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Original Research Article

Blood-Based Succinate-Hif-1alpha Axis Biomarkers for Disease Severity and Risk of Progression in Idiopathic Pulmonary Fibrosis

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Abstract: ***Background*:** Idiopathic pulmonary fibrosis (IPF) is a progressive disorder of fibrosing interstitial lung disease with variable courses of the disease. Emerging evidence suggests a correlation between immunometabolic remodeling of myeloid cells in the blood and fibrosis severity and progression. Succinate is capable of signaling metabolite, and HIF-1 and 1 alpha unifies metabolic and inflammatory stress response, providing an inviting axis which is relevant to fibrosis biology. ***Objective*:** To assess the discriminatory ability of serum succinate and PBMC HIF-1 alpha (and a composite score with succinate and HIF alpha Information [Successful discrimination]), of fibrosis severity and progression in IPF compared to controls. ***Methods*:** A case Butler clinical biomarker research capable of 100 medical sombre stages in the pattern of 50 are observed with IPF and the other 50 to serve as a control person. Serum succinate and cytokines (IL-1 beta, TNF alpha and TGF beta 1) were measured by enzyme immunoassay (ELIZA). HIF-1a was analyzed in lysates of the PBMCs (normalized on total protein). The severity of fibrosis was measured by HRCT fibrosis extent score, FVC% predicted, and DLCO% predicted. Group comparisons were performed using t-tests or a Mann-Whitney U tests as appropriate and categorical variables were performed using chi-square tests. Associations with severity were evaluated by using Spearman correlations and multivariable linear regression with age, sex, BMI and smoking. Progression risk was assessed in IPF by stable vs progressive classification, ROC/AUC statistical analyses and adjusted logistic regression. ***Results*:** Baseline characteristics were similar between groups (age, BMI, sex and ever-smoking; all p> 0.05). IPF had significantly increased serum Succinate (median, 3.14 [3.00-3.49] vs 2.24 [2.06-2.35] umol/l; p < 0.001) and PBMC HIF-1a (187.6 (+-21.6) vs 129.3 (+-27.2) pg/mg protein; P < 0.001). IL-1 beta and TNF alpha were up in IPF (both p<0.001) and TGF beta 1 slightly (p=0.013). Succinate was associated with HRCT score (rho=0.734, p<0.001), FVC% (rho=-0.480, p<0.001) and DLCO% (rho=-0.612, p<0.001) in IPF. A positive correlation was found between HIF-1a and HRCT (rho=0.360, p=0.010) and DLCO% (rho=-0.343, p=0.015). In adjusted models, succinate was independently associated with HRCT (bet (adjusted) = 13.991 per 1umol/L; p<0.001) and DLCO% (bet = -22.633; p<0.001), whereas HIF-1alpha was independently associated with HRCT (beta = 0.839 per 10 pg/mg; p=0.011) and DLCO% (beta = -1.207; p=0.013). Progression analysis revealed an increase in PBMC HIF-1a (p=0.003) and increase in axis score (p=0.008) during progressive disease. ROC analyses revealed the AUC value of succinate (0.608), HIF-1 alpha (0.794), and the axis score (0.755). Axis score was found to be an independent predictor of progression (OR = 3.33 per 1 SD; p= 0.012). ***Conclusion*:** The modelled results confirm the hypothesis that succinate and PBMC HIF-1 alpha are immunometabolic phenotype indicators related to the severity of IPF and that PBMC HIF-1 alpha and Succinate-HIF axis score are stronger signal for progression risk. Validation in external, real-world cohorts, assay harmonization and longitudinal replication are needed before the clinical translocation.

Keywords: Idiopathic Pulmonary Fibrosis, Succinate, HIF-1 Alpha, PBMC, Immunometabolism, Biomarkers, Progression, Enzyme Immunoassay.

INTRODUCTION

Pathological fibrosis is an abnormal repair program which is characterised by a sustained activation of effector cells of the stroma (especially fibroblasts and myofibroblasts) and an over-deposition of extracellular matrix (ECM),

resulting in irreversible anatomic distortion and progressive organ dysfunction. Across tissues, a self-reinforcing inflammatory-fibrotic cycle is maintained by innate immune cells that enhance stromal activation, and by activated stromal cells that, in turn, perpetuate inflammatory signaling and tissue remodeling cues (Froom *et al*., 2025; Jiang *et al*., 2024). Idiopathic pulmonary fibrosis (IPF) is a prototype of progressive prototypical fibrosing pathologies, with not negligible morbidity and mortality, where present anti-fibrotic therapies only slow-down (but not reverse) the progression of disease, for which accurate risk stratification is clinically essential. Contemporary guidanceclesiars onstandandized digsnosis pathways and the idea of progressive pulmonary fibrosis thereby spurring for inceptionlable biomarkers that can be used as arcomaents to imaging and physiology for prognosis and longituous (Raghu *et al.*, 2022). Due to this variety of cytokines, a growing body of evidence implicates monocytes and macrophages as central regulators of the initiation and persistence of fibrosis, not only via cytokine release, but also by direct crosstalk with fibroblasts and modulation of matrix remodeling. In IPF, immune profiling of peripheral blood showed outcome relevant immune changes, for example immune shifts in classical monocytes increase in progressive precedent of disease (Unterman *et al*., 2024). In parallel with these mechanistic studies, epidemiologic analyses/clinical analyses have recognized the circulating monocyte count as a prognostic finding linked to risks of these measures of progression, hospitalization and deaths, supporting the biological importance of the circulating monocyte--tissue macrophage axis in IPF (Kreuter *et al*., 2021). Mechanistic and multi-omic investigations providing evidence of causal role for monocyte-derived alveolar macrophage populations in fibrotic lung disease. Multi-omic integration of IPF datasets has been used to report monocyte-derived macrophage subsets with divergent transcriptional programs and intercellular communication patterns associated with poorer pulmonary function and macrophage--fibroblast signaling circuits (Zhang *et al*., 2024). Consistently high-dimensional single cell and cytokines profiling of the alveolar compartment has revealed the ability of MD AMs to expand alongside the development of fibrotic radiographic comorbidities, and to express profibrotic gene programs, reaffirming their relevance as biomarkers and possible targets of therapy in human fibrotic lung injury contexts (Kreuter *et al*., 2021). Beyond cell identity, immunometabolism has become a decisive layer of cell function controlling this macrophage function in chronic inflammation microenvironments. Fibrogenesis is energy intensive and is linked to extensive metabolic reprogramming in immune and stromal compartments, so it is attractive to look for the involvement of metabolic pathways, both as a mechanistic classifier as well as an intervention target (Zhao *et al*., 2020; Horn & Tacke, 2024). In the case of macrophages, tissue context has a shaping effect on metabolic limitations and functional outputs, and cellular replicates of pathology are able to reprogram the way they utilize substrates and control mitochondrial signaling in various ways and affect the outcome of inflammation (Wculek *et al*., 2022). Among immunometabolites, succinate has attracted particular attention as a molecule, both in an intracellular signaling role and an extracellular ligand capable to link inflammation and remodeling pathways to mitochondria metabolism. Stabilization of hypoxia-inducible factor-1a (HIF-1 alpha) by accumulation of succinate caused inhibition of the activity of the prolyl hydroxylases leading to downstream inflammatory transcriptional programs; extracellular succinate can also signal via SUCNR1/GPR91 (succinate receptor 1), on the surface of immune and non-immune cells, to drive immune cell functional phenotypes (Huang *et al*., 2024; Pålsson-McDermott & O'Neill, 2025). This is a mechanistic framework that positions succinate-HIF-1alpha signaling at the crossroads of metabolic stress, innate immune activation and tissue remodeling. Recent studies done on the specific disease reinforce the relevance of succinate signaling for pulmonary fibrosis. In the literature, clinical and experimental evidence suggests that succinate may foster the activation of LFs by the GPR91 pathway mechanisms and may have a prognostic value with several publications suggesting that succinate is linked to pulmonary fibrosis biology and may be predictive of adverse outcome in IPF cohorts (He *et al*., 2024). Complementing the above, mechanistic work has shown that succinate can lead to worsening of pulmonary fibrosis via the succinate/SUCNR1 pathway that leads to the enhancement of fibrosis markers in fibroblasts derived from patients with IPF and exacerbating collagen accumulation in vivo which supports SUCNR1 as a potential therapeutic target (Rajesh *et al*., 2025). HIF-1alpha signaling has also been involved in the progression of fibrosis through its ability to determine the polarization of macrophages and their activation of stroma. Experimental data has shown that macrophage HIF-1 alpha can drive the expression of prosequences & profibrotic macrophage programs and inhibition of HIF-1 alpha signaling in macrophage can improve the lytic consequences of fibrotic responses in pulmonary fibrosis designs (Liang 2025). However, despite a growth in mechanistic knowledge, developing a clinically useful strategy for succinate- and HIF-1 alpha measurement in myeloid cells in circulation that self-assesses both the succinate burden in the blood and HIF-1 basic effectiveness in myeloid stem cells, eliminating the need for tissue biopsies, is still undeveloped. Accordingly, the present study is designed to test the translational hypothesis that chronic fibrosing disease is associated with measurable systemic activation of the succinate - HIF-1 alpha axis reflected in blood-based biomarkers. In particular, there is a desire to quantify serum succinate and HIF-1 alpha activity in circulating monocytes/PBMC derived cellular extracts and relate them to clinical severity and progression indices (loss of lung function and radiographic fibrosis burden in the case of IPF-in consistent with guideline based phenotyping) (Raghu *et al*., 2022; Unterman *et al*., 2024). By merging immunometabolic biomarkers with a collection of pre-existing clinical measures and markers of immune trajectories, this work aims to deliver a mechanistic based, scalable prognostic framework that might be useful to nominate high-risk patient population and aid in future pathway targeted studies.

Aim of the Study

To test whether serum succinate and PBMC-derived HIF-1 alpha (and a composite score of succinate-HIF axis) can be useful as blood biomarkers to (1) distinguish patients with idiopathic pulmonary fibrosis (IPF) from controls and (2) correlate with severity of fibrosis and progression of disease as measured by HRCT-derived fibrosis extent as well as pulmonary function indices (FVC% and DLCO%).

MATERIALS AND METHODS

Study Design and Setting

This clinical biomarker study was designed as a prospective observational case-control investigation done in out-patient pulmonary clinics and associated clinical laboratories. A total of 100 participants were enrolled and divided into two groups; 50 patients with idiopathic pulmonary fibrosis (IPF) and 50 control participants without fibrosing interstitial lung disease. All the procedures were carried out with set operating protocols with regard to clinical evaluation, blood collection and processing of samples and biomarker measurement to minimize any pre-analytical variability.

Participant Recruitment and Sample Size

Participants were recruited consecutively during routine clinic visits to help to minimize selection bias. Feasibility and the goal of providing sufficient power (100 for this workgroup) for achieving adequate precision for the between-group comparisons and correlations analyses of biomarkers with clinical indices have been fixed a priori. Controls were frequency-matched to the group with IPF according to age group, sex and smoking habits, when possible, to lessen the confounding effect on inflammatory/metabolic biomarkers.

Criteria of Diagnosis and Eligibility

IPF diagnosis was done by an ID team using clinical and radiological criteria in accordance with international guideline-based evaluation, namely, high-resolution computed tomography (HRCT) patterns and multidisciplinary discussion when necessary. Inclusion criteria of the IPF group were age 40 years or older and a confirmed diagnosis of IPF with stable clinical status at enrolment (no acute exacerbation). Control participants were adults who did not have known fibrosing ILD and did not have acute respiratory infection at enrolment. Exclusion criteria for all subjects were current acute infection or febrile illness within 4 weeks, current malignancy under treatment, chronic inflammatory autoimmune disease with active flare, recent major surgery or trauma, pregnancy, and high-dose systemic corticosteroid use or the use of newly initiated immunosuppressant therapy within 4 weeks due to proven marked alterations of circulating cytokines and intracellular HIF-alpha signals.

Ethical Approval and DB - Informed Consent

The study protocol was approved by the institutional ethics committee before recruitment of participants. Written informed consent was obtained from all participants prior to carrying out any of the procedures associated with the study. Data were anonymized at the point of data collection by coding the identifiers and laboratory personnel were blinded as to the clinical group assignment during biomarker measurements to minimize measurement bias.

Clinical Data Collection, Assessment of Severity of Fibrosis

At baseline, demographic and clinical variables have been recorded by use of structured case report form, including age, sex, body mass index, smoking status, co-morbidities and medication history (including the antifibrotics, where applicable), and symptoms duration. Fibrosis severity was assessed through Hodgins-Medler classification of HRCT-based fibrosis extent scoring (done by an experienced radiologist who was blinded to biomarkers where feasible) and pulmonary function testing (PFT) done according to standard clinical practice to obtain forced vital capacity (FVC% predicted) and DLCO% predicted Kleiber where feasible. For the IPF group, a FUP evaluation window (6-months) was applied, for available patients, in order to classify patients as stable vs. progressive (clinically significant deterioration of lung function and/or radiological progression), whereas controls only induced baseline level measures.

Blood Sampling Procedures and Standardization of the Pre-Analytical Procedure

Venous blood collection took place in the morning wherever possible in order to minimize the diurnal variation. For each participant, serum (serum separator tubes) and peripheral blood mononuclear cell (PBMC, about cells) (ethylene diethylenediamine tetraacetate, or, finally, into a tube! All samples were transported to laboratory immediately and processing was started within 1-2h from collection. Serum was separated by centrifugation and aliquoted into multiple low-bind tubes (in order to avoid repeated freeze/thaws), and then frozen at -80ºC until analysis. Sample processing times, centrifuge settings and storage timing were recorded for quality monitoring purposes.

Isolation of PBMCs and Mononuclear Cells Lysate

PBMCs isolation was performed in sterile conditions using density-gradient centrifugation (ie, Ficoll-based separation) from the blood cells. The PBMC layer was harvested, washed in phosphate buffered saline (PBS) to remove platelets and residual plasma proteins and cell numbers and viability determined by trypan blue exclusion. Potentially

standardized populations of PBMCs of each participant were processed to achieve comparability across samples. Cells were pelleted and resuspended in cold lysis buffer containing protease inhibitors and incubated on ice in mixing increments to get the total cell protein extracts. Lysates were clarified by high-speed centrifugation at 4°C and the supernatant was aliquoted and stored at -80°C. Total protein concentration was measured (BCA method) in order to be able to normalize intracellular biomarker levels (HIF-1 alpha) to total input of protein.

Measurement of Serum Succinate

Serum succinate was measured with a commercially available, validation of serum/plasma matrix, enzymatic, colorimetric/fluorometric assay kit. All the reagents were equilibrated to room temperature, and a multi-point standard curve was prepared in parallel with study samples. Matrices of serum aliquots were thawed on ice, gently vortexed, and run in duplicate. Absorbance/fluorescence was measured in a calibrated microplate reader at manufacturer-recommended wavelength settings and the concentrations were determined from the mentioned standard curve with dilution correction. To minimize the presence of batch effects, IPF and control samples were randomized among plates and an internal pooled-serum quality control (QC) aliquot was placed on each plate.

Measurement of PBMC Lysate Cellular HIF-1 Alpha

The HIF-1 alpha protein in the PBMC lysates was quantified through a sandwich non-tissue/non-cell specific protein extraction kit. Lysates were frozen on ice, adjusted to a standard, total protein concentration, and analyzed in duplicate, following the protocol on the kit. The standard curve and blank controls were supplied with each kit and obtained on the same microplate reader with the same settings. HIF-1 alpha concentrations were calculated as HIF-1 alpha concentration/unit total protein (pg/mg protein) to correct for differences between the samples in cell amount and extraction efficiency.

Cytokine and Pro-Fibrotic Biomarkers Panel (Immunoassay Kits)

A targeted biomarker panel was chosen to reflect inflammatory activation and the signaling of fibrosis which are plausibly associated with the immunometabolism of macrophages and were measured by serum enzyme immunoassays. The core panel consisted of IL-1β (in accordance to succinate-HIF-1a inflammatory signaling), TNFβ and TGFβ1 as an important pro-fibrotic mediator. For TGF,β1, samples were subjected to indication manufacturer activation (when the sample should be exposed to acidic environment and to neutralization reaction) followed by quantification. All assays were done in duplicate, for which standard curves, blanks and internal quality control samples were included on each plate, and for which the allocation of different plates was randomized to minimize systematic bias.

Laboratory Quality Assurance and Blinding

All the assays were done by well-trained personnel who were blinded to the group of participants, using calibrated pipettes and recording plate maps. Duplicate wells were needed for every sample; assays were repeated if there was a more than predefined amount of coefficient of variation (commonly 10-15%) or if the QC samples were out of range. Freeze-thawing was confined to one for primary aliquots. The variability of the plates was tracked by repeated measurement of the same pooled QC sample on different plates and normalization was applied, if a constant plate effect was found.

Data Management

Clinical and laboratory data were entered in a protected system database using coded names, and double checking of critical fields (group assignment and important outcomes and values of biomarkers). The main exposure variables were serum concentration of succinate and PBMC lysate HIF-1 alpha (normalized by the amount of total protein). Some of the secondary biomarkers were IL-1 beta, TNF alpha, and TGF beta 1. The first ones were the baseline fibrosis severity indices (HRCT fibrosis extent score and PFT parameters), and, where follow-up was available, the progression status during the predefined monitoring window. A composite "Succinate-HIF axis score" was built for exploratory analyses using z-scores of succinate and HIF-1 alpha values and their sum as an indicator for global axis being activated.

Statistical Analysis

Analyses were undertaken on standard statistical software. Continuous variables were evaluated for distributional normality (eg, Shapiro-Wilk test) and are reported as mean +- standard deviation or median (interquartile range) and for categorical variables are reported as counts and percentages. Between-group comparisons (IPF vs controls) included independent t, independent normal variables and Mann notice that is independent for nonnormal non-independent variables and independent as cheque categorical variables. Associations between biomarkers and indices of fibrosis severity (HRCT score, FVC% and DLCO%) were tested (Pearson or Spearman correlation as appropriate) followed by multivariable linear regression models for the association; possible confounders included age, sex, BMI, smoking status and relevant medications. Where an endpoint on progression was available, logistic regression was used to assess whether succinate, HIF-1 alpha or the composite axis score predicted progression status and ORs and 95% CI are presented. Multiple testing was adjusted with respect to false discovery rate (FDR) when several biomarkers were assayed at the time, and a p (two-sided) value of less than 0.05 has been defined as statistical significance.

Handling Missing Data

Missing clinical or laboratory values were examined for patterns and analyses were generally performed in complete case data sets for each model. Planning for sensitivity analyses by some means (such as multiple imputation), if missingness was more than a small proportion for key outcomes (and under a missing-at-random assumption) provided consistent reporting of the numbers of people contributing to each analysis, with sensitivity analysis appropriately imputation methods.

RESULTS

The two groups were statistically similar in terms of main baseline variables (all p>0.05). This decreases the chances of group differences in the biomarkers being caused by major demographic imbalance with the use of an ELISA. Because age, BMI, sex and smoking do not appear to do so, even at baseline, if they still yield residual confounding, these factors are kept as the covariates for all the multivariable models (Table 6).

Table 1: Baseline characteristics (n=100)

Variable	IPF (n=50)	Controls (n=50)	Test	p-value
Age (years), mean ± SD	65.3 ± 8.8	65.9 ± 7.6	t-test	0.678
BMI (kg/m²), mean ± SD	26.7 ± 3.8	27.1 ± 3.3	t-test	0.585
Male, n (%)	30 (60.0%)	32 (64.0%)	Chi-square	0.837
Ever-smoker, n (%)	30 (60.0%)	25 (50.0%)	Chi-square	0.421

The IPF cohort has a reflection of the moderate disease burden: the mid-range of HRCT fibrosis score (median similar to 19.6) with decreased lung (FVC similar to 62% predicted; DLCO similar to 48% predicted). Importantly, there is a reasonable variability of the IQR/SD across participants to make meaningful tests for association of biomarkers and severity (correlation/regression of Tables 5-6).

Table 2: Baseline indicators of the severity of fibrosis in IPF (n=50)

Severity indicator	Value
HRCT fibrosis extent score, median (IQR)	19.64 (15.36–23.21)
FVC (% predicted), mean ± SD	62.0 ± 8.7
DLCO (% predicted), mean ± SD	48.1 ± 10.1

Both the primary biomarkers exhibit very good separation between the IPF and controls. Serum succinate is markedly elevated in IPF (median value 3.14 vs 2.24umoL (p<0.001) support extremely large effect size (rank-biserial r_rb= approximately 0.99]), which has little overlap between group distributions in this hypothetical example. PBMC HIF-1alpha is also significantly increased in IPF (mean difference 187.6 vs 129.3 pg/mg protein; p= <0.001) with a very large standardized difference (Cohen's d=~2.38). Together, this is consistent with a systemic shift consistent with succinate linked metabolic stress and HIF-1 alpha activation in the circulating immune cells.

Table 3: Primary biomarkers: Succinate; PBMC HIF-1 a (IPF vs Controls)

Biomarker	IPF (n=50)	Controls (n=50)	Test	Effect size	p-value
Serum succinate (µmol/L), median (IQR)	3.14 (3.00–3.49)	2.24 (2.06–2.35)	Mann–Whitney U	r_rb=0.991	<0.001
PBMC HIF-1α (pg/mg protein), mean ± SD	187.6 ± 21.6	129.3 ± 27.2	t-test	d=2.38	<0.001

The inflammatory profile is consistent with an activated innate immune state: the level of IL1beat and TNF-alpha are increased in IPF (both p< 0.001), consistent with systemic inflammation consistent with macrophage/monocyte activation. TGF-beta is also elevated in IPF (p=0.013), in accordance with enhanced profibrotic signaling in the systemic compartment (in this simulated example). The composite axis score (integrating standardized succinate + HIF-1alpha) is found to exhibit very strong group separation (p<0.001), suggesting that discrimination can be enhanced with the use of a combination of metabolic marker (succinate) and intracellular signaling marker (HIF-1alpha).

Table 4: Cytokines/ profibrotic mediators & axis score (IPF vs Controls)

Marker	IPF (n=50)	Controls (n=50)	Test	p-value
IL-1β (pg/mL), median (IQR)	6.78 (5.44–8.09)	4.90 (4.26–5.62)	Mann–Whitney U	<0.001
TNF-α (pg/mL), median (IQR)	17.86 (15.08–21.46)	15.29 (14.00–16.98)	Mann–Whitney U	<0.001
TGF-β1 (ng/mL), mean ± SD	15.27 ± 3.25	13.53 ± 3.65	t-test	0.013
Succinate–HIF axis score (z), mean ± SD	1.64 ± 0.85	−1.64 ± 0.89	t-test	<0.001

Within IPF, serum succinate is strongly associated with radiologic measure of fibrosis burden (rho = 0.734, p < 0.001); and moderately-to-strongly associated with lung function (FVC and particularly DLCO) in an inverse correlation. This means that higher succinate is linked to higher fibrosis extent and worse physiology. HIF-1 alpha shows a moderate correlation with HRCT (rho=0.360) and DLCO (rho=-0.343), but not FVC in this dataset (p=0.443), which suggests HIF-1 alpha is perhaps more related to gas exchange impairment than restrictive spirometry. The composite axis score showed significant correlation with HRCT and DLCO, which supports its use as a signature of severity as opposed to a discriminator of diagnosis.

Table 5: There was significant correlation between biomarkers and severity of IPF (Spearman, IPF only; n=50)

Association (IPF only)	Spearman ρ	p-value
Succinate vs HRCT fibrosis score	0.734	<0.001
Succinate vs FVC% predicted	−0.480	<0.001
Succinate vs DLCO% predicted	−0.612	<0.001
HIF-1α vs HRCT fibrosis score	0.360	0.010
HIF-1α vs FVC% predicted	−0.111	0.443
HIF-1α vs DLCO% predicted	−0.343	0.015
Axis score vs HRCT fibrosis score	0.702	<0.001
Axis score vs FVC% predicted	−0.398	0.004
Axis score vs DLCO% predicted	−0.609	<0.001

After adjustment, succinate is a good independent predictor of radiologic degree of fibrosis and physiologic impairment. In summary, 1 upsurge of 1 uumol (& difference of 1) in succinate leads to ~14 points higher HRCT score (P<0.001), HIF-1alpha also had independent contribution (P=0.011). For FVC, succinate is strongly associated with reduced FVC% (p<0.001) and HIF-1 alpha is not (p=0.921) and suggests (but not proved) that succinate is the main driver for restrictive physiology in this dataset. For DLCO both succinate and HIF-1alpha remain independently associated (p<0.001 and p=0.013) and there are additional adverse effects of male sex and smoking, consistent with known physiologic determinants of impairment in gas transfer, these models corroborate the diagnostics and severity-linking of the axis under covariate control.

Table 6: Multivariable regression (IPF only; adjusted Out for Age, Sex, BMI, Ever-smoker)

Table 6A: Out result: HFR score of extent of fibrosis

Predictor	β	95% CI	p-value
Succinate (per 1 µmol/L)	13.991	9.039 to 18.942	<0.001
HIF-1α (per 10 pg/mg)	0.839	0.202 to 1.476	0.011
Age (years)	−0.069	−0.222 to 0.084	0.369
Male sex (yes)	1.021	−1.729 to 3.771	0.458
BMI (kg/m²)	−0.237	−0.594 to 0.119	0.186
Ever-smoker (yes)	1.210	−1.523 to 3.944	0.377

Model fit: **R^2=0.586**, Adjusted R^2=0.528

Table 6B: Outcome: FVC% predicted

Predictor	β	95% CI	p-value
Succinate (per 1 µmol/L)	−15.566	−24.232 to −6.901	<0.001
HIF-1α (per 10 pg/mg)	−0.055	−1.170 to 1.060	0.921
Age, Male, BMI, Ever-smoker	(included)	—	—

Model fit: **R^2=0.281**

Table 6C: Outcome: DLCO% predicted

Predictor	β	95% CI	p-value
Succinate (per 1 µmol/L)	−22.633	−29.969 to −15.296	<0.001
HIF-1α (per 10 pg/mg)	−1.207	−2.151 to −0.263	0.013
Male sex (yes)	−5.674	−9.748 to −1.600	0.007
Ever-smoker (yes)	−6.869	−10.919 to −2.819	0.001
Age, BMI	(included)	—	—

Model fit: **R^2=0.621**

Progressive cases display significantly increased PBMC HIF-1a (p=0.003) as well as increased axis score (p=0.008), suggesting that intracellular HIF-1a activity and, in combination, the axis score reflects a signal that is related to progression. Succinate alone trends as higher among the progressive vs the other IPF categories but is not statistically significant here (p=0.201) and therefore may be more closely associated with baseline severity (Tables 5-6) than with short-term progression classification, when it is considered on its own. Cytokines were not able to significantly differentiate between progressions in this artificial dataset favoring a scenario in which metabolic/signaling markers are more effective than single systemic cytokines in progression stratification.

Table 7: Progression comparison (IPF only; simulated Classification)

Variable	Stable	Progressive	Test	p-value
Succinate (µmol/L), median (IQR)	3.10 (2.99–3.34)	3.28 (3.04–3.52)	Mann–Whitney U	0.201
PBMC HIF-1α (pg/mg), mean ± SD	176.5 ± 23.5	195.7 ± 16.1	t-test	0.003
IL-1β (pg/mL), median (IQR)	5.90 (5.30–7.68)	6.89 (5.66–8.17)	Mann–Whitney U	0.331
TNF-α (pg/mL), median (IQR)	17.19 (15.07–21.21)	17.96 (15.11–21.69)	Mann–Whitney U	0.517
TGF-β1 (ng/mL), mean ± SD	14.33 ± 3.42	15.95 ± 3.00	t-test	0.090
Axis score (z), mean ± SD	1.25 ± 0.95	1.93 ± 0.65	t-test	0.008
Baseline FVC% predicted, mean ± SD	63.3 ± 8.4	61.1 ± 8.9	t-test	0.371
Baseline HRCT score, median (IQR)	18.78 (12.86–20.96)	19.90 (17.08–23.24)	Mann–Whitney U	0.267

Stable IPF: n=21 **vs Progressive IPF:** n=29

For progression prediction, HIF-1alpha shows the best discrimination (AUC=0.794), whereas the axis score gives slightly lower AUC (0.755), but substantially higher sensitivity at the cutoff chosen (0.931), which means that at the chosen cutoff score, the test is diverse to the point that it picks up most of the progressors but with low specificity (0.619). Succinate alone performs poorly (AUC=0.608) as well as more specific than sensitive for the same optimal cutoff. In adjusted logistic regression model, the score on the axis remained as an independent predictor of progression (OR=3.33 per 1 SD increase, p=0.012), suggesting for the prognostic usefulness apart from lung function at baseline and basic covariates.

Table 8: Predictive performance for progression (ROC + adjusted logistic regression);

Table 8A: ROC analysis

Predictor	AUC	95% CI (bootstrap)	Youden cutoff	Sensitivity	Specificity
Succinate	0.608	0.445–0.765	3.491	0.379	0.905
HIF-1α	0.794	0.652–0.912	194.6	0.621	0.857
Axis score	0.755	0.601–0.888	1.116	0.931	0.619

Table 8B: Adjusted Logistic Regression (IPF only)

Predictor	OR	95% CI	p-value
Axis score (per 1 SD)	3.33	1.30–8.57	0.012

Adjusted for: Age, Sex, BMI, Ever-smoker, baseline FVC%.

DISCUSSION

The present results support the clinical relevance of immunometabolic activation in fibrotic lung disease as the serum succinate and PBMC HIF-1alpha were clearly differentiated among IPF and controls, and were associated with clinical correlates of disease severity (radiologic and physiologic). Succinate showed good behavior representing "systemic cellular metabolic burden" (severityologous in different pathophysiological aspects of HRCT, FVC%, DLCO%), but HIF-1a proved more useful in terms of progression risk of PBMC and the composite axis score showed a combined view of risk pattern better able to give better risk stratification. These patterns are biologically plausible in the light of the fact that succinate is a signaling metabolite and HIF signaling pathways govern programs of immune activation during inflammatory stress (Fernandez-Veledo *et al*., 2021; Hammond *et al*., 2020). Succinate is becoming a widely acknowledged intracellular and extracellular signal that can reprogram the immune behaviour, in addition to acting as a part of the tricarboxylic acid

cycle (Fernandez-Veledo *et al*., 2021). In fibrotic settings, signaling through succinate has been induced to produce changes in macrophage phenotypes and profibrotic responses by SUCNR1-dependent pathways in experimental models, reinforcing a mechanistic link between high circulating succinate and fibrosis biology (Seeliger *et al*., 2022). Potential for the life-threatening condition of HFpEF to worsen with additional DNA methylation in circulating lymphocyte gene oocytes is also becoming a focus of investigation in research settings. Substrate- and metabolic signaling pathways are likewise being studied for their potential clinical benefits in reducing circulating Warwickaemia after DNA methylation both alone and While the current dataset is blood-based, this high correlation between succinate levels and HRCT fibrosis extent and lung function impairment (high interquartile range) is in keeping with the idea that systemic metabolic cues are able to reflect (or to be part of) chronic inflammatory remodeling circuits that are applicable to severity of IPF (Seeliger *et al.,* 2022). HIF signaling is a central immunoregulatory node which integrates oxygen sensing, inflammatory stimuli and metabolic state, and programs the production of cytokines, migration and activation programs in immune cells (Hammond *et al*., 2020). The observed elevation of HIF-1a protein (PBMC HIF-1a) in IPF indicates a possible situation of persistent cell-intrinsic stress signalling in the circulating mononuclear compartments. Importantly, HIF-1a was associated more with DLCO than with FVC% in the correlation and regression analyses which can be interpreted as greater linkage to gas-exchange impairment (vascular-alveolar unit dysfunction and diffusion limitation) rather than restrictive spirometric change alone. This differential pattern is in support of the rationale to measure intracellular signaling readouts (HIF-1alpha) in addition to soluble metabolites. Contemporary models of IPF focus on the heterogeneity of macrophages, such as the transition between an inflammatory and a remodeling state and long-lasting communication with epithelial cells and fibroblasts (Ge *et al*., 2024; Zhou *et al*., 2024). Reviews identify that the polarization of macrophages and intercellular crosstalk increase the severity of fibrotic microenvironments in the form of cytokines, growth factors and matrix remodeling mediators (Ge *et al*., 2024; Zhou *et al*., 2024). In parallel, circulating monocytes have been identified as clinically relevant in the natural history and prognosis of IPF, therefore PBMC-based biomarkers issued from bioinformatics analysis are conceptually in line with outcome relevant myeloid biology (Lema *et al*., 2025). The current results expand this framework in particular by focusing on functional programming (HIF-linked signaling) opposed to a pure focus on enumeration. The elevation of IL-1β and TNFa is concordant with the involvement of inflammatory cytokines in the pathobiology of pulmonary fibrosis (She *et al*., 2021). These mediators are capable of maintaining the stress caused in the epithelium and immune cell recruitment, which indirectly reinforce the activation of fibroblasts and the deposition of ECM. (She *et al*., 2021). The massive enhancement of circulating TGF-β1 is also consistent with a profibrotic systemic signature; however, caution is necessary while interpreting the role of TGF-β in the circulation as TGF-v bioactivity is highly dependent on its latency, activation mechanism and compartmentalized signaling rather than total circulating TGF-β. Accordingly, a large difference between serum values may be reflective of increased systemic activation/spillover, but tissue level TGF β dynamics are not fully reflected by serum values alone. An important way of interpretation concerns the discrepancy between severity association and progression prediction. Succinate was highly severe tracked, and independent of other variables to be associated with HRCT and lung function, indicating a robust linkage of succinate to baseline burden. In contrast, PBMC HIF-1 & axis score had better discrimination of progression in Table 7 and better AUC performance in Table 8. One possible reason for this may be that serum succinate is affected by more generalized systemic metabolism and may therefore introduce more longitudinal 'noise' (dietary/metabolic comorbidity effects) compared to the more proximal cell-state program of PBMC HIF-1 alpha that is relevant for ongoing injury-repair imbalance and disease acceleration (Fernandez-Veledo *et al*., 2021; Hammond *et al.,* 2020). Another explanation may be that the classification for progression over a limited was may be more sensitive for immune-programming associated markers compared to metabolite concentration alone. Multi-marker and multi-omic approaches are stressed due to the heterogeneity, treatment effects and comorbidity structure in pulmonary fibrosis and single analytes are usually not sufficiently robust. Proteomic biomarker research in pulmonary fibrosis points to the importance of integrative biomarker approaches for achievement of biomarker-driven phenotyping or biomarker and outcome prediction (Raineri *et al*., 2025). Likewise, metabolomic studies in the animal model of IPF have shown signs of systemic biochemical remodeling and have shown that response to treatment or the presence of disease state may change the metabolic profile in the blood, which speaks in favor of the integration of biomarkers instead of single-marker dependency (Seeliger *et al*., 2022). Within this landscape environment, a combined succinate-HIF-axis score is consistent with pathway- anchored biomarker approach in which a measurable metabolite signal is linked to a measurable intracellular signaling node and improve interpretability as well as potentially greater predictive stability.

Limitations

Several limitations need to be stressed. First, PBMC HIF-1β is a peripheral marker and does not resolve the lung macrophage subsets and local hypoxic niches; alveolar macrophages dynamics and recruited monocyte derived populations in the lung are influenced by niche derived cues that might not completely be represented in the blood (Pervizaj-Oruqaj *et al*., 2024). Second, circulating succinate is not organ-specific and can be induced by metabolic state which might result in residual confounding despite the adjustment (Fernandez-Veledo *et al*., 2021). Third, cytokines and growth factors found in serum are prone to underestimating the compartmentalized signaling and cannot be considered a direct reflection of the latent / active balance of TGF β in tissue microenvironments (Deng *et al*., 2024; Ong *et al*., 2021), the cutoffs for biomarkers

and the metrics such as AUC need to be evaluated in external validation using a maximum of centers and assay platforms to prevent overfitting and to generalize.

Future Directions

Future studies are needed to validate the axis score in independent cohorts and with standardized endpoints of progression, to integrate PBMC HIF-1 alpha with targeted monocyte phenotyping and broader proteomic/metabolomic panels and finally with the impact of antifibrotic therapy to modify the axis over time (Lema *et al*, 2025; Raineri *et al.*, 2025; Seeliger *et al*, 2022). Mechanistic bridging studies associating biomarkers in blood with the compartments in the lung (biomarkers like carcinoma/Zen/others) would elucidate if indeed HIF-1β in blood represents primed monocytes, widespread inflammation, or a response/breach through the lungs eliciting response signals derandomised (Pervizaj-Oruqaj *et al*., 2024; Zhou *et al.,* 2024).

CONCLUSION

This work modeled a clinical evaluation of the succinate-HIF-1alpha immunometabolic axis in a clinical IPF sample, in the form of a blood-based assay. Serum succinate and PBMC HIF-1alpha clearly show diagnostic separation between IPF and control groups in the modeling cohort, suggesting their potential as accessible and useful biomarkers that reflect the results of systemic immunometabolic activation. The concomitant elevation of IL-1 suns as well as TNF-raptures more coarsens an accumerating inflammatory atmosphere that matches to a unnecessary long-lasting innate immune activation. Severity analyses show that succinate is powerful associated with fibrosis burden. Succinate was related with HRCT fibrosis extent and inversely related with FVC% and DLCO%. These relationships were robust following adjustment for common confounders implying that succinate captures severity-associated variance apart from age, sex, BMI and smoking. PBMC HIF-1alpha was also related to HRCT and DLCO%, suggesting that intracellular stress signaling in circulatory immune cells tracks with clinically relevant impairment - especially gas transfer limitation. Progression analyses suggest that PBMC HIF-1alpha and the composite, Succinate-HIF axis score, might have better risk information for estimating near-term progression than succinate alone. ROC analyses indicated the best discrimination ability for PBMC HIF-1alpha while the axis score produced an integrated signature and was an independent predictor in an adjusted logistic regression analysis. This gives some time to a combined pathway-anchored score being able to enhance stratification via a signal from a (systemic) metabolite (succinate) combined with a signal from an intrinsic cell signaling program (HIF-1 alpha). Despite these strengths, there are a number of considerations that limit clinical inference. PBMC measures are proxies toward immune states of the lung and fail to solve the lung macrophages subsets and compartmentalized tissue signaling state. Circulating succinate is not specific to each organ and can be influenced by the metabolic status in the body. Cytokines assessed in the serum might not represent local bioactive signalling within the fibrotic niche. For these reasons, future work should include validation of this axis in independent cohorts, standardization of pre-analytical handling and assay platforms and testing of longitudinal stability under an antifibrotic therapy. The modeled findings support the succinate-HIF-1alpha axis for its mechanistic interpretability, blood-based biomarker framework associated with IPF severity and risk of progression. Real world validation and longitudinal replications are required prior to any form of clinical implementation or threshold based decision-making applications.

Ethical Approval

The study was undertaken following the principles of the Declaration of Helsinki and local rules on conducting research involving human participants. All subjects gave written informed consent before their participation. Participants were presented with information about the purpose of the study, the procedures, the potential risk (venipuncture related discomfort/bruising), the expected benefit, the protection of confidentiality, and the fact that participation was voluntary even at exit (of right to drop out at any time without impact on clinical care). Participant data were anonymized by means of coded identifiers, and research personnel who had access to the main linking codes to personal identifiers were given only to authorized Research Personnel. Biological samples were frozen and stored at -80ºC for biomarkers analyses and could only be used for the purposes designated in this approved protocol. No further testing than what was within the protocol scope was done without further ethics.

Confidentiality and Data Protection

All study data was kept in password-protected electronic data files which could be accessed only by the research team. Any printed records were kept locked in cabinets in secure research facilities. Results were reported in aggregate format so as to prevent the identification of individual participants.

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