostic biomarkers of ILD[1,2] were found at significantly elevated or reduced levels across all ILD subtypes vs healthy controls

Table 2: Plasma proteins significantly elevated across all ILD subtypes vs healthy donor plasma (p<0.05)

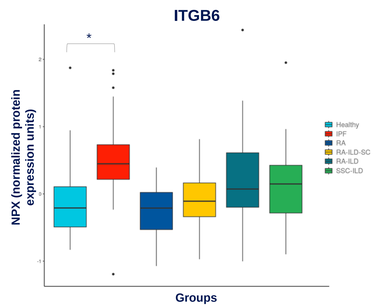
Protein ID	IPF vs Healthy Log2 Fold Change	RA-ILD vs Healthy Log2 Fold Change	SSc-ILD vs Healthy Log2 Fold Change
AREG	1.20	0.75	0.77
BPIFB1	0.96	1.34	0.77
CCL18	0.66	0.86	0.44
CEACAM6	1.08	1.08	0.66
COL24A1	0.59	0.50	0.55
EVPL	1.50	1.64	1.35
FOLR1	0.44	0.44	0.37
GDF15	1.22	0.99	1.28
ICAM1	0.53	0.44	0.38
IL17C	0.62	0.71	0.87
IL2RA	0.34	0.39	0.45
KRT19	1.47	1.29	1.26
MAMDC2	0.26	0.46	0.45
MMP7	0.73	0.55	0.50
MSLN	1.01	0.84	0.99
NOS3	0.76	1.72	1.35
OCLN	0.70	0.71	0.38
PIGR	0.63	0.84	0.63
PLAUR	0.51	0.60	0.40
PRSS8	0.68	0.64	0.53
RBFOX3	0.54	0.78	0.78
SFTPA2	0.95	1.45	2.00
SPINT1	0.53	0.44	0.33
TNFRSF6B	0.49	0.56	0.90
WFDC2	1.01	1.29	0.97

Tables 1 & 2: Comparative analyses between plasma donor groups were performed for biomarkers of ILD progression/outcome previously validated using the Olink platform[1,2]. All proteins listed were found at significantly different concentrations between ILD donor groups and healthy donors ($p < 0.05$)

Table 3: Plasma proteins significantly reduced across all ILD subtypes vs healthy donor plasma (p<0.05)

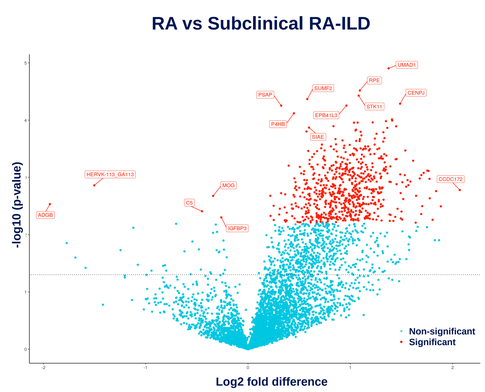
Protein ID	IPF vs Healthy Log2 Fold Change	RA-ILD vs Healthy Log2 Fold Change	SSc-ILD vs Healthy Log2 Fold Change
ADAMTS13	-0.49	-0.61	-0.48
AGER	-0.74	-1.05	-0.83
CD1C	-0.47	-0.81	-0.47
FASLG	-0.76	-0.70	-0.63
L1CAM	-0.32	-0.40	-0.46

Figure 2: Plasma ITGB6 was elevated in donors with IPF, SSc-ILD, and RA-ILD relative to healthy subjects



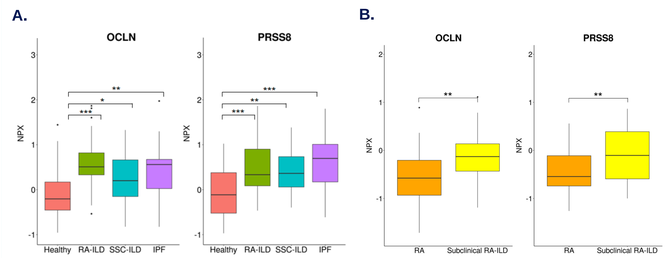
Plasma levels of ITGB6 shown in normalized protein expression (NPX) units for patients with RA-ILD, SSc-ILD, IPF, subclinical RA-ILD, RA, and healthy donors; * $p < 0.05$

Figure 3: Proteomic screening comparing plasma from RA patients with and without radiologist-identified subclinical RA-ILD[3]



Volcano plot presenting log2 fold change of plasma proteins between RA patients with and without radiologist-identified subclinical RA-ILD. Red dots indicative of significance (q<0.05; adjusted t-test).

Figure 4: PRSS8 and OCLN, two plasma proteins increased across all ILD subtypes vs healthy donors, were also increased in patients with subclinical RA-ILD, providing potential early diagnostic plasma biomarkers of ILD



Plasma levels of OCLN and PRSS8 shown in normalized protein expression (NPX) units for **(A)** patients with RA-ILD, SSc-ILD, and IPF vs healthy donors, and **(B)** patients with subclinical RA-ILD vs RA ;
* $p < 0.05$, ** $p < 0.01$, ** $p < 0.001$.

CONCLUSIONS

- **Proteomic screening of plasma from patients with IPF, RA-ILD, and SSc-ILD identified biomarkers consistently dysregulated across multiple ILD subtypes**
- **Comparison of proteins between RA patients with and without subclinical ILD identified potential early diagnostic biomarkers**
- **Further studies are required to evaluate the utility of these biomarkers for informing clinical decision making in ILD**

References: [1]Bowman, W.S. et al. Lancet Respir Med 2022; [2]Oldham, J. et al. Am J Respir Crit Care Med 2024; [3]Matson, SM et al. Am J Respir Crit Care Med 2021

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Disclosures: RA, EHB, JS, LS, and MD are current or former employees and shareholders of Pliant Therapeutics