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Early View

Research letter

Proteomic profiling of peripheral blood and bronchoalveolar lavage fluid in interstitial lung diseases: An explorative study

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Proteomic profiling of peripheral blood and bronchoalveolar lavage fluid in interstitial lung diseases: An explorative study

To the Editor:

Interstitial lung diseases (ILDs) are a diverse group of disorders, which differ significantly with respect to etiopathogenesis, clinical, radiological, and pathological picture. Idiopathic pulmonary fibrosis (IPF) and sarcoidosis represent two distinctive examples of ILDs (1).

In IPF, the most significant pathobiological mechanism is linked to the uncontrolled activity of various growth factors resulting in accumulation of myofibroblasts and excessive secretion of extracellular matrix (ECM) proteins, mainly collagen. As a consequence, we observe irreversible and progressive fibrotic lung disease, leading to death within 3–5 years in 50% of patients (2).

On the other hand, sarcoidosis has an immunoinflammatory systemic pathology, with Th1 lymphocyte predominance and excess production of interferon gamma, leading to granuloma formation in various organs. Löfgren's syndrome is a clinical phenotype of acute sarcoidosis, with a very low risk of lung fibrosis, a high rate of spontaneous resolution, and a good overall prognosis (3).

No studies on simultaneous proteome analysis of serum and bronchoalveolar lavage fluid (BALF) in IPF and sarcoidosis have been published to date. It is noteworthy that the comparative studies of different ILDs using BALF are few (4). Proteomics using panels targeted at various biological processes and other techniques that are more affordable than mass spectrometry, such as proximity extension assay (PEA), are becoming more readily available (5). Briefly, 92 oligonucleotide labeled antibody probe pairs are allowed to bind to their respective target proteins, if present in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA polymerization event. This is then amplified, and subsequently detected and quantified using real-time PCR. The method is validated and provides high sensitivity, specificity and scalability (5). To investigate the utility of PEA technology for BALF samples obtained from patients with ILDs, we performed an explorative study to see if the proteome signature differed in the systemic and lung

compartments between IPF and sarcoidosis, two ILDs with differing predominant mechanisms: fibrotic versus immunoinflammatory.

A total of 25 newly diagnosed ILD patients, including 15 patients with acute sarcoidosis (Löfgren's syndrome) (median age 40 years (range 25–56), 8 females) and 10 patients with IPF (median age 71 years (range 60–80), 1 female) were studied. Sarcoidosis and IPF were diagnosed in accordance with international recommendations (2,3). Peripheral blood and BALF samples were collected from all study participants. Bronchoscopy with BALF was performed in accordance with the Polish Respiratory Society recommendations (6). Both serum and BALF supernatant samples were frozen at -80 °C until further proteomic evaluation. Biological samples were analysed with a dedicated proteomics panel for 92 inflammatory proteins using PEA technology (Olink Proteomics, Uppsala, Sweden). The validation information of the protein measurements included in the Olink Inflammation Panel is available from https://www.olink.com/content/uploads/2019/04/Olink-Inflammation-Validation-Data-v3.0.pdf. The complete list of the analysed proteins, see the Olink Proteomics web page for the Inflammation panel (www.olink.com/products/inflammation/biomarkers/). The study protocol was approved by the Ethics Committee of the Medical University of Lodz and the study was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent for study participation.

For serum, a total of 81 out of 92 proteins were detectable (levels above the limit of detection in at least 75% of the individuals). For BALF, the corresponding number was 49 out of 92 proteins (see Figure 1). All samples passed quality control except one BALF sample from a patient with sarcoidosis. The protein measurements from this BALF sample were removed from further analyses.

Multiple linear regression models were used with each of the 130 proteins (log-transformed) as dependent variables, a patient group (sarcoidosis or IPF) as an independent variable, and adjusted for age, sex, smoking status, and the percentage of lymphocytes in BALF. The results were adjusted for multiple testing using the Benjamini-Hogberg method (7) to control for false discovery rate (FDR). The significance was set to FDR-corrected p values < 0.05.

Significant differences (adjusted for sex, age, smoking status, and lymphocyte percentage) were found for the following 16 proteins in BALF: matrix metalloproteinase (MMP)-1, MMP-10, interleukin (IL)-6, IL-8, transforming growth factor-alpha (TGF-alpha), TNF-

related apoptosis-inducing ligand (TRAIL), osteoprotegerin (OPG), oncostatin M (OSM), chemokine (C-X-C motif) ligand (CXCL) 5, CXCL9, CXCL10, *monocyte chemoattractant protein* (MCP)-1, MCP-3, MCP-4, macrophage inflammatory protein (MIP)-1β, and eotaxin-1, and for two proteins in serum: stem cell factor (SCF) and CUB-domain containing protein 1 (CDCP1) (Figure 1). All differentially expressed proteins had higher levels in IPF than sarcoidosis, except SCF (Figure 1).

Pearson's correlation coefficients between the levels of proteins and the percentage of lymphocytes in the sarcoidosis group were tested. A total of 22 proteins were significantly related after adjustments for multiple testing: OSM, IL-12 beta, IL-18, CXCL-11, T-cell surface glycoproteins CD5 and CD8 alpha chain, tumour necrosis factor receptor superfamily member 9 (TNFRSF9) (all proteins measured in BALF, all r values were between 0.8 and 1.0), CXCL-9, CXCL-10, MCP-2, MCP-4, C-C motif chemokine (CCL) 3, CCL4, CCL19, IL-18, IL-18 receptor 1 (IL-18R1), and TNF-beta (TNFB) (IL-18 and TNFB from serum, others from BALF; r values 0.7–0.8), CCL20, TRAIL, OPG, TNF, and hepatocyte growth factor (HGF) (all proteins measured in BALF, $r < 0.7$).

Pearson's correlation coefficients between the levels of proteins in BALF and serum were tested for the proteins that could be detected in both BALF and serum ($n = 49$). One protein was significantly related after adjustments for multiple testing: CCL19.

In the present study, we successfully demonstrated the utility of PEA technology for comprehensive proteomics of BALF and identified differences in the proteome from the lung compartment between systemic immunoinflammatory ILD, sarcoidosis, and fibrotic ILD, IPF. Only two of the 81 measurable proteins in serum differed significantly between sarcoidosis and IPF: SCF and CDCP1. SCF may be crucial for the regulation of inflammatory response in the lungs of patients with ILDs (8). Recent studies suggest that cell surface glycoprotein CDCP1 acts as co-receptor of the TGF-β1 signalling pathway (9); however, its precise role in fibroproliferative diseases remains to be elucidated.

On the other hand, almost one third (16 of 49) of the measurable proteins in BALF differed significantly between sarcoidosis and IPF in our study. Our finding of increased MMP-1 in BALF of patients with IPF is in line with the previous findings of its elevated levels in serum, BALF, and lung tissue in IPF, compared with controls and other forms of ILDs (10). Similarly, increased levels of IL-6 and IL-8 were previously found in patients with various ILDs compared with controls (11). We noted overexpression of TRAIL, OPG, and OSM in

BALF from IPF subjects, which may suggest involvement in the TGF-β1-independent accumulation of ECM proteins in IPF (12–14). We also noted a number of upregulated CC and CXC chemokines which may play significant roles in cell trafficking in pulmonary fibrosis (15).

Further research should include other fibroproliferative ILDs to check whether there are quantitative differences in the proteomes within that heterogeneous group. It seems that PEA technology offers a good alternative to mass spectrometry for proteomics studies utilizing different biological samples, including BALF, and may be applied to study a larger population of patients with ILDs. Some of the proteins of interest might show a different profile if plasma is used instead of serum, but this was outside the scope of the present analyses. The small sample size of the two investigated groups might explain the large confidence intervals noted for the different proteins.

In summary, this small explorative study demonstrates, for the first time, the feasibility and usefulness of PEA technology for BALF proteomics in patients with ILDs. Despite the small sample size, we noted significant differences in levels of BALF proteins between subjects with IPF and those with sarcoidosis. Future, larger, prospective studies should focus on the biological relevance and clinical significance of our findings, with regard to disease prognosis, response to therapy, or novel pathophysiological pathways in ILDs.

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Conflicts of interest

None of the authors has any conflicts of interest to declare with regard to the present work.

Figure legend

Figure 1. Difference in the 92 investigated protein levels expressed between the group of IPF and sarcoidosis expressed as regression coefficient (circle) and 95% confidence interval (line) in BALF (left panel) and serum (right panel). Significant differences (FDR-corrected p-values <0.05) are plotted in red. If the confidence interval is on the right side of the dotted line (0), this means that subjects with IPF had higher levels of that protein than subjects with sarcoidosis. The list of the proteins is found at https://www.olink.com/products/inflammation/.

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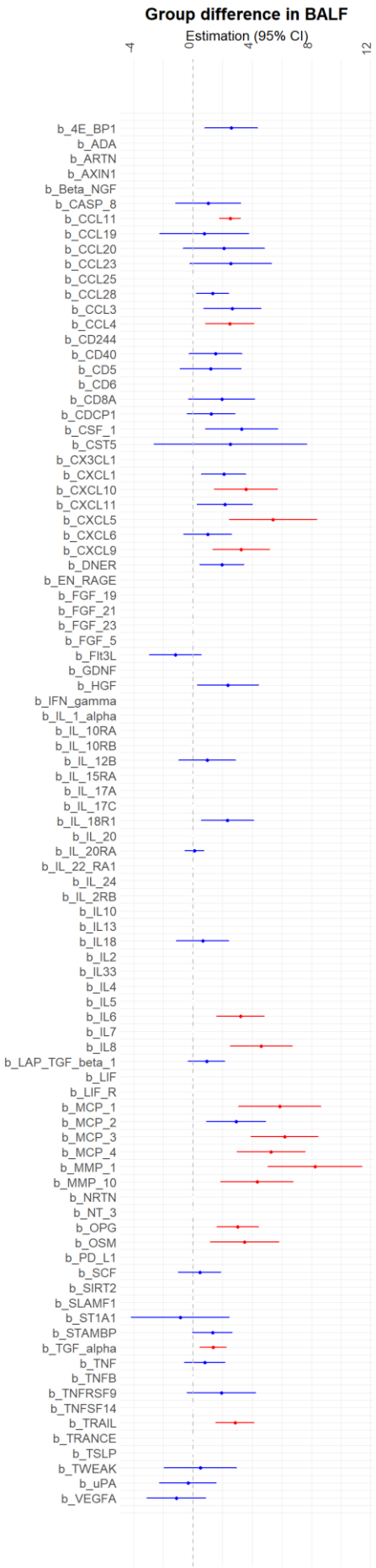
Group difference in BALF
Estimation (95% CI)
-4
0
4
8
12
b_4E_BP1
b_ADA
b_ARTN
b_AXIN1
b_Beta_NGF
b_CASP_8
b_CCL11
b_CCL19
b_CCL20
b_CCL23
b_CCL25
b_CCL28
b_CCL3
b_CCL4
b_CD244
b_CD40
b_CD5
b_CD6
b_CD8A
b_CDCP1
b_CSF_1
b_CST5
b_CX3CL1
b_CXCL1
b_CXCL10
b_CXCL11
b_CXCL5
b_CXCL6
b_CXCL9
b_DNER
b_EN_RAGE
b_FGF_19
b_FGF_21
b_FGF_23
b_FGF_5
b_Flt3L
b_GDNF
b_HGF
b_IFN_gamma
b_IL_1_alpha
b_IL_10RA
b_IL_10RB
b_IL_12B
b_IL_15RA
b_IL_17A
b_IL_17C
b_IL_18R1
b_IL_20
b_IL_20RA
b_IL_22_RA1
b_IL_24
b_IL_2RB
b_IL10
b_IL13
b_IL18
b_IL2
b_IL33
b_IL4
b_IL5
b_IL6
b_IL7
b_IL8
b_LAP_TGF_beta_1
b_LIF
b_LIF_R
b_MCP_1
b_MCP_2
b_MCP_3
b_MCP_4
b_MMP_1
b_MMP_10
b_NRTN
b_NT_3
b_OPG
b_OSM
b_PD_L1
b_SCF
b_SIRT2
b_SLAMF1
b_ST1A1
b_STAMBP
b_TGF_alpha
b_TNF
b_TNFB
b_TNFRSF9
b_TNFSF14
b_TRAIL
b_TRANCE
b_TSLP
b_TWEAK
b_uPA
b_VEGFA
Group difference in Serum
Estimation (95% CI)
-5
0
5
s_4E_BP1
s_ADA
s_ARTN
s_AXIN1
s_Beta_NGF
s_CASP_8
s_CCL11
s_CCL19
s_CCL20
s_CCL23
s_CCL25
s_CCL28
s_CCL3
s_CCL4
s_CD244
s_CD40
s_CD5
s_CD6
s_CD8A
s_CDCP1
s_CSF_1
s_CST5
s_CX3CL1
s_CXCL1
s_CXCL10
s_CXCL11
s_CXCL5
s_CXCL6
s_CXCL9
s_DNER
s_EN_RAGE
s_FGF_19
s_FGF_21
s_FGF_23
s_FGF_5
s_Flt3L
s_GDNF
s_HGF
s_IFN_gamma
s_IL_1_alpha
s_IL_10RA
s_IL_10RB
s_IL_12B
s_IL_15RA
s_IL_17A
s_IL_17C
s_IL_18R1
s_IL_20
s_IL_20RA
s_IL_22_RA1
s_IL_24
s_IL_2RB
s_IL10
s_IL13
s_IL18
s_IL2
s_IL33
s_IL4
s_IL5
s_IL6
s_IL7
s_IL8
s_LAP_TGF_beta_1
s_LIF
s_LIF_R
s_MCP_1
s_MCP_2
s_MCP_3
s_MCP_4
s_MMP_1
s_MMP_10
s_NRTN
s_NT_3
s_OPG
s_OSM
s_PD_L1
s_SCF
s_SIRT2
s_SLAMF1
s_ST1A1
s_STAMBP
s_TGF_alpha
s_TNF
s_TNFB
s_TNFRSF9
s_TNFSF14
s_TRAIL
s_TRANCE
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