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Review Article

Pulmonary Fibrosis, Models and its Diagnostic Markers

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Abstract

Idiopathic pulmonary fibrosis is an uncommon lung illness with no known cause that leads to death quickly. The incidence of IPF has been progressively increasing over the previous two to three decades. Two anti-fibrotic medicines, pirfenidone and nintedanib, have recently been shown to be successful in slowing disease progression and have been approved as therapies. Although the median survival time is about 2 - 3 years, there is a wide range of illness progression from sluggish to rapid deterioration. Biological markers are heightened indicators of physiological/pathological processes or pharmacological response to therapeutic therapies that may be objectively quantified. Various models of pulmonary fibrosis like as Bleomycin, Asbestosis and some others and diagnostic markers like TGF-β, ICAM, Il-13, TGF-α, TNF-α, MMP-1, MMP-7. This lung fibrosis becomes a common problem nowadays.

Keywords: *Fibrosis; Extracellular Matrix; Bleomycin*

Introduction

Idiopathic pulmonary fibrosis is an uncommon lung illness with no known cause that leads to death quickly [1]. According to epidemiological research, the incidence of IPF has been progressively increasing over the previous two to three decades [2]. Despite the fact that the aetiology and pathophysiology of IPF are still unknown, two anti-fibrotic medicines, pirfenidone and nintedanib, have recently been shown to be successful in slowing disease progression and have been approved as therapies [3,4]. Due to a lack of precise indications of disease progression and easy short-term evaluations of therapy response, clinical management of IPF remains challenging [5]. Although the median survival time is about 2 - 3 years, there is a wide range of illness progression from sluggish to rapid deterioration [1].

Biological markers, also known as biomarkers, are heightened indicators of physiological/pathological processes or pharmacological response to therapeutic therapies that may be objectively quantified [6]. Biomarkers are desperately needed in IPF as differential diagnostic tools, predictors of disease progression, and therapeutic response [7]. In the case of IPF, an early diagnosis is critical in order to slow the disease's course as much as possible [8,9] (Figure 1).

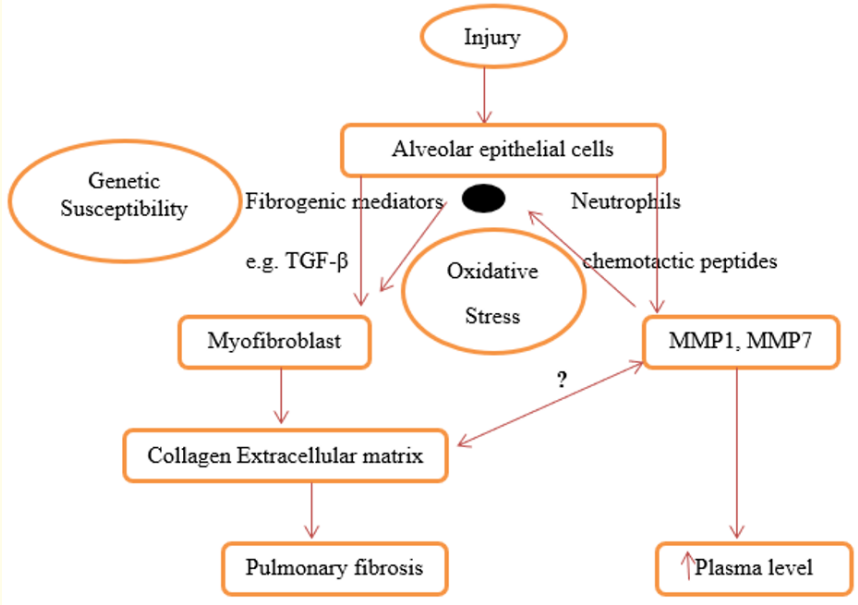

Figure 1: *Mechanism of IPF (doi:10.1371/journal.pmed.0050098.g001 modified).*

Models of pulmonary fibrosis

Domestic animals including cats, dogs, and horses can acquire spontaneous pulmonary fibrosis, which has many of the same histological characteristics as human IPF. The West Highland white terrier is a dog breed that is especially prone to canine IPF [10]. With coarse crackling audible on thoracic auscultation, pulmonary hypertension, and/or airway collapse, the condition shares many clinical symptoms with human IPF. Ground glass opacification and traction bronchiectasis have been seen in dogs, along with subpleural and peribronchiolar fibrosis, honeycombing, and alveolar epithelial alterations similar to those seen in humans [10]. Interstitial fibrosis with fibroblast/myofibroblast accumulation, honeycombing, and type II pneumocyte hyperplasia are associated with spontaneous IPF-like illness in cats [11,12]. Horses and donkeys can develop an IPF-like disease, however unlike cats and dogs, where the disease appears to be spontaneous, equine IPF is connected to infection with equine herpes virus 5 [13].

Mice with a genetic defect can develop spontaneous pulmonary disease and age-related lung disease [14]. More typically, mice in pulmonary fibrosis models have been subjected to cytotoxic drugs (e.g. bleomycin [BLM]), profibrotic cytokines (e.g. transforming growth factor beta [TGF-b] and interleukin [IL]-13), or pharmacological treatments (e.g. phorbol myristate acetate [PMA]). However, because these produced mouse models frequently involve young adult mice and provoke acute lung injury with inflammatory responses that lead to tissue fibrosis, they do not follow the development of human IPF. The main issue is that each model is based on a single recognised injury that causes an immune response with slightly different cellular constitution and duration. Furthermore, unlike the chronic, progressive, and ultimately fatal condition seen in human IPF, these animal models are more realistic of an acute lung injury, with fibrosis that in most cases resolves. Regardless, these models have shed light on the pathways that contribute to collagen modulation, fibrosis pathogenesis, and potential therapies [15].

Bleomycin (BLM)

BLM, an anti-cancer medicine that causes DNA damage in target cells, is the best-studied and most extensively used agent for inducing pulmonary fibrosis in mice and rats [16,17]. BLM is normally given as a single dose in saline or PBS via intratracheal, intranasal, intraperitoneal, oropharyngeal, or intravenous routes, with the concentration varying depending on the route and the species and strain of animal utilised.

The animals frequently lose weight during the first few days after a single BLM challenge, which is linked to acute lung injury. Weights begin to increase after 5 - 7 days and animals eat and behave normally. The maximum lung weight is usually reached 7 days following BLM therapy [18]. Lung injury has a similar histologic pattern in all species, however it varies significantly depending on the route of administration [19]. The first injury mostly affects the bronchioles, with areas of microvascular leakage and early hyperplastic alterations in type II pneumocytes in inflammatory cell influx sites. The injury is more widespread by day 7 after BLM treatment, involving the distal lung parenchyma, with multiple inflammatory foci and oedema present within alveolar septa. The lungs demonstrate a more developed regional interstitial fibrosis by day 14, with an increase in macrophages, localised lymphocytosis, and lymphoid expansion. With significant collagen deposition and remodelling of the alveolar unit, focal alveolar re-epithelialization is common, as is a low-grade focal 'honeycombing' reaction, which is associated with emphysematous alterations. There is evidence of focused condensation of ECM by days [20]. Macrophage and lymphocyte margination is prominent, especially in the fibrotic regions' periphery, and regional re-epithelialization of the alveolar septa is noticeable. There is bronchiolar and peribronchiolar fibrosis, as well as inflammation and emphysematous alterations, by 120 days, with significant portions of normal lung. These changes have been seen in other research and are likely to represent changes seen in people with IPF. Although the first source of injury is the endothelium of capillaries and bigger arteries, as well as the perivascular lung structures of the subpleural parenchyma, intravenous or intraperitoneal treatment of BLM results in a similar pattern of lung fibrosis. Single-cell RNA sequencing (scRNAseq) was utilised by Aran and colleagues to characterise the heterogeneity of macrophages after BLM-induced fibrosis in mice. They discovered a profibrogenic macrophage population with a characteristic gene profile ($CX3CR1^+$, $CCR2^+$, $MHCII^+$), which was intermediate between monocyte-derived and alveolar macrophages and produced high quantities of platelet-derived growth factor AA, which guides fibroblast proliferation. Depletion of $CXCR3^+$ macrophages in mice from day 8 after BLM injection resulted in a large reduction in $SiglecF^+$ macrophages and fibroblasts in the lungs, as well as a drop in collagen formation. These data imply that $CX3CR1^+$ macrophages play a key role in lung fibrosis. They were also able to demonstrate the presence of a similar population of transitional macrophages in human IPF patients' lung tissues. Reyfman and colleagues compared IPF patients' lung biopsy samples to healthy lung tissue acquired from transplant donors using RNAseq. They compared immune cells, epithelial cells, and fibroblasts scRNAseq data sets. They discovered a similar new profibrogenic macrophage population in IPF patients and were able to create a pulmonary fibrosis single-cell atlas. For the first time, this novel and unique resource revealed considerable variability of alveolar macrophages and lung epithelial cells in IPF patients.

A continuous or repetitive delivery method of BLM appears to produce more fibrosis in the lung, and a fibrotic phenotype more closely resembling IPF than the single BLM delivery method [20]. Heterogeneous areas of inflammation and fibrosis, with persistent deposition of collagen and collapse of alveolar structures, likely leading to reduced lung function 5 - 6 weeks after BLM exposure, support the progressive nature of the pulmonary lesion in this model, thereby more closely resembling the human UIP pattern [21]. Aged mice have also been utilised in several fibrosis models, including BLM-induced lung fibrosis, in an attempt to better mimic human IPF [22]. Within these models, however, there is minimal evidence on the influence of ageing on the immune response.

'Humanized' immunodeficient mice have proven to be a useful tool for studying the function of human cells/tissues in lung fibrosis pathobiology. To explore the profibrotic capability of these mesenchymal cells, these investigations were limited to the transfer of human

IPF vs control lung fibroblasts. The C.B.-17 SCID/beige [23,24] mouse strain as well as the NODscid-IL2Rγc$^{-/-}$ [NSG] mouse strain were employed as recipients [25] Pierce., *et al*. were the first to establish that human IPF fibroblasts may generate pulmonary fibrosis in recipient mice, but not control fibroblasts, mirroring the disease pathology found in human IPF patients. Furthermore, they used specific antibodies to neutralise human CCR7 or CCL21 proteins and shown that immunotherapy might slow down the progression of lung fibrosis in animals Jones., *et al*. looked into the role of the MAP3K19 enzyme, which has been found to be elevated in patients with IPF. They used C.B-17SCID/bg mice to transfer cultured human IPF fibroblasts and found that targeting the MAP3K19 enzyme with siRNA or a small molecule inhibitor could reduce lung fibrosis in recipient mice. Based on the expression of the check point molecule programmed death ligand-1, Geng., *et al*. [25] used the humanised mouse model to identify two different fibroblast populations from the IPF lung (PDL-1, also called CD274). In comparison to PD-L1^{-} fibroblasts, PD-L1^{+} fibroblasts showed increased motility and invasive characteristics. PDL1^{+} cells caused more pulmonary fibrosis in NSG mice than PDL1^{-} fibroblasts did in NSG animals. They also discovered that antibody-mediated PD-L1 targeting or CRISPR-mediated PD-L1 knockdown both reduced lung fibrosis in NSG recipient mice [25]. By transplanting T cells from an IPF patient to an NSG mouse, Habiel and colleagues demonstrate that CD28null T cells play a role in the aetiology of pulmonary fibrosis. 63 - 65 days after injection, the mice developed unresolved lung remodelling. The loss of BAL surfactant protein C, as reported by the authors, suggests that this is related to injury to type II alveolar epithelial cells [26].

It's also important to think about the mouse strain to employ, because there's evidence that the genetic background of a mouse can influence the degree of lung fibrosis after BLM treatment. Because of the reproducibly high amounts of inducible lung collagen deposition that are sustained for at least 12 weeks, C57BL/6J mice are the most widely used strain for BLM therapy. Other inbred strains, including as DBA/2, have been shown to have more persistent fibrosis in numerous exposure experiments. Inbred stains such as A/J, C3Hf/KAM, or C3H/HeJ, on the other hand, are protected from BLM-induced fibrosis. Scientists looked into if there were any BLM susceptibility genes for lung fibrosis because of the differences in fibrotic response between mouse strains. The bleomycin-induced pulmonary fibrosis 1 (BLMpf1) locus was discovered on chromosome 17 after a series of genetic crosses. Using subcongenic mice with 17 C3H/HeJ alleles in the BLMpf1interval on a C57BL/6J background, the locus was further narrowed down to a 0.71 Mb area.

Asbestosis

Asbestos exposure is another model that accurately represents a common kind of human lung fibrosis. Several pathologic findings distinguish the asbestos-induced lung fibrosis model from IPF, including asbestos bodies incorporated within the fibrous tissue, fewer myofibroblast foci, and bronchial wall fibrosis. In some circumstances, the UIP pattern may also be present [27]. Some of these characteristics are replicated in animal inhalation models, which have aided our understanding of the pathophysiology of both asbestosis and IPF [28]. The formation of fibrosis is mediated by a single intratracheal dose of asbestos fibres; nevertheless, the model has significant flaws, as fibrosis is more typically central than subpleural and is unevenly distributed between lungs. Inhalation models produce a more peripheral pattern, but disease progression can be delayed, especially when chrysotile fibres are used. Intratracheal animal models using amphibole fibres exhibit the same dynamics as BLM models, with fibrosis appearing on day 7 and peaking on day 14. The formation of fibrotic damage in inhalation models can take up to a month. Asbestos fibre deposition causes fibrosis by causing alveolar epithelial cell death, macrophage M2 polarisation, and activated T lymphocyte overproduction of pro-fibrotic cytokines, all of which lead to myofibroblast development and extracellular matrix production.

Monocrotaline

Monocrotaline (MCT) is a pyrrolizidine alkaloid that is both pneumotoxic and hepatotoxic after being metabolically activated by the liver [29]. A single dosage of MCT administered subcutaneously causes pulmonary hypertension and respiratory distress syndrome that is morphologically and functionally similar to human circumstances [30]. When the diseases have fully appeared, animals are routinely euthanized 3 - 6 weeks following injection. With bleeding and oedema, the lungs reveal a strong inflammatory reaction. Reduced alveoli,

thicker alveolar septa, and an increase of inflammatory cells, primarily macrophages, are among the parenchymal alterations. In the adventitia of small arteries and arterioles, there is a narrowing of the lumen, thickening of the wall, hyperproliferation of the media, inflammatory cells, and increased collagen [31]. Mice given frequent subcutaneous MCT injections for up to 18 weeks and subsequently euthanized after 28 weeks showed severe interstitial pneumonia and pulmonary fibrosis, with a significant increase in collagen deposition in the alveolar septa after 8 weeks of treatment. However, the levels utilised in this investigation were extremely lethal, and several mice died during the course of the experiment. MCT treatment caused perivascular infiltration of macrophages, mast cells, and T cells in a rat model of MCT-induced pulmonary arterial hypertension (PAH), which was reduced by inhibiting CXCL12, a key homeostatic chemokine that controls leukocyte migration into a variety of tissues, including the lung [32,33]. Cuttica and colleagues looked at the function of T-cell-mediated immunological disease in the development of PAH in mice after exposure to MCT. They found that $Rag1^{+}$ animals, which are devoid of mature B and T cells, are protected from vascular injury. In the same paradigm, they found evidence for the role of $CD4^{+}$ T lymphocytes in driving pulmonary vascular remodelling [34].

Fluorescein isothiocyanate

A single intratracheal instillation of fluorescein isothiocyanate (FITC), a skin sensitising hapten capable of triggering particular immune responses, causes fibrosis in the lungs of mice and rats [35]. The distribution of FITC in the lung may be directly visualised using this technique, which is a benefit. However, because the molecule is highly intractable, this drug is difficult to deliver and requires sonication for a better dispersion in the lungs and a more uniform and repeatable harm [36].

The animals acquire a pattern of harm compatible with acute lung injury after receiving FITC. Haemorrhage, alveolar wall oedema, eosinophilic alveolar exudate, a significant infiltrate of mononuclear cells and neutrophils, primarily around the bronchioles, and bronchial epithelial cell hyperplasia are all examples of this by 21 days, there is patchy focal loss of normal lung architecture with focal interstitial fibrosis that lasts for at least five months and is characterised by a mainly mononuclear cell infiltrate. The infiltration and scarring were both limited to peribronchial FITC deposition regions. The involvement of lymphocytes in FITC-induced lung fibrosis was investigated by Christensen., *et al*. in two mouse strains: C57BL/6 and BALB/c. There appeared to be no difference in fibrosis onset between the two strains, implying that pathogenesis was not affected by background specific genes. FITC-induced lung fibrosis was also thought to be triggered by particular immunity to the fluorescein hapten at first. $CD3^{+}$ T cells and $B220^{+}$ B cells co-localized within lymphoid clusters in the lungs six months after FITC treatment, near to areas of active tissue fibrosis, similar to the pattern seen in human IPF lung. However, lung fibrosis was still seen in FITC-treated T-cell-depleted SCID and $Rag1^{-/-}$ mice, indicating that the adaptive immune system is not implicated in fibrogenesis in these animals. In the absence of the chemokine receptor CCR2, which is responsible for monocyte infiltration, FITC therapy reduced fibrosis, showing that while the adaptive immune response may not be necessary, the activation of innate immune responses may be key in generating FITC-induced lung fibrosis [37].

Radiation-induced pulmonary fibrosis

In humans, thoracic radiation exposure can cause severe inflammatory reactions that result in alveolitis or even fibrosing alveolitis. Tissue destruction, epithelial and fibroblast cell proliferation and lung interstitium remodelling characterise radiation-induced pulmonary fibrosis. Mice were given a single 18 Gy dose of radiation to replicate the pathologic alterations that occur in humans after exposure to radiation. Lung fibrosis developed within 6 months of therapy in this model. Fox and colleagues exposed C57BL/6, A/J, and C3HeJ mouse strains to a single complete thoracic radiation dose of 18 Gy and found that C57BL/6 mice were the most prone to developing lung fibrosis compared to the other strains. However, in comparison to the other lung fibrosis models reviewed, the radiation model is time expensive and requires a high level of surveillance since irradiation causes lymphopenia, which makes the animals more susceptible to infection [38].

Age-related models

IPF is a type of age-related illness, and many of the markers of ageing, such as genomic instability, telomere attrition, epigenetic changes, unregulated cellular bioenergetics, and cellular senescence, have lately been proposed as characteristics of the fibrotic lung [39]. According to studies, aged mice are more vulnerable to pro-fibrotic stimuli such as BLM than younger mice [40]. Given that IPF is more common in the elderly, this is of special interest. The spontaneous age-dependent development of pulmonary fibrosis has been linked to transgenic deletion of senescence-related genes like as RAGE and relaxin, showing the central role of ageing in disease susceptibility [41]. On the other hand, the role of "virome" as a pro-fibrotic mediator has been further investigated in the context of the aged-related development of lung fibrosis, with researchers demonstrating that only aged mice (> 15 months) develop γ-herpesvirus-68-induced lung fibrosis through a mechanism involving alveolar epithelial cell reprogramming to produce pro-fibrotic factors and increased TGF-β signaling in lung fibroblast [42]. Torres-Gonzalez., *et al*. [43] also found that gamma herpesvirus induced endoplasmic reticulum stress, type II lung epithelial cell death, and activation of profibrotic pathways in ageing animals.

The presence of herpes viral genomes in IPF lungs, as well as the epidemiological link between viral infections and IPF acute exacerbations, could explain this finding [44].

Cytokine overexpression models

Lung fibrosis is caused by the overexpression of cytokines such as TGF-β, TGF-α, IL-13, TNF-α, and IL-1β, utilising gene-transfer and transgenic techniques. These essential cytokine overexpression models will be briefly discussed.

TGF-β overexpression

TGF-β is a powerful profibrotic cytokine seen in most types of lung fibrosis. As a result, models that rely on TGF-β overexpression are particularly useful for deciphering the downstream signalling cascades in a variety of cell types. TGF-β overexpression can be accomplished in epithelial cells via adenoviral administration or doxycycline-regulated transgenic expression. Significant elevations of active TGF-β were found in the lung by day 1 in the initial rat models of adenoviral overexpression, reaching a peak concentration of 13 ng/ml by day 7. Notably, the concentration of latent TGF-β peaked in these trials around Day 7 at a value of 72.3 ng/ml, implying that constitutively active transgenic expression stimulated endogenous production. TGF-β expression in the lungs was accompanied by mononuclear cell infiltration (Days 3 - 7), which was followed by alveolar consolidation (Days 8 - 10). By Day 14, lung collagen concentrations had grown by a factor of two. Following these initial findings, adenoviral vectors were employed to show a dose-dependent rise in fibrosis in mice. This paradigm was also strain-dependent, as C57Bl/6 mice were more susceptible to TGF-β overexpression than Balb/c mice [45]. Apoptosis of epithelial cells and alterations in soluble mediators that mirror real illness are other hallmarks of this model. Adenoviral-mediated TGF-β overexpression is particularly distinct in that it causes long-term scarring, which may more closely resemble the fibrotic alterations seen later in IPF. The treatment of mice with doxycycline resulted in a rapid up-regulation of TGF-β (approx.1 ng/ml) within the lungs after 12 hours in the doxycycline-inducible, club cell (Clara cell) 10 (CC10) promoter - driven model of TGF-β over expression. Over the course of two months, induction resulted in a 2-fold increase in lung collagen concentrations. The degree of fibrosis continued to worsen over the course of the doxycycline treatment, despite the fact that it was not progressive per se. Overexpression of TGF-β has been linked to death of airway and alveolar cells, myofibroblast accumulation, and the activation of the epithelial-to-mesenchymal transition in these animals. The contributions of variably activated macrophages to disease development have also been clarified using the overexpression model [46].

TGF-α overexpression

TGF-α, which is identical to TGF-β, is overexpressed in the lungs of IPF patients. In animal models, TGF-α overexpression in the lung epithelium causes lung fibrosis. Furthermore, TGF-α overexpression causes pulmonary hypertension and hence is useful in determining the pathogenic changes in vascular architecture. The importance of regulating interaction between lung epithelial cells and mesenchymal cells is demonstrated in this model. TGF-α overexpression in the lung epithelium, for example, causes a long-term up-regulation of the mitogen-activated protein kinase (MEK)/extracellular regulated kinase signalling pathway in the lung mesenchyme. Lung remodelling is partially reversed when TGF-α inducible expression is stopped, allowing researchers to investigate the mediators involved in this reversal process [47].

IL-13

The overproduction of IL-13 in the lungs has been achieved via a transgenic technique in which the IL-13 gene is produced constitutively by the CC10 promoter. Both airway and parenchymal fibrosis develop in these transgenic animals (as indicated by trichrome staining and the Sircol assay). The development of airway and parenchymal scarring is mediated by an increase in TGF-β activity, despite the fact that the time course of collagen accumulation in this animal is unknown. TGF-β activity is required to modulate the profibrotic effects of IL-13, as evidenced by a reduction in collagen production following treatment with a soluble TGF-β receptor. Furthermore, giving IL-13 overexpressing animals aprotinin, a serine protease inhibitor, reduced TGF-β activity and limited scarring [48].

IL-1β

Lung fibrosis was also induced by a single injection of an adenoviral vector encoding the IL-1β gene. In comparison to rats given a control vector, injecting 5×10^8 plaque-forming units (pfu) of adenoviral (Ad). From day 21 to day 60, IL-1β caused a gradual rise in lung collagen, as determined by hydroxyproline. An early neutrophilic inflammatory response in bronchoalveolar lavage fluid and an increase in lung macrophages were also seen after the IL-1β vector were given, and this influx of inflammatory cells was visible on lung histology. Lung histology showed an increase in α - smooth muscle actin (α-SMA) positive cells inside fibroblast foci by day 14. TGF-β concentrations, TGF-β activity, and platelet derived growth factor (PDGF) concentrations were all enhanced by IL-1β overexpression, suggesting that they could be potential downstream mediators of IL-1β profibrotic actions [49].

TNF-α

TNF-α gene transfer through adenovirus has also been shown to cause lung fibrosis. TNF-α overexpression elicited an early inflammatory response, similar to IL-1β, with an influx of neutrophils, macrophages, and lymphocytes. Although the extent of collagen build-up was not assessed in this initial investigation, lung sections revealed an accumulation of α -SMA - positive cells beginning on day 7 and increasing in number by day 14. TNF-α gene transfer was also linked to an increase in TGF-β levels. Although this work revealed TNF-α as a profibrotic molecule, a subsequent investigation of transgenic mice overexpressing this cytokine via the surfactant protein - C promoter suggested that it may have antifibrotic characteristics when combined with bleomycin or TGF-β [50]. In the study that showed that TNF-α overexpression inhibited fibrosis, concentrations of the antifibrotic mediator prostaglandin E2 were elevated in the bleomycin-damaged lungs, suggesting a mechanism of fibrosis protection. TNF-α may play a multifaceted role in both generating and preventing lung fibrosis, according to these research.

Biomarkers

Alveolar epithelial markers

The Krebs von den Lungen-6 (KL-6) antigen, as well as surfactant protein A and D (SP-A and SP-D), are the major biomarkers linked to alveolar epithelial cell injury (or malfunction). Serum KL-6 had the highest accuracy for diagnosing interstitial lung illnesses (IPF and ILD associated with connective tissue diseases) when compared to SP-A and SP-D [51].

Krebs von den Lungen6 antigen (KL6)

KL-6 is a glycoprotein with a high molecular weight that belongs to the human trans-membrane mucin 1 family (MUC1). Type II pneumocytes express that glycoprotein mostly on the external membrane surface [52]. It was first discovered in non-IPF interstitial lung disorders (ILDs) and was found to be elevated in IPF, possibly indicating a link to an increased risk of IPF-related death. KL-6 enhances lung fibroblast migration, proliferation and survival, and hence may have a role in the pathophysiology of IPF. A serum baseline level of more than 1000 U/ml appears to be linked to a poorer result. Our findings show that blood KL-6 levels in IPF patients are much higher than in healthy participants, however we found no evidence of lower KL-6 levels in patients treated with pirfenidone or nintedanib [53]. Serum KL-6 may be linked to therapy response, although more longitudinal research is needed to prove this.

Surfactant protein A and D (SPA and SPD)

Surfactant proteins are lipoprotein complexes that type II pneumocytes produce and secrete to reduce surface tension at the air - liquid interface. They also play a part in the defence of the lung host. Serum SP-A and SP-D levels are known to be raised in patients with ILD, with IPF patients having higher levels [54]. Surfactant lipids may also protect against intraluminal fibrogenesis by promoting fibroblast death and lowering collagen deposition, according to certain data. In this setting, SP-A and SP-D have been demonstrated to be predictive of survival when tested early in the disease's course. Their role in the physiopathological process of IPF is highlighted by a family type of IPF linked to surfactant protein C mutations [55].

Fibrogenesis and extracellular remodelling markers

Matrix metalloproteinases (MMP)-1 and -7, Lysyl oxidase-like 2 (LOXL2), and Periostin are the most common fibrogenesis biomarkers found in IPF.

Matrix metalloproteinases1 and 7 (MMP1 and MMP7)

Matrix metalloproteinases are zinc-dependent proteases that are involved in the breakdown and remodelling of extracellular matrix components. In comparison to hypersensitivity pneumonitis, sarcoidosis, and COPD, MMP-1 and MMP-7 appear to be primarily overexpressed in plasma of IPF patients, suggesting that they may be beneficial in differential diagnosis [56]. They also play a role in inflammation and appear to play a role in the pathophysiology of pulmonary fibrosis. MMP-7, which is known to be considerably elevated in epithelial cells both at the gene and protein levels and is thought to be active in hyperplastic epithelial cells and alveolar macrophages in IPF, is certainly the most researched. Higher MMP-7 concentrations have also been linked to disease severity as measured by forced vital capacity (FVC) and DLCO (percent pred). As a result, MMP-7 is one of the most important individual biomarkers in IPF.

In the serum of people with IPF, the concentrations of protein fragments produced by MMP activity are higher than in healthy people. Increased neoepitope concentrations were linked to disease progression, which was defined as death or a drop in FVC of more than 10% 12 months after study enrolment and the pace of rise predicted survival [57]. We can speculate that because neoepitopes are produced by proteases, they may be linked to lung remodelling and fibrosis.

Lysyl oxidaselike 2 and periostin

Lysyl oxidase-like 2 (LOXL2) is seen in fibrotic lungs and is hypothesised to be involved in matrix remodelling and fibrogenesis. The amount of LOXL2 in the blood is linked to the course of IPF disease (as determined by the traditional regression tree approach). Despite this, a trial of anti-LOXL2 targeted medication failed to slow the course of IPF. Periostin, an extracellular matrix protein that promotes to

lung fibrosis, is found in high concentrations in the blood and lung tissue of IPF patients. It's detected in the "fibroblast foci" in lung tissue, which are common in IPF. Periostin levels in the blood are also linked to a composite score that measures disease progression [58].

Chemokines

CC chemokine ligand 18

CCL18 (CC motif chemokine ligand 18) is a tiny chemo attractant protein produced from alveolar macrophages. Antigen-presenting cells such as monocytes, macrophages, and dendritic cells are the primary producers of CCL18. Alveolar macrophages are thought to be the main source of CCL18 in the lung in the setting of pulmonary fibrosis and have a role in the pathogenesis of pulmonary fibrosis. CCL18 levels in the blood are elevated in IPF, however this is not a disease-specific finding. CCL18 is inversely linked with pulmonary function tests (TLC and DLCO) in patients with IPF. Patients with blood levels of CCL18>150 ng/ml were found to be independently linked with death in IPF patients in a prospective analysis (HR 1.98, 95% CI 2.49 - 25.51, $p = 0.005$). Furthermore, one of the particular anti-fibrotic medications for IPF, pirfenidone, significantly reduced CCL18 expression in macrophages [59]. As a result, CCL18 may be of relevance as a prognostic tool in IPF.

Interleukin8 (IL8)

IL-8 is a cytokine that is known to be raised in the serum of IPF patients and is a strong chemoattractant for neutrophils. A study found a negative connection between IL-8 and pulmonary function tests (DLCO, TLC, VC) [60] as well as survival [61], suggesting that it could be useful as a biomarker in IPF.

Growth factors and adhesion molecules

YKL40

YKL-40 is a chitinase-like protein that affects cell proliferation and survival and has been linked to liver fibrosis in the past. YKL-40 has also been extensively researched in a variety of ILDs. Its method of action is unknown, however it appears to have a promitogenic effect on lung fibroblasts in animal models and enhances macrophage activity in COPD patients. Furthermore, YKL-40 levels have been observed to be higher in IPF patients' fibrotic regions, particularly in macrophages and bronchial cells. Although YKL-40 was not found to be specific for IPF in a prospective investigation, a serum level of more than 79 ng/ml was linked to a worse prognosis (HR 10.9, 95% CI 1.9 - 63.8, $p < 0.01$) [62]. As a result, we believe that YKL-40 is useful for diagnostic and prognosis stratification, and that it should be explored.

Insulinlike growth factor 2 (IGFBP2)

IGFBP-2 belongs to a family of six insulin-like growth factor (IGF) binding proteins that has recently been discovered in IPF. Cell proliferation and differentiation are thought to be aided by IGF and IGFBPs. IGFBP-1 and IGFBP-2 are higher in newly diagnosed IPF, and IGFBP-2 is reduced in patients treated with anti-fibrotic medication, but still elevated, as compared to healthy persons, according to a cross-sectional study of 50 patients with IPF [63]. However, we found no link between IGFBP-2 and measures of decreased lung function. In contrast to IGFPB-1 and -2, IGF-1 and -2 levels in the serum of untreated IPF patients were found to be lower. Longitudinal studies are also required to assess their use as biomarkers in IPF.

ICAM1 and ICAM2

Intracellular adhesion molecule-1 and -2 (ICAM-1 and ICAM-2) levels are also raised (albeit not to the same extent) in serum from IPF patients. ICAM-1 has been reported to be overexpressed on pulmonary epithelial cells in IPF patients [64], but ICAM-2 has been found to be inversely related to DLCO. Its clinical value is unknown at this time, and longitudinal studies are needed to find out.

VEGF

The glycoprotein vascular endothelial growth factor (VEGF) is expressed in alveolar epithelial cells. In one longitudinal investigation of 41 IPF patients, increased serum VEGF, but not CRP, was found to be linked with disease severity as measured by the alveolo-arterial difference of O_2. Serum levels of VEGF in severely hypoxemic IPF were found to be similar to those reported in non-small cell lung cancer in this investigation. Serum VEGF was linked to an increase in VC loss over time. Patients with VEGF levels exceeding 207 pg/ml had a worse 5-year survival rate. Nintedanib, which is utilised as a particular IPF medication, targets VEGF [65].

Conclusion

This review paper concludes all the detail of pulmonary lung fibrosis. With the present treatment and how to induce lung fibrosis with different models. It's also mentioned different diagnostic markers of pulmonary lung fibrosis. Nowadays lung fibrosis is a common burden in the world.

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Authors Contributions

Mohd Rafi Reshi, Saman Anees and Maaz Naqvi equal contribution in the planning and drafting of the full manuscript. Muzammil Muzaffar, Arifa Hassan and Huma Parveen also help in making some part in the manuscript. All authors approved the final version of the manuscript.

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