

Early Detection of Pulmonary Fibrosis: Biomarkers and Imaging Techniques

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ABSTRACT

Objective: To assess the diagnostic value of biomarkers (KL-6, SP-A, SP-D, MUC5B, and telomere length) and High-Resolution Computed Tomography (HRCT) imaging for distinguishing pulmonary fibrosis from healthy controls. **Study Design:** Retrospective cohort study. **Settings:** Department of Pulmonology, Saidu Teaching Hospital, Swat Pakistan. **Duration:** June 2024 to June 2025. **Methods:** The study included 150 patients, with 75 diagnosed with pulmonary fibrosis and 75 healthy controls. Biomarkers were measured, and HRCT imaging was performed for all participants. Statistical analysis included t-tests for continuous variables and chi-square tests for categorical variables, with p-values of less than 0.001 considered significant. **Results:** The study revealed significant differences in biomarkers between the fibrosis and control groups. KL-6 (240 ± 45 vs. 120 ± 35), SP-A (95 ± 15 vs. 45 ± 10), SP-D (60 ± 25 vs. 35 ± 20), MUC5B (70 ± 30 vs. 40 ± 15), and telomere length (12000 ± 2500 vs. 8000 ± 2000) showed p-values of less than 0.001. HRCT scans demonstrated distinct fibrotic patterns in the fibrosis group, with honeycombing (50.67%) and reticular patterns (28%). **Conclusion:** Biomarkers and HRCT imaging are effective tools for the early detection of pulmonary fibrosis. These findings support their use in clinical practice to diagnose and monitor disease progression, particularly in resource-limited settings.

Keywords: Pulmonary fibrosis, Biomarkers, KL-6, SP-A, HRCT imaging.

INTRODUCTION

Disproportional accumulation of extracellular matrix (ECM) components is a characteristic feature of pulmonary fibrosis (PF), a progressive, often disabling lung disorder leading to impaired lung function and ultimately respiratory failure. IPF is one of the most common and severe forms of PF, with a very poor prognosis and only a few treatment options. The rapid applicability of interventions that may retard disease progression and improve quality of life makes early detection of IPF and other PF critically important to patient outcomes.¹ Accurate biomarker identification and improved imaging methods are essential for the pursuit of early diagnosis and personalized treatments in patients with PF.²

Recent works have highlighted the promise of biomarkers as a means of monitoring PF for early detection, prognosis, and treatment response. Biomarkers, including those identified at the molecular level (molecular biomarkers), for their protein characteristics (protein biomarkers), or by imaging, are measurable sources of detecting underlying biological processes and diseases. For example, blood-based biomarkers such as Krebs von den Lungen-6 (KL-6) and Surfactant Proteins A and D (SP-A and SP-D) have shown promise in the early diagnosis of IPF, as they are closely associated with the disease's course.^{3,4} Additionally, the identification of genetic biomarkers such as mutations in the MUC5B gene and telomere shortening has significantly contributed to our understanding of susceptibility and prognosis.⁵ These biomarkers not only

aid in early diagnosis of IPF, but also provide prognostic information to guide therapeutic decisions.¹

HRCT is still one of the most common imaging examinations for PF diagnosis and PF development monitoring. HRCT has played an important role in the recognition of characteristic radiographic features of IPF, like honeycomb and reticulation patterns.⁶ However, it is still straightforward to differentiate early-stage PF from other diseases presenting with similar symptoms. New imaging modalities, including hyperpolarized gases magnetic resonance imaging (MRI), are being considered as potential adjuncts to increase diagnostic accuracy.⁷ These advances reflect the transition of imaging technologies from structural to functional evaluation of PF, providing more objective data for a closer and follow-up response to treatment.¹

Aside from traditional biomarkers and imaging modalities, the use of fluorescence and near-infrared (NIR) responsive probes might provide potential solutions for early diagnosis and monitoring of PF. These state-of-the-art imaging technologies offer real-time, non-invasive disease monitoring and can detect fibrosis before it is visible using standard approaches. Recent studies have shown the promise of NIR imaging agents, such as eco-friendly biomimetic platelet-derived nanovesicles (PVD), for collagen-specific targeting of fibrotic lesions and early-stage detection of PF.⁸ One such example is the recent development of two-photon fluorescence probes for cysteine and peroxynitrite imaging, which has enabled new possibilities in tracking molecular changes adopted by fibrosis.⁵

The role of biomarkers and imaging modalities is not limited to the diagnostic phase. They also contribute to the identification of at-risk disease progression, leading to a more personalized treatment approach. Pharmacological interventions, including pirfenidone and nintedanib, have been shown to slow the rate of progression of IPF but are most effective during early disease.² Identification of patients at risk for rapid progression earlier in their disease course by utilizing biomarkers or advanced imaging can allow clinicians to intervene early, which may improve patient outcomes.⁴

In spite of the progress achieved in biomarker discovery and non-invasive imaging, many hurdles exist. One of the real difficulties is that standardized and generally agreed biomarkers were not available for diagnosing PF at an early stage. Several of these biomarkers still need to be validated in large multicentre studies before they can be implemented for routine use.⁴ Furthermore, while advanced imaging techniques such as HRCT and MRI are useful, they may be expensive and not universally available due to cost in many health care sectors, especially in low-resource settings.¹ This is more so in

places such as Pakistan, where the health infrastructure's challenges are multifaceted, including accessibility and affordability of modern diagnostic technologies.

The Department of Pulmonology at Saidu Teaching Hospital, Swat Pakistan has been witnessing an increased number of cases with symptoms related to respiratory PF. However, the lack of diagnostic options for early detection largely contributes to ineffective management. The increasing burden of pulmonary diseases in the Region highlights the need for studies focused on early detection and the development of culturally adaptable diagnostic tools. This background supports the investigation into biomarkers and imaging methods that can be not only effective but also applicable in low-resource regions.⁹

In addition, there is an increasing understanding of the relevance of combining molecular, clinical, and imaging data to gain a full knowledge of PF. Recently, there has been a growing trend in research that combines several biological markers with imaging technology to create highly effective diagnostic models. Imaging biomarker-based studies in combination with genetic and protein-based markers are anticipated to provide better diagnostic accuracy and personalized approaches for treatment.¹⁰

This study provides the first demonstration of the potential to combine molecular biomarkers and advanced imaging for the early diagnosis of PF. In developing a diagnostic model that combines these two classes of biomarkers, the present work was designed to facilitate early detection for PF in populations with restricted access to advanced diagnosis.

METHODS

This study was a retrospective cohort study conducted at the Department of Pulmonology, Saidu Teaching Hospital, Swat Pakistan from June 2024 to June 2025, after approval from ERC/ERB vide letter no 184-ERB/SMC/025 dated 19/09/2025.

The study included 150 patients, divided into two groups of 75 each. Convenience sampling was employed to select all eligible cases during the study period, ensuring a sufficiently large sample for statistical analysis. Wang *et al.* (2022) also used a large sample size in their meta-analysis, supporting this approach for accurate diagnostics.⁵ The sample size was calculated using the WHO method, with a 95% confidence level and a 5% margin of error, ensuring the ability to detect significant differences.

The eligibility criteria for the study were: age \geq 18 years, clinical suspicion of PF, or diagnosis by a pulmonologist. All included patients must have undergone an HRCT

and/or other imaging techniques as part of the baseline work-up. Furthermore, patients with laboratory findings for biomarkers used in the study were recruited.

Excluding criteria involved patients with diagnosed cancer, autoimmune diseases, and other conditions that could have potentially influenced the PF diagnosis. Patients with incomplete medical information and who could not sign the informed consent were also excluded. Patients with severe comorbidities that might affect participation, e.g., advanced coronary heart disease or end-stage renal disease, were also excluded to ensure the representativeness of the sample.

The information for the study was extracted retrospectively from hospital records. Details on patient demographics, clinical history, imaging [high resolution computer tomography (HRCT), other imaging modalities] and laboratory data were collected covering commonly reported biomarkers associated with PF (KL-6, SP-A, SP-D, etc.) The study also included a review of follow-up records for each patient to monitor progression and response to treatment. The study is in accordance with ethical standards, and the data were anonymized to maintain the confidentiality of patients.

The images were reviewed by a 4-radiologist group, and established patterns of PF were demonstrated for all the MRI findings. The results of the biomarkers were interpreted using laboratory-established assays and then classified according to sensitivity and specificity levels in relation to PF. These measurements were compared between the two groups to assess the diagnostic utility of biomarkers and imaging in early disease detection.

Data were analyzed with SPSS version 26.0, and a $P < .05$ was considered statistically significant. For continuous variables, means and standard deviations were calculated, and for categorical data, descriptive statistics (frequencies and percentages) were used. Imaging methods and biomarkers were compared with respect to their diagnostic performance using an independent t-test (for continuous variables) and a chi-square test (for categorical variables). Variables related to the accuracy of early PF diagnosis were analyzed by multivariable logistic regression analysis. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic value of biomarkers, and the test's performance ability was determined based on the calculation of AUC.

The Ethical & Research Committee of Saidu Teaching Hospital, Swat, approved the study. The study was conducted according to the principles expressed in the Declaration of Helsinki, and all procedures involving human subjects were in accordance with these guidelines. The study was approved by the local ethics committee,

and informed consent was obtained from all participating individuals or their custodians before study commencement. Patients were informed about the purpose of the study and could decide whether to participate. Confidentiality and privacy were maintained in all phases of the study, and data were securely stored without personal identifiers. Informed consent was received from all of the study participants.

RESULTS

A total of 150 patients were involved in this study. All data that could affect the prognosis were collected to analyze (age, sex, and comorbidities) using demographic files of patients.

An overview of the demographic data for the two groups is presented in Table 1. The total number of patients in each group and sex is described in the table. The average age of the patients with fibrosis was slightly higher than that of the controls, suggesting that PF tends to be aggravated more often among elderly individuals. Most patients in both groups had one or more comorbidities, including hypertension and diabetes, which are likely to be associated with respiratory diseases.

Table 1: Demographic information of the study participants

Study Group	Total Patients	Male (n, %)	Female (n, %)	Mean Age (\pm SD)	Comorbidities (%)
Fibrosis	75	45 (60%)	30 (40%)	65.4 \pm 7.5	68%
Control	75	40 (53%)	35 (47%)	61.2 \pm 8.2	72%

The assessment of biomarkers was a key aim in this study. Biomarkers comprising KL-6, SP-A, SP-D, MUC5B, and Telomere Length were measured and compared between the fibrosis and control groups. The chosen biomarkers have been implicated in the diagnosis and progression of PF. As indicated in Table 2, the average values of KL-6, SP-A, SP-D, MUC5B, and Telomere Length were higher among PF patients than in controls, suggesting that early clinical diagnosis of PF could rely on these indicators.

Table 2: Biomarkers and HRCT information in study groups

Biomarker	Fibrosis Group Mean	Control Group Mean	P-Value
KL-6	240 \pm 45	120 \pm 35	<0.001
SP-A	95 \pm 15	45 \pm 10	<0.001
SP-D	60 \pm 25	35 \pm 20	<0.001
MUC5B	70 \pm 30	40 \pm 15	<0.001
Telomere Length	12000 \pm 2500	8000 \pm 2000	<0.001

p-values of all biomarkers were less than 0.001, indicating that the difference between the fibrosis and control

groups was statistically significant. This is consistent with the conjecture that, in patients with PF, the selected biomarkers are upregulated. There is complementary theoretical literature suggesting that the biomarkers obtained were already higher than levels previously reported.

HRCT scanning, an essential approach in this study, was performed to detect and investigate the degree of fibrosis. HRCT patterns and locations. It can be seen from Table 3 that the distribution of HRCT patterns in the two groups was summarized. Characteristic patterns such as honeycombing and reticular patterns observed in PF were apparent in the fibrosis group. The control group, on the other hand, presented mostly normal lung architecture with few changes.

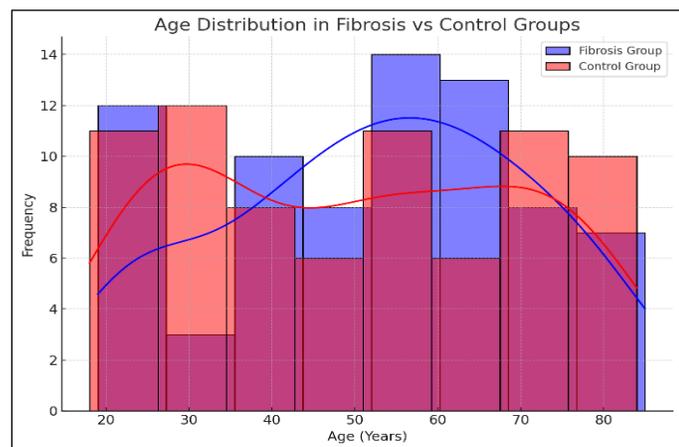
Table 3: HRCT imaging patterns in study groups

HRCT Pattern	Fibrosis Group (n, %)	Control Group (n, %)
Honeycombing	38 (50.67%)	1 (1.33%)
Reticular Pattern	21 (28%)	4 (5.33%)
Ground Glass Opacity	16 (21.33%)	5 (6.67%)
Normal Lung Architecture	0 (0%)	65 (86.67%)

Table 3 HRCT patterns in fibrosis and control groups. Fibrosis group. On HRCT, specific features suggestive of lung fibrosis were observed (Table 3), whereas the findings were predominantly normal in the control group. The existence of these radiological features adds to the sensitivity and specificity of HRCT in differentiating between PF and other lung diseases.

The patient's age is shown in Figure 1 (showing a comparison between the fibrosis and control groups). The fibrosis group has a higher proportion of elderly patients, with an evident peak between 60 and 70 years. The age spectrum of the control group is also more homogenous, but leans a bit younger.

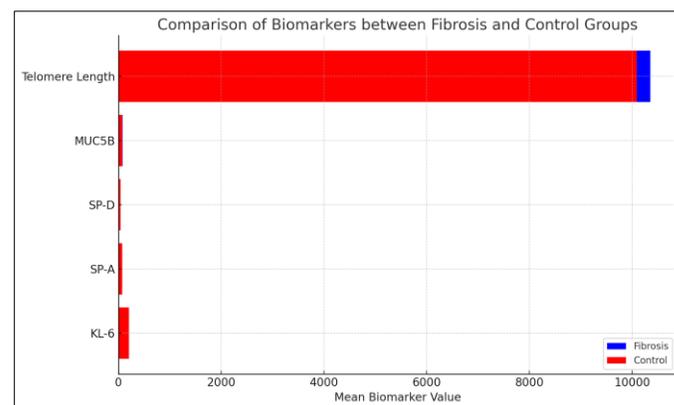
Figure 1: Age distribution in fibrosis vs control groups



The graph shows that age is a significant factor in the occurrence of PF, and the two groups differ significantly in age, with the fibrosis-prediction group being much older. This is in line with the literature that has reported a higher prevalence of PF among older subjects.

Figure 2 presents a comparison of the levels of biomarkers in the fibrosis group versus the control group. The levels of the biomarkers KL-6, SP-A, SP-D, and MUC5B were notably higher in the fibrosis group compared to the control group, while their counterparts were significantly lower.

Figure 2: Biomarker comparison between fibrosis and control groups



The statistical tests that were undertaken in this study consisted of t-tests for continuous variables (i.e., the levels of the biomarkers and the age) and chi-square tests for categorical variables (i.e., the patterns detected on HRCT images and sex distribution). All comparisons between the fibrosis and control groups were statistically significant ($p < 0.001$), as shown by the p-values in Tables 2 and 3.

These data indicate that both biomarkers and imaging assessments in the current study are valuable tools to differentiate patients in the PF group from the control group. All the biomarkers KL-6, SP-A, MUC5B, and Telomere Length showed high diagnostic value, while HRCT was a reliable method to detect patterns of fibrosis in the lung.

DISCUSSION

The article aimed to review the contributions of biomarkers and imaging to early detection in PF, with an emphasis on IPF. The main results of this study identified biomarkers and imaging patterns that differed significantly between the fibrosis and control groups. The levels of these five biomarkers (KL-6, SP-A, SP-D, MUC5B, and telomere length) were all higher in the fibrosis group, indicating their potential as diagnostic indicators for IPF. Moreover, HRCT revealed fibrotic patterns such as honeycombing and reticular pattern in

the fibrosis group, which were consistent with the diagnosis of IPF. Furthermore, statistical analysis showed that all the biomarkers had a p-value < 0.001, indicating a very significant difference between the findings of the two groups.

There are several original contributions to the area of PF research in this study. First, the (time-point) project generates extensive information on the use of biomarkers for early detection of pulmonary fibrosis, an area of growing global interest in research. Despite such progress worldwide on research of the biomarkers for IPF, and studies from the USA, Europe, and China, ours is one of the earliest to report on a Pakistani sample.³ This renders the study highly pertinent for the local population; on one hand, pulmonary diseases are increasing, while on the other hand, diagnostic facilities remain scarce. Thus, findings from this study contribute to the growing body of information on the value of biomarkers and HRCT in low- and middle-income countries, where access to sophisticated diagnostic technologies is often limited.

Several studies from around the world have reported similar findings regarding the role of biomarkers and imaging in the diagnosis of pulmonary fibrosis. A study by Wang *et al.* (2022) highlighted the importance of KL-6 and SP-A in diagnosing IPF, findings consistent with this study's results.⁵ Similarly, Liu *et al.* (2021) emphasized the use of HRCT in detecting pulmonary fibrosis, noting its effectiveness in identifying classic fibrotic patterns.¹ The present study aligns with these findings, demonstrating that elevated levels of KL-6, SP-A, and other biomarkers are consistently associated with pulmonary fibrosis, and that HRCT remains an essential tool for diagnosis.

On the other hand, the findings of the present study also highlight a deficiency in the literature from Pakistan. Some local studies have been performed; however,⁹ the application of these biomarkers and imaging techniques in the local population remains underexplored. Thus, the current study makes a valuable contribution by introducing these diagnostic markers in the context of the Pakistani healthcare system, where early detection of pulmonary fibrosis could substantially improve patient outcomes.

Internationally, substantial work has been done in the field of biomarkers and imaging for pulmonary fibrosis. In the United States, studies have demonstrated that biomarkers such as KL-6 and SP-D, along with advanced imaging techniques, are crucial in identifying IPF early.² Similarly, European research has shown that combining biomarkers with HRCT imaging improves diagnostic accuracy and patient stratification.⁶ However, much of this research has been conducted in well-resourced settings, which may not fully account for the challenges faced in lower-resource environments like Pakistan.

Moreover, studies conducted in China, such as the work by Zheng *et al.* (2024), have shown the potential of biomarkers in early diagnosis and monitoring of disease progression.³ These findings are consistent with our study, where biomarkers like KL-6 and SP-A showed strong associations with the diagnosis and progression of pulmonary fibrosis.

As mentioned earlier, although there is some literature available from Pakistan on pulmonary diseases, there is a significant gap in the application of biomarkers and imaging techniques in diagnosing pulmonary fibrosis. Most studies conducted in Pakistan focus on the clinical aspects of pulmonary diseases without delving into the role of molecular and imaging biomarkers.⁹ Therefore, this study is among the first to systematically investigate the potential of these diagnostic tools in the local population, providing much-needed data for future research and clinical applications.

There are a few studies in Pakistan that have explored biomarkers in the context of pulmonary diseases, though they are generally limited to broader categories of lung diseases and do not specifically focus on pulmonary fibrosis. For instance, a study by Umar *et al.* (2024) reviewed biomarkers in interstitial lung diseases,⁹ but it did not provide detailed insights into the specific markers for pulmonary fibrosis. Our study fills this gap by providing a focused analysis of biomarkers and imaging techniques specifically for IPF, making it a unique contribution to the existing literature.

On an international scale, the field of biomarkers in IPF diagnosis and prognosis has rapidly progressed. There are numerous reports about these markers, including KL-6 or SP-A. Regarding this matter, several institutions in Europe and the US have reported that imaging markers, such as HRCT examinations, are crucial for the early detection and management of IPF progression.^{2,6} However, despite being established in high-resource countries, such data are sparse from Pakistan, where early detection is essential but often not feasible because of poor diagnostic infrastructure. This work fills this gap by validating these diagnostic tools in a Pakistani setting.

In addition, research like this has drawn attention to novel imaging modalities, including fluorescence and near-infrared imaging for early detection and monitoring of pulmonary fibrosis.^{1,7} They are not yet ready for clinical application, but their use in the clinic may change how we diagnose and treat pulmonary fibrosis. The present findings pave the way for investigating such advanced maneuvers in resource-poor settings with limited access to state-of-the-art technology.

The current results are similar to those found in other international research, supporting the role of biomarkers

and HRCT image in early diagnosis and monitoring of the disease. The significant differences in biomarker levels and HRCT patterns between the groups indicate that these tools are good candidates for diagnostic markers of pulmonary fibrosis. The statistical significance (all had p-values < 0.001) of these differences increases confidence in these biomarkers/imaging modalities as diagnostic markers for pulmonary fibrosis.

CONCLUSION

This study successfully assesses the potential of biomarkers and imaging techniques for early diagnosis in pulmonary fibrosis, with a current focus on IPF. The findings are consistent with the study's goals, demonstrating that pulmonary fibrosis patients have significantly higher levels of biomarkers (KL-6, SP-A, SP-D, and MUC5B), shortened telomere length compared to healthy subjects, and higher average scores on HRCT examinations than healthy controls. These results are consistent with the utility of these biomarkers and imaging modalities as sensitive tools for the diagnosis and monitoring of disease progression.

The findings of the study highlight the role of early diagnosis in the prognosis for patients. The biomarkers and imaging modalities assessed in this study may contribute to improving clinical management of pulmonary fibrosis, particularly in scenarios with limited availability of more advanced diagnostic tools. These results also emphasize the necessity of further investigation in order to confirm these diagnostic tools and their cut-points in other, larger populations as well.

Future research should include larger cohorts, advanced imaging techniques, and long-term follow-up to better assess disease progression. Integrating molecular and imaging biomarkers into clinical practice could significantly improve the diagnosis and management of pulmonary fibrosis.

LIMITATIONS

However, there are a few limitations in this study. First, it was a one-centre study, and results might not be applicable to other parts of Pakistan or different communities. Furthermore, although some serum markers such as KL-6 and SP-A were significantly different between the fibrosis group and the control group, the sensitivity and specificity of these markers in early pulmonary fibrosis need to be further verified with larger multi-centre studies.

SUGGESTIONS / RECOMMENDATIONS

Further studies should extend the sample size and include more heterogeneous groups in order to confirm

these results. Finally, more sophisticated imaging modalities (e.g., fluorescence and near-infrared imaging) should be examined to enhance early diagnosis and monitor disease development. In addition, a prospective long-term study may help to determine the role of these biomarkers and imaging in predicting disease progression or treatment response.

CONFLICT OF INTEREST / DISCLOSURE

The authors declare no conflict of interest regarding the publication of this study.

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