

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,500

Open access books available

136,000

International authors and editors

170M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Biomarkers in Idiopathic Pulmonary Fibrosis

Sanja Stankovic, Mihailo Stjepanovic and Milika Asanin

Abstract

Numerous published papers are investigating the utility of biomarkers in Idiopathic Pulmonary Fibrosis (IPF) diagnosis, treatment, and outcome prediction. This chapter will summarize our current knowledge about biomarkers associated with alveolar epithelial cell damage and dysfunction (Krebs von den Lungen, surfactant proteins, the mucin MUC5B, CA 15-3, CA 125, CA 19-9, defensins, Clara cell protein (CC16), telomere shortening), biomarkers associated with fibrogenesis, fibroproliferation and extracellular matrix (ECM) remodeling (MMPs and their inhibitors, osteopontin, periostin, insulin-like growth factors, fibulin-1, heat shock protein 47, lysyl oxidase-like 2, circulating fibroblasts, extracellular matrix neoepitopes) and biomarkers related to immune dysfunction and inflammation (C-C chemokine ligand-18, C-C chemokine 2, YKL-40, C-X-C motif chemokine 13, S100A4, S100A8/9, S100A12, autoantibodies to heat shock protein 72, toll-like receptor 3, soluble receptor for advanced glycosylated end products, endothelial damage (vascular endothelial growth factor, interleukin 8, endothelin 1). The future directions in incorporating IPF biomarkers into clinical practice will be reviewed.

Keywords: idiopathic pulmonary fibrosis, biomarkers, extracellular matrix, remodeling and fibroproliferation, alveolar epithelial cell dysfunction, immune dysfunction diagnosis, prognosis

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic fibrotic lung disease of unknown etiology, progressive and irreversible interstitial lung disease (ILD). IPF is the most common form of idiopathic interstitial pneumonia. It affects around 3 million people worldwide [1]. The increasing count of IPF cases is evident. The prognosis for patients with IPF is poor, with a median survival of 3–5 years if untreated [1]. IPF generally affects adults over 50 years, mainly in their sixth or seventh decade, but the earlier onset was noted in familial IPF. According to the epidemiological data, the incidence rates in Europe and North America are between 2.8 and 19 cases per 100,000 people per year [2]. The number of cases older than 65 years of age is about 400 per 100,000. The IPF has a prevalence of 8.2 cases per 100,000 and belongs to the rare diseases group [3]. The first IPF manifestation is shortness of breath (up to 85% of cases), chronic non-productive cough (up to 75%), tiredness, loss of appetite, and progressive exertional dyspnea, followed by an impaired quality of life [4]. More rarely, it can be an acute exacerbation (AE), acute episodes of sudden, rapid worsening of the disease of dyspnea over just a few weeks, and a consequent significant increase in mortality risk [5].

The pathogenesis of IPF is not completely understood. For many years, IPF was principally an inflammatory disease, given the increase in inflammatory cells in the lungs. Dramatic advances in the understanding of IPF pathogenesis mechanisms over the past decade were based on proteomics data. It discovered proteins in terms of prognosis, diagnosis, and IPF progression. Today, we think about IPF as an epithelial-driven disease. IPF originates from unknown microinjuries resulting from recurrent exposures of the lung epithelium to stimuli or predisposition, followed by initiation of alveolar epithelial cells (AECs) dysfunction, fibroblast recruitment, and proliferation and progression of fibrosis through fibroblast differentiation, myofibroblasts proliferation, and accumulation of extracellular matrix and remodeling [6].

Usually, pulmonary function tests reveal reduced total lung capacity, low carbon monoxide diffusing capacity, and arterial hypoxemia. Although the course of the disease is variable, IPF has a poor prognosis, mortality is high, and reported median survival is from 2.5 to 5 years from the time of diagnosis [7, 8].

The most frequent cause of death is respiratory failure. Although there is no identified cause for the IPF, men are more frequently affected than women. Genetic and environmental factors may contribute to the development or worsen the prognosis of IPF. A history of smoking increases the risk of developing IPF. Occupational and environmental risk factors for IPF are agricultural exposure, dusts from metal, asbestos, wood, chemicals, air pollution, etc. Although IPF is a disease that is limited to the lungs, numerous comorbidities have been increasingly recognized in patients with IPF, such as cardiovascular, pulmonary hypertension and ischemic heart disease, gastroesophageal reflux, lung cancer, chronic obstructive pulmonary disease/pulmonary emphysema, depression, sleep apnea, and diabetes [9].

Diagnosis of IPF is challenging because the initial symptoms are vague, non-specific, often mild, and may be attributed to advancing age or other diseases. Frequently the diagnosis is complex, requiring a multidisciplinary evaluation as recommended by international guidelines. The diagnosis of IPF continues to be a diagnosis of exclusion of other known causes for pulmonary fibrosis. High-resolution computed tomography (HRCT) plays a central role in the diagnosis of IPF. The presence of the HRCT pattern of usual interstitial pneumonitis is the hallmark of IPF diagnosis. In the case of the inconsistent pattern of UIP, significant inter-observer variability, surgical lung biopsy is necessary despite possible complications: triggering of the pneumothorax, pulmonary collapse, etc. Specific combinations of HRCT patterns and histopathology patterns in patients subjected to lung tissue sampling (transbronchial lung cryobiopsy or surgical lung biopsy) are an important part of the diagnosis.

In summary, the required criteria for diagnosing IPF is the combination of exclusion of known causes of ILD and presence of UIP pattern on chest HRCT or exclusion of known causes of ILD and specific HRCT/histology combinations. In the case of atypical HRCT presentation, lung biopsy is recommended. However, not all patients are eligible due to age and comorbidity limits. The average time from the symptoms' onset to the correct diagnosis is approximately 1.5 years [10–12].

Current guidelines also support the use of clinical, radiological, and physiologic evaluations to estimate IPF disease severity and predict disease progression [12]. These include quality of life questionnaires and quantitation of IPF exacerbation frequency; serial measurements of forced vital capacity (FVC), diffusing capacity for the lungs for carbon monoxide (DLCO), and 6-min walk test (6MWT) distances; and sequential HRCT scans when indicated. Composite scoring systems such as the Composite-Physiologic Index (CPI) and Gender Age Physiology (GAP) index, which incorporate demographic and physiological data, may represent more accurate prognostic models [13, 14].

IPF patients usually respond poorly to therapy. The treatment is based on the use of antifibrotic drugs (nintedanib or pirfenidone), which slow down the disease progression, but they do not significantly improve the survival of these patients. Lung transplantation is the only treatment option that increases survival in IPF. Early intervention may help improve clinical outcomes [15].

2. Biomarkers

A growing body of knowledge highlights IPF diagnosis, and providing accurate prognostic information is difficult using the currently available clinical, radiological, and physiologic findings. Furthermore, pulmonary function tests, clinical assessments, and imaging are very good for some cases, but not good for others. For example, lung biopsy is often not feasible in an elderly population with comorbidities, etc. [16].

With the development of new treatments for IPF, it is critical to identify patients at an earlier stage of disease and rapidly identify those patients who will progress to worse clinical outcomes. That's why there has been an emergence of molecular biomarkers. Compared to today's diagnostic methods, an optimal biomarker for discriminating patients with IPF from healthy subjects or non-IPF patients should be less invasive, more rapid, and reproducible, easier to obtain from patients.

At the same time, we are the witnesses that non-invasive biomarkers can provide very important information for the clinical assessment of patients. Although considerable advances have been made in the last decade in revealing IPF pathogenesis, this is not the case with IPF biomarkers. Similar to the previous guidelines, current existing guidelines such as 2021 German Respiratory Society (DGP), 2018 American Thoracic Society (ATS), European Respiratory Society (ERS), Japanese Respiratory Society (JRS), American Latin Thoracic Association (ALAT) guidelines strongly recommend not to measure any serum biomarker for IPF diagnosis and distinguishing IPF from other interstitial lung diseases in patients with newly detected ILD of apparently unknown cause who are clinically suspected of having IPF. Also, no guidelines on prognostic biomarkers are available [12, 17–20].

Although there is no molecular biomarker in widespread clinical use for IPF, advancements in this field have been achieved; a growing body of literatures indicates a fascinating field of IPF biomarkers has reported changes in the level of various biomarkers in IPF patients, which implies the potential to become a new tool for clinical practice of IPF.

IPF biomarkers include:

- a. predisposition biomarkers for identification of patients at risk for developing IPF
- b. diagnostic biomarkers for identification of IPF patients and differentiation of IPF patients from healthy controls or patients with other ILD or another lung disease
- c. prognostic biomarkers for staging disease severity, monitoring disease progression, herald worsening of IPF or the onset of an acute exacerbation or more accurate prediction of mortality
- d. therapeutic biomarkers that are a reliable measure of efficacy and safety during treatment

- e. biomarkers used as a surrogate endpoint in clinical trials helping predict clinical benefit based on epidemiologic/therapeutic/pathophysiologic evidence [21–23].

It is very well known that the ideal biomarker should be noninvasive, easily measured by a single, readily available test, to have high sensitivity/specificity, to be reproducible, accurate, widely available, and cost/effective [24].

Before considering the clinical implementation of the biomarker candidate, it must be evaluated critically with respect to key analytical and clinical characteristics. Criteria to be satisfied for definitive clinical implementation of biomarker related to the test such as adequate assays for its measurement, its predictive value defined in specific clinical contexts, optimal cut-off(s), and known timing of measurement (release kinetics) [25, 26].

Biomarkers should be measured from body fluids or tissues (serum, urine, exhaled breath condensates bronchoalveolar lavage fluid (BALF) transbronchial biopsy, surgical lung biopsy, etc.) with a recommendation to use easily obtainable body fluids or tissues. Although airway biomarkers could be obtained non-invasively via exhaled breath, is simple to collect and unlimited in quantity, most studies used bronchoscopy to obtain these biomarkers via BALF [27].

Additionally, incremental marker value should be examined, and the data about the effect on patient management and outcome and cost-effectiveness should be available. Also, validation across sexes, ages, ethnicities, and disease severity to assure generalizability is very welcome.

This chapter will summarize our current knowledge about IPF biomarkers associated with alveolar epithelial cell damage and dysfunction, biomarkers related to extracellular matrix remodeling and fibroproliferation, as well as biomarkers related to immune dysfunction.

3. Markers of alveolar epithelial cell damage and dysfunction

Markers that belong to this group are the most studied biomarkers and offer the most convincing data. The increase in serum levels of these markers can be attributed to an increase in the production of these proteins by regenerating alveolar type II cells and/or to an enhanced permeability following the destruction of the alveolar-capillary barrier [28].

3.1 Krebs von den Lungen-6 (KL-6) antigen

Krebs von den Lungen-6 (KL-6) antigen is a high molecular weight glycoprotein belonging to the group of human transmembrane mucins, expressed on type II pneumocytes, bronchial epithelium, as well as in glandular epithelium, including breast and pancreatic epithelium [22].

It was originally studied as a potential tumor marker in adenocarcinoma, whereas today's research is mainly based on KL-6 as a diagnostic and prognostic biomarker in ILD [22]. It shows marked inter-individual variability in serum levels.

Although few studies have revealed the KL-6 role as a diagnostic marker for IPF and found a higher value of KL-6 in patients IPF compared to controls. KL-6 was approved in Japan more than twenty years ago as a diagnostic biomarker in ILD [29].

Serum concentrations of KL-6 depend on the polymorphism of the MUC1 gene encoding its synthesis, which accounts for the different values in people of different ethnicities [29]. For these reasons, validation in the non-Asian population is necessary for this biomarker to be internationally used in patients with IPF [30].

However, KL-6 has been mostly studied as a prognostic biomarker. KL-6 values are predominantly increased in ILD, characterized by damage to AECs and progressive thinning of the interstitium, including IPF. A serum cut-off value of ≥ 1000 U/ml is associated with a poorer prognosis of patients with ILD and a higher risk of death [30].

KL-6 fluctuations in the follow-up of IPF patients have also been reported to be potentially useful in predicting functional disease progression [31]. Few studies examined the prognostic significance of serial measurements of KL-6 levels in IPF. Sokai et al. [32] found that serial measurements of serum KL-6 may provide additional prognostic information than physiological parameters in patients with IPF. Wakamatsu et al. [33] found that patients with both initial serum KL-6 values < 1000 U/mL and no serial increase in KL-6 had more favorable prognoses than those with serial increases in KL-6 or initial serum KL-6 values ≥ 1000 U/mL. Bennett et al. [34] revealed that higher KL-6 levels in BALF are related to the more severe and extended disease.

As previously discussed, the course of IPF varies widely, and some patients experiencing acute exacerbations of IPF, but the risk factors contributing to AE are unclear. It was noticed that basal values of KL-6 are significantly higher in patients who develop AE compared to patients with stable IPF [30]. Qui et al. [35], in systematic review and meta-analysis, investigated the risk factors for AE in IPF patients. The meta-analysis included seven articles involving 14 risk factors for AE in IPF patients, and poor pulmonary function, mechanical procedures, higher serum KL-6, and secondary pulmonary hypertension were associated with increased risks of AE in IPF patients.

Meta-analysis of 10 studies in IPF found that KL-6 had the strongest association with diagnosis of lung fibrosis compared with the three other examined markers (SP-D, SP-A, and MMP7) until for prognostic studies (decline in forced vital capacity and/or mortality) in IPF, KL-6 showed significant prognostic value [36].

Recently published systematic review and meta-analysis [37] was evaluated the robustness of available evidence for the use of KL-6 measurements in blood to predict prognosis in IPF patients. Twenty-six studies were included in the systematic review, and 14 studies were mainly performed on Asian patients in meta-analysis. The meta-analysis found that IPF patients with increased KL-6 concentrations had a significantly increased risk of developing AE, but the relation of KL-6 concentrations with mortality was not found.

3.2 Mucin 5B

Secreted mucins are the most abundant glycoprotein component of mucus. Secreted mucins (MUC2, MUC5AC, MUC5B, MUC6–8, and MUC19) are secreted into the extracellular space [38] MUC5B is among the major best-described, secreted gel-forming mucins. The main tissues expression of MUC 5B is; respiratory tract, submandibular glands, endocervix. Mucin 5B is one of the main components of respiratory secretions, and it participates in defense of the respiratory system from infections [39, 40]. However, the accumulation of this gel-forming glycoprotein further contributes to impaired gas exchange and complicates the clinical features of IPF patients [41]. The over-expression of mucin 5B in a study in mice showed a negative effect on mucociliary clearance, so inhaled harmful substances remain in the airways longer and initiate damage, and consequently tissue repair with fibrotic changes [42].

In 2011, a genome-wide linkage study identified a locus on chromosome 11 that was significantly associated with IPF risk. A common single nucleotide

polymorphism (SNP) (rs35705950) in the promoter of the gene encoding for Mucin 5B (MUC5B) is associated with an increased risk for IPF [43, 44]. Meta-analysis of Zhu et al. [45] revealed a strong association between the MUC5B promoter rs35705950 polymorphism and the risk of IPF, and confirmed that the minor T allele is significantly associated with an increased risk of IPF compared.

The same polymorphism has been associated with higher concentrations of MUC5B and its distribution, predominantly in the epithelial cells of small airways [46]. Mutations in this gene are not the only cause of increased mucin expression. Recent data indicate that increased DNA methylation is also associated with increased MUC5B expression [47]. This association has not been clarified yet and is certainly a topic for future research.

It was also shown that mucin 5B could be a good prognostic marker. Namely, the mutation in the promoter region of the MUC5B gene is associated with a lower risk of lethal outcome [48]. It has not yet been found how the same mutation leads simultaneously to an increased risk of disease. Yet, it is associated with a better prognosis and a higher degree of survival.

3.3 Oncomarkers

Certain similarities between IPF and lung cancer have already been identified. Both diseases primarily affect the lower parts of the lung lobes; risk factors such as smoking, exposure to harmful substances in the living and work environment, viral infections, and others are also common. There are also certain similarities in pathogenetic mechanisms, such as genetic and epigenetic changes, dysfunctions at the molecular and cellular levels, and activation of certain signaling pathways [49]. All the above indicates the possibility of using certain tumor markers in IPF when assessing the severity of the disease and predicting the outcome [50].

Carbohydrate antigen 19-9 (CA 19-9) is a marker of epithelial damage, widely used as a serum tumor marker of carcinoma of the pancreas and digestive system [51]. An increase in the concentration of this marker has been observed in patients with IPF, but the significance of determining it is still speculative.

Another widely used tumor marker that indicates the severity of the disease in IPF is CA 15-3. This glycoprotein, or the most significant tumor marker of breast cancer, is elevated in patients with pulmonary fibrosis. It is useful in predicting the severity of the disease, and after lung transplantation, there is a decrease in its concentration [50].

Carcinoembryonic antigen (CEA), a glycoprotein used as a serum tumor marker of colon, rectum, stomach, pancreas, lung, and breast cancer, also stands out as a useful marker in IPF [52]. The values of this analyte are elevated in IPF and are associated with the severity of the disease [52].

Yin and Lloyd [53] recently identified MUC16 as a transmembrane mucin corresponding to the CA125 antigen, long known as a marker for ovarian cancer. Recently, CA125 was identified as a serum biomarker for disease progression and death in IPF patients.

In the analysis from the PROFILE study, examining 123 serum proteins in IPF patients, Maher et al. [54] singled out primarily CA19-9, CA125, and SP-D as three markers with the greatest potential for routine use in clinical practice. Although these three biomarkers are all secreted in small amounts by the pulmonary epithelium in states of health, SP-D being secreted by alveolar type 2 cells and CA19-9 and CA-125 by the bronchial epithelium, they are secreted abundantly by the metaplastic epithelium of IPF patients. Mahler et al. [54] indicate that the potential of these parameters is reflected precisely in their ability to distinguish patients from healthy people (SP-D) reliably, predict disease progression (CA 19-9), and dynamically reflect

disease progression and overall mortality (CA 125) [54]. By examining the concentrations of CA19-9 in the final stage of IPF, Balestro et al. [55] got results consistent with previous research. Namely, most patients at this stage of the disease had CA19-9 values above the threshold (37kU/L). As confirmed by the results of several studies on different populations, CA19-9 is a reliable marker of disease progression [50, 54, 55].

The direct mechanisms of the increase in the concentration of tumor markers in idiopathic pulmonary fibrosis remain unclear. Nevertheless, research results are consistent in that these already widely used markers are useful in assessing the severity and progression of IPF [49, 50]. The great potential of these molecules is reflected, among other things, in the fact that they are already in routine use, as well as that there are commercial tests for their determination, unlike many of the aforementioned potential markers of the IPF.

3.4 Surfactant proteins

Surfactant proteins are lipoprotein complexes synthesized and then secreted exclusively by alveolar epithelial cells, bronchial epithelial cells, as well as Clara cells [56]. They are encoded by SFTPA, SFTPB, SFTPC, and SFTPD genes [57]. Their role is to reduce surface tension in the alveoli and prevent their collapse [58].

Surfactant proteins themselves, as well as mutations in the genes encoding these proteins, have been described as potential biomarkers in IPF [22]. Mutations in the genes for surfactant proteins (SP) C and A2 have been associated with the development of oxidative stress and damage to the endoplasmic reticulum, but an additional profibrotic stimulus is necessary to induce the development of pulmonary fibrosis [59–61].

However, SP-A and SP-D are the most studied surfactants in IPF, as well as surfactants studied for the longest time. The mechanisms by which SP-A and SP-D from pneumocytes enter the circulation are hyperplasia of AECs and thus increased synthesis of these proteins, and loss of AEC integrity i.e., increased permeability of the basement membrane of the pulmonary epithelium to the interstitium [58].

In the serum of patients with IPF, there was a significant increase in the concentration of SP-A and D, while in contrast, their concentration in BAL was lower compared to healthy, control subjects [58]. In addition, an increase in SP-D has been found in patients with acute exacerbations of the disease [62]. This surfactant protein may be useful in detecting patients who are more prone to disease progression and poorer outcomes [54]. There is evidence that SP-D is a biomarker that can be used for differential diagnosis of interstitial lung disease, as its level is higher in IPF than in other ILDs [63].

Wang et al. [64], in a meta-analysis of 21 articles, evaluated the use of serum SP-A and SP-D for differential diagnosis and prognosis of IPF. Serum SP-A levels were significantly higher in patients with IPF than in patients with non-IPF ILD. In the AE of IPF, serum SP-A/D was higher than those in the stable stage.

Studies, therefore, show that these proteins, as well as KL-6 and matrix metalloproteinase-7 (MMP-7), are predictive markers; however, in some studies, only SP-A and SP-D are independent predictors of mortality [65]. In addition, SP-D has proved to be a more sensitive marker than SP-A, with a sensitivity of 77% (SP-A sensitivity is 33%). However, these markers are not specific to IPF, but their increase is also observed in other interstitial lung diseases. Also, a study conducted in South Korea has shown that the application of these biomarkers in IPF, combined with clinical parameters, does not significantly contribute to the assessment of outcome compared to the application of clinical parameters alone. However, if KL-6 is included in the assessment, the contribution of biomarkers to clinical parameters becomes significant [65].

Compared with SP-A and SP-D in the serum of patients with IPF, the data for SP-B are limited. SP-B is a component of pulmonary surfactant, produced by alveolar epithelial cells, which is synthesized as a preproprotein [66]. The maturation process of this protein involves primarily the removal of the signal peptide, followed by the glycosylation of the C-terminal region, and finally, the cleavage of the N- and C-terminal propeptides [67]. Mature surfactant protein B is hydrophobic and strongly associated with phospholipids rich in surfactants. At the same time, its precursors, proSP-B, and C-proSP-B are more hydrophilic so that they can be found in the supernatant of bronchoalveolar lavage [68]. In healthy subjects, concentrations of both mature and SP-B precursors are almost undetectable in serum [69]. The study of Khan et al. [68] has been studied SP-B precursor, C-pro-SP-B, as a new biomarker in serum of patients with different chronic lung diseases, including ILDs. The highest C-proSP-B levels were detected in the serum IPF patients. In a multivariate analysis, C-proSP-B levels were able to discriminate IPF patients from patients with all other pulmonary diseases ($p < 0.0001$). SP-B pre-proteins might serve as a biomarker in pulmonary diseases with alveolar or interstitial damage in IPF.

3.5 Clara cell secretory protein (CC16)

Clara cells are exocrine bronchiolar cells with several different physiological functions, including a protective and regenerative role, as well as a role in maintaining pulmonary homeostasis [70]. These cells' protective and regulatory function is achieved through the secretion of various surfactants, glycosaminoglycans, enzymes, and other proteins [70]. In addition, these cells are involved in the biotransformation of many harmful substances that enter the lungs through the inhaled air [71].

CC16 is a 16 kDa homodimeric secretory protein of Clara cells with anti-inflammatory and antioxidant properties and has been studied as a potential therapeutic agent in various lung diseases [70]. It is encoded by the SCGB1A1 gene. Low serum CC16 values are associated with decreased lung function in children, accelerated decline in lung function in adults, and an increased risk of death, primarily in lung cancer [72].

In contrast, significantly high values of CC16 have been observed in the serum and bronchoalveolar lavage of patients with IPF [72]. Also, CC16 values are high in other interstitial lung diseases, such as sarcoidosis, although the values are significantly higher in IPF [72]. It is assumed that the activation of Clara cells after the alveolar epithelium damage leads to elevated serum concentrations of CC16. However, the exact role of CC16 in the alveolar repair process has not been thoroughly tested [70]. Although CC16 is a potential biomarker in various lung diseases, further studies are needed since CC16 values do not correlate with disease severity; there are no reference values, nor can it be used independently in diagnostics since it is a non-specific marker [70].

3.6 Telomeres

Telomeres are repetitive nucleotide sequences at the ends of chromosomes, whose role is to protect chromosomes from degradation [73]. As DNA polymerase cannot completely replicate the DNA strand, wherein a sequence of about 50 nucleotides is lost during each replication, the importance of telomeres is reflected in the fact that during replication, these non-coding parts of chromosomes are lost. The loss of telomere parts is compensated by the telomerase enzyme, which

incorporates guanine-rich sequences at the ends of chromosomes during cell replication. However telomeres become shorter during repeated replications, resulting in cell aging and apoptosis [74].

It has been found that approximately one-third of patients with familial IPF have shortened telomeres, and/or mutations in the gene encoding telomerases [75]. When examining telomere length in peripheral blood leukocytes in patients with IPF, it was found that 40% of patients with familial IPF and a quarter of patients with sporadic IPF have shortened telomeres, below the 10th percentile [76]. In a 2014 cohort study involving over three hundred patients with IPF, it was found that telomere length in peripheral blood leukocytes was an independent predictor of mortality [77]. It was also found that telomere shortening in peripheral blood leukocytes as a surrogate marker for telomere mutations, so telomere length in peripheral blood may be examined in the family of a carrier of these mutations, instead of carrying out genetic analysis, which would indicate a risk factor for familial IPF [78].

3.7 $\alpha v \beta 6$ integrin

Integrins are receptors found on the surface of cells, and they have a role in their binding to the extracellular matrix, in the interconnection of cells, and their migration, proliferation, and innate immune response [79]. Structurally they are heterodimers, made of different α and β subunits, and the $\alpha v \beta 6$ integrin itself consists of αv and $\beta 6$ subunits. The $\beta 6$ subunit is expressed only in epithelial cells, so the whole integrin is present only. This integrin is extremely important for the pathogenesis of IPF, as it can activate transforming growth factor beta (TGF- β), which is involved in the interaction of lung epithelial cells and fibroblasts [80]. In patients with IPF, higher concentrations of this integrin have been found in lung tissue [81]. Also, higher concentrations of integrin are associated with a poorer prognosis [82].

4. Markers of fibrogenesis and extracellular matrix remodeling

4.1 Matrix metalloproteinases (MMP)

Matrix metalloproteinases (MMP) are zinc-dependent proteases, which degrade the extracellular matrix. They can modulate the proliferation, migration, and apoptosis of smooth muscle cells, endothelial cells, and some types of immune system cells. So far, 23 members of this family have been discovered, encoded by 24 genes, where two genes serve to encode the same matrix metalloproteinase - MMP-23 [83]. Under physiological conditions, the activity of these enzymes, collectively called matrixins, is regulated at the level of transcription, activation of their inactive zymogen precursors, interaction with extracellular matrix components, and finally inhibition by endogenous inhibitors [84]. Matrixins are divided into seven categories: collagenases, gelatinases, stromelysins, membrane-type MMP, matrilysins, metalloelastases, and other types of matrixins [85].

Although MMPs are expected to prevent fibrotic changes due to their many functions and role in ECM degradation, these enzymes can have both a profibrotic and an antifibrotic role [85]. More details on members of the MMP-7 and MMP-1 matrix families, specifically elevated in the serum of patients with IPF, will be provided below.

4.1.1 MMP-7 (matrilysine)

This metalloproteinase is expressed in alveolar epithelial cells, phagocytes, and fibrocytes. An increase in MMP-7 levels has been observed in patients with IPF, and this enzyme has been confirmed as a biomarker of IPF [86]. The expression of this matrixin in the lung epithelium in IPF is further increased by osteopontin, a marker that will be discussed later [87]. Two SNPs have been identified in the promoter of the MMP-7 gene, which causes increased transcription, and are associated with the development of idiopathic pulmonary fibrosis [88]. In addition, as an enzyme that effectively removes tissue pathway factor inhibitor (TPFI), MMP-7 creates a procoagulant environment in the alveolar space, which has been observed in many fibrotic diseases, including IPF. Although this enzyme is also involved in the regeneration of lung epithelium after damage, in studies in mice lacking the MMP-7 gene, it was not possible to induce pulmonary fibrosis (PF) with bleomycin, suggesting that this metalloproteinase nevertheless promotes the development of PF [89]. This fact singles out MMP-7 as a potential new therapeutic target.

White et al. study tested the differentiation of IPF from a heterogeneous comparator group that included various other ILDs [63]. In another study, the serum MMP7 levels of IPF patients were compared to a group of patients with other ILD. Serum MMP7 values had a median sensitivity, specificity, accuracy, and diagnostic odds ratio of 71.7, 64.4, 68.4, and 4.7%, respectively [90]. MMP7 indicates a correct IPF diagnosis in more than half of the patients, suggesting an incorrect classification in about one-third of patients. Based on these data, the diagnostic value of these serum biomarkers is currently considered insufficient to support clinical use [17].

The Bosentan Use in Interstitial Lung Disease (BUILD)-3 trial that assessed potential prognostic capabilities of few biomarkers showed that MMP-7 is higher than healthy controls. Baseline MMP-7 levels were good predictors of worsening and could predict changes in FVC as early as month 4. MMP-7 shows the potential to be a reliable predictor of lung function decline and disease progression [91].

Despite the promising data regarding MMP-7 as a prognostic biomarker of IPF, it is not included in clinical practice due to the lack of reproducible, uniform cut-off values in different studies. There are major discrepancies between different studies about collection matrices; for example, EDTA collection tubes suppress MMP activity while PBMC layers are sometimes [10% of cases) contaminated by neutrophils, therefore significantly affecting predictive cut-off thresholds [92].

4.1.2 MMP-1 (collagenase type I)

This type of matrixin degrades the extracellular matrix collagen; it is not expressed in healthy tissue but during physiological and pathophysiological processes [87]. Along with MMP-7, MMP-1 is the most studied matrixin in IPF. The combination of these two matrixins in the diagnosis of IPF has a positive predictive value of up to 91% (for concentrations of MMP-7 > 2.6 ng/mL and MMP-1 > 8.9 ng/mL). Additionally, elevated values of these two MMPs can reliably distinguish IPF from other ILDs [86].

4.2 Osteopontin

Osteopontin (OPN) is an acidic phosphorylated glycoprotein secreted by various cells, including osteoclasts, activated T-lymphocytes, and activated macrophages [93]. Osteopontin is a multifunctional cytokine involved in various biological processes, including cell adhesion, chemotaxis, and reparative processes [87]. In this regard, the

biological role of osteopontin in the pathogenesis of cardiovascular diseases, diabetes, glomerulonephritis, and several types of cancer is suggested [93, 94].

The function of osteopontin in the occurrence of pulmonary fibrosis was tested in experimental mouse models, where the role in promoting the migration, adhesion, and proliferation of fibroblasts in the bleomycin-induced pulmonary fibrosis was demonstrated [93, 94]. In addition, analysis of lung biopsy samples of patients with IPF showed that osteopontin is a cytokine with the highest expression [93].

Osteopontin serum values are significantly higher in AE of IPF, compared to values in stable IPF, which is associated with a poorer prognosis [87, 95]. Although OPN is increased in serum and BALF of IPF patients [96], it is not specific in differentiating IPF from other ILDs [93].

The studies did not show the correlation between OPN concentration and SP-A and KL-6 concentrations, which can be explained by the different origins of these markers. Serum values of KL-6 and SP-A better reflect a later phase of the fibrosis process, i.e., the remodeling phase [93]. Although OPN values are highest in patients with IPF, no significant differences were observed compared to the values in patients with other ILD subtypes, indicating the limited use of this biomarker in differential diagnosis [94].

4.3 Periostin

Periostin is an extracellular matrix protein from the fascicline family, and it is involved in the pathogenesis of various diseases accompanied by increased levels of inflammation and fibrosis [97]. Studies have shown that periostin is a protein that is highly expressed in the lungs of patients with IPF [97, 98]. The highest level of periostin expression in the lungs is in fibroblasts, in the areas of active fibrosis [97]. Stimulation of periostin synthesis in fibroblasts is influenced by various factors, including TGF- β and IL-4/IL-13 [98]. Experimental mouse models have shown that suppression of the periostin gene or administration of neutralizing antibodies protects to a large extent against bleomycin-induced pulmonary fibrosis [99]. Also, periostin acts in cooperation with inflammatory cytokines, such as TNF- α , by activating NF- κ B, which is accompanied by the production of inflammatory cytokines and chemokines, leading further to the development of pulmonary fibrosis [97].

All this indicates the importance of the biological role of periostin in the pathogenesis of PF. However, elevated serum levels of periostin are also observed in other inflammatory diseases, which is why there is a need to develop a test that will enable greater diagnostic specificity [98]. There is a test designed to determine specifically periostin monomers, which is a better diagnostic marker compared to total periostin [98]. In addition, both total and monomeric periostin are better predictive markers of short-term deterioration of IPF compared to conventional markers KL-6, SP-D, and LDH [98]. The potential role of periostin in the treatment of patients with IPF should also be noted since experimental mouse models have shown that suppression of periostin expression or administration of neutralizing antibodies may result in improvement in the fibroproliferative phase [99].

4.4 Lysyl oxidase 2-like protein (LOXL2)

Lysyl oxidase (LOX) and lysyl oxidase-like proteins (LOXL) represent a group of enzymes with important roles in extracellular matrix remodeling, including covalent binding of elastin and collagen [100]. The LOXL proteins promote collagen accumulation and deposition, participating in ECM stabilization. In addition to the enzymatic function, LOX also has a function in regulating the transcription of elastin and collagen III genes [101].

Four LOX isoenzymes (LOX1-LOX4) encoded by genes located on different chromosomes have been identified [101]. Changes in LOX expression, i.e., increased LOX activity, have been associated with the mechanisms of fibrotic changes in certain lung, liver, and kidney diseases [101]. Increased LOX expression was observed in experimental mouse models in bleomycin-induced pulmonary fibrosis [101].

Also, elevated serum concentrations of LOXL2 in patients with IPF have been associated with a higher risk of disease progression but cannot be correlated with disease severity [101, 102]. Given its role in the pathogenesis of pulmonary fibrosis, the applicability of LOXL2 as a potential therapeutic target was examined. However, the study of the use of a monoclonal anti-LOXL2 antibody (sintuzumab) in the treatment of patients with IPF was discontinued in the second phase of the clinical trials due to the lack of efficiency [101]. One of the potential reasons for failure is the impossibility of adequate penetration into the lung tissue, but there were not enough data for a complete evaluation [101]. In any case, further testing of the diagnostic, predictive and prognostic value of LOXL2 as a biomarker in IPF is necessary.

4.5 Insulin-like growth factors and their binding proteins

IGFs are hormones or growth factors primarily synthesized in the liver. For the most part, they are bound to some of their binding proteins (IGF-BP), which modulate their effects and bioavailability [103]. The IGF binding protein family consists of six members, which also originate primarily from the liver. IGF and IGF-BP are synthesized locally in many tissues to achieve their autocrine and paracrine effects, respectively [104].

Studies have shown a significant increase in circulating concentrations of these binding proteins in newly diagnosed IPF patients. In contrast, in those patients who started using antifibrotic drugs, lower levels of GFBP-2 were found than in patients who do not receive this type of therapy [105]. IGFBP-2 values do not return to the levels of healthy subjects, even with the use of antifibrotic therapy [105].

As IGFs are very strong growth factors, their significant increase in the process of fibrosis, and even lung fibrosis, is expected. However, Guiot et al. [105] found a decrease in the concentration of these analytes in the serum of IPF patients. These surprising results can be explained in several ways. It is possible that IGF-BP, by binding to the extracellular matrix in the lungs with fibrotic changes, locally releases IGF and thus enables its effects in such an environment. On the other hand, an increase in the concentration of binding proteins to insulin-like growth factors means that these factors bind to a greater extent, thus reducing their effectiveness, which can also have a protective role in IPF [106–108].

4.6 Fibulin 1

Fibulin 1 (Fbln1) is a secretory glycoprotein with a significant role in embryonic morphogenesis and alveolar septal formation [109]. Four isoforms of this protein (Fbln1a/b/c/d) have been isolated, differing from each other in C-terminal sequences [110]. However, the identification of individual variants is difficult due to the unavailability of antibodies specific to certain isoforms [111]. Fbln1 has an important role in tissue repair and has been associated with several different respiratory diseases [111]. The importance of the Fbln1c form in the pathogenesis of various respiratory diseases is especially emphasized, which is achieved through the stimulation of fibroblast proliferation and remodeling of the extracellular matrix [110, 111]. Experimental mouse models have shown that the inhibition of Fbln1c expression reduces the proliferation of smooth muscle cells and fibroblasts and collagen deposition around the small airways [111]. In addition, mouse models have

shown a significant role of Fbln1c in chronic inflammation, where the inhibition of Fbln1c expression reduces the influx of inflammatory cells into the bronchoalveolar lavage and the synthesis of cytokines and chemokines in the lungs [111]. Accordingly, Fbln1 is mentioned as a potential biomarker and therapeutic target in respiratory and other diseases involving inflammation and remodeling [111].

Elevated values of Fbln1 in the serum and lungs of patients with IPF compared to healthy subjects suggest a role of Fbln1 in the pathogenesis of this disease [109]. High values of Fbln1 in the lungs are a consequence of increased production in smooth muscle cells and fibroblasts; apart from that, under the influence of TGF- β , exogenously synthesized Fbln1 is incorporated into the extracellular matrix [111]. The high serum concentration of Fbln1 correlates with decreased lung function and is associated with acute exacerbation of the disease [109, 112]. Fbln1 values are higher in patients with IPF compared to other ILDs. Still, they are in correlation with pulmonary function in other types of disease, suggesting that Fbln1 may be a predictive marker of disease progression in other ILDs, such as idiopathic nonspecific pneumonia [109].

4.7 Neopeptides

Excessive deposition of the extracellular matrix is critical to the pathogenesis of IPF. Collagen is the main component of the extracellular matrix, whose synthesis and degradation take place in a balanced way in healthy lungs, while in IPF, this balance is disturbed [113, 114]. During synthesis, the procollagen is cleaved, and during the degradation of collagen molecules, MMPs cut parts of this molecule, which reveals different neopeptides in all these processes [115].

Peptides formed during synthesis and newly formed neopeptides are released into the circulation and detected in the blood. Studies have shown that serum concentrations of neopeptides of collagen synthesis PRO-C3 and PRO-C6 (collagen type 3 and type 6) are higher in patients with IPF compared to healthy subjects of the same age. Their elevated concentration is associated with IPF progression [115]. The concentration of collagen degradation markers (C1M, C3M, C6M, and CRPM) is also elevated in IPF. Longitudinal changes in serum concentrations of these neopeptides follow the progression of fibrosis and can predict mortality in individuals with IPF in three months [116]. Biomarkers of collagen synthesis and degradation have the potential to improve clinical trials in IPF, prognostic evaluation, and make decisions on therapy [115].

4.8 Heat shock protein 47 (HSP47)

HSP47 is a protein necessary for the synthesis and secretion of collagen molecules. Increased expression of HSP47 is closely related to excessive production and accumulation of collagen, so these data indicate a significant role of this molecule in fibrotic processes and its correlation with the activity of such diseases. It has been shown that a significant increase in the concentration of HSP47 occurs during the acute exacerbation of the disease, compared to the stable form of IPF. Additionally, this biomarker has been found to be superior to better known and studied markers of pulmonary fibrosis, such as KL-6 and SP-A and D [117]. It was assumed that, as HSP47 concentrations in the exacerbation phase of the disease are higher than during stable disease, this distinction would also exist between patients with a stable form of the disease and healthy subjects. However, these assumptions have been refuted in the research conducted [117, 118].

The precise role of HSP47 in the pathogenesis of IPF has not been determined, but this molecule is likely responsible for the additional effect of pirfenidone in the

inhibition of fibrotic processes. In addition to direct suppression of type I collagen expression, it is possible that pirfenidone partially achieves its anti-fibrotic effect by suppressing the expression of HSP47 depending on TGF- β 1 [119].

4.9 Circulating fibroblasts and fibrocytes

The lungs are characteristic of IPF patients in the regions of the so-called fibroblast foci, where ECM production is most active. In these foci, the predominant cells are myofibroblasts, where under the effect of various cell mediators, the proliferation of these cells takes place, with the inhibition of their apoptosis [120]. Myofibroblasts are cells that phenotypically correspond to the stage between fibroblasts and smooth muscle cells [121].

There are two hypotheses on the origin of myofibroblasts: traditional – that they are formed from fibroblasts after their activation by inflammatory stimuli and more recent – that they are formed by differentiation of alveolar epithelial cells [122].

Fibrocytes are cells originating from the monocytic lineage. In case of tissue damage, migrate to the site of damage attracted by chemotactic factors and then differentiate into fibroblast-like cells. They are present in the circulation and can produce ECM. Fibrocytes express different markers, and these are primarily CD45 leukocyte markers and type I collagen. During its differentiation, it has been found that CD45 expression gradually decreases while type I collagen expression remains unchanged. It has also been found that their differentiation is accelerated under the effect of TGF- β [123]. Although they have a protective role in the process of tissue remodeling and damage repair, it is considered that fibrocytes are involved in the progression of pulmonary fibrosis. Studies show that in the blood of IPF patients, an increased number of circulating fibrocytes is associated with a poor disease outcome [124, 125]. It has been found that, compared to healthy subjects, in patients with IPF, there is a significantly higher number of circulating fibrocytes, identified precisely as CD45+, collagen type I+ cells. In addition, in patients with AE of the disease, these cells are present in ten times greater numbers than in the case with a stable state [125].

5. Markers of immune system dysfunction and inflammation

Although IPF is primarily not an inflammatory disorder, inflammatory and immune-mediated pathways are activated in IPF patient's lungs.

5.1 CC chemokine ligand 18 (CCL18)

CC chemokine ligand 18 (CCL18) is a protein secreted by myeloid lineage cells: monocytes, macrophages, and dendritic cells. In patients with idiopathic pulmonary fibrosis, alveolar macrophages produce large amounts of CCL18 [126, 127]. Th2 cytokines lead to alternative activation of alveolar macrophages, which thus activated have a role in tissue and fibrosis healing [128, 129]. Alternatively, activated macrophages produce CCL18, which leads to increased collagen production by pulmonary fibroblasts, and collagen then stimulates alveolar macrophages to produce CCL18 by a positive feedback loop. In this way, the process of fibrosis is continuously maintained [126].

Increased serum concentrations of CCL18 in IPF are negatively correlated with pulmonary function tests and associated with disease progression [126, 127]. In a prospective study of 72 patients, significantly higher mortality was observed in the group of patients with a CCL 18 concentration above 150 ng/mL [130]. It was

also found that pirfenidone used in the treatment of IPF significantly reduces the expression of CCL18 in macrophages [130].

Data obtained from meta-analysis Elhai et al. showed that CCL18 has a significant prognostic value [36]. Based on previous research, it can be concluded that CCL18 is a good prognostic marker in IPF.

In a posthoc analysis of phase 3 ASCEND and CAPACITY trials [131], concentrations of IPF biomarkers in IPF patients who received pirfenidone 2403 mg/day or placebo were investigated, and their associations with changes in FVC and disease progression over one year. CCL18 was consistently prognostic for absolute change in percentage of FVC% and was the most consistent predictor of disease progression across IPF cohorts.

5.2 CC chemokine ligand 2 (CCL2)

CC chemokine ligand 2 (CCL2) is one of the chemokines involved in the recruitment of mononuclear phagocytes, thereby promoting inflammation and the development of tissue fibrosis [132]. Additionally, the recruitment of fibrocytes into the lungs most likely occurs because of interactions between chemokine ligands (including CCL-2) and their receptors [133]. More than 20 years ago, it was established that significantly higher serum concentrations of this chemoattractant are present in patients with idiopathic pulmonary fibrosis [134]. A recently published paper, which focused on examining the prognostic potential of various chemokines, found significantly higher concentrations of CCL-2 in patients with both acute exacerbations of IPF and a stable form of the disease, compared to a control group of subjects [135]. The same study concluded that CCL2 levels, among other chemokines, showed neither correlation with lung function nor patient survival [135].

5.3 CXC chemokine 13 (CXCL13)

CXC chemokine 13 (CXCL13) is a protein secreted by dendritic cells and the main mediator in attracting B lymphocytes to inflammatory lesions. Antigen-stimulated B lymphocytes undergo a process of gradual maturation, so these cells, as well as altered, differentiated B lymphocytes, are present in patients with IPF [136]. Increased CXCL13 mRNA has been isolated in the lungs of patients with IPF compared to control subjects, and serum levels of CXCL13 were increased in patients with IPF compared to control subjects. Elevated CXCL13 protein levels are associated with increased mortality in patients with IPF. The highest levels of CXCL13 were found in IPF patients with acute exacerbations or pulmonary hypertension [137].

5.4 Toll-like receptor 3

The toll-like receptor is a transmembrane glycoprotein receptor expressed predominantly endosomal. Recent studies show an association between Toll-like receptors and aberrant fibrogenesis characteristic of idiopathic pulmonary fibrosis [138]. These receptors recognize molecular patterns that can be potentially dangerous and promote adequate immune response [138]. The Toll-like receptor 3 L412F polymorphism is associated with defective TLR3 activation, which causes mortality in IPF [139]. The association of this mutation with accelerated decline in lung function and consequent early death has been proven. This information can be critical in identifying patients with a rapidly progressive phenotype [140]. Toll-like receptor 3 belongs to the group of receptors that have a significant role in innate immunity. It mediates the innate immune response to tissue injury or infection by inducing NF- κ B activation and type 1 interferon production [141]. Toll-like

receptors recognize patterns from bacterial, viral, protozoal, and fungal pathogens, which are most important for their survival [141]. The Toll-like receptor 3 is a receptor that recognizes viral double-stranded RNA (dsRNA) and regulates the pro-inflammatory response and IFN-1 production [142]. In studies on fibroblasts in IPF, the unregulated proliferation of primary fibroblasts was observed and decreased production of IFN- β mediated by TLR3 receptors [139]. Activation of TLR3 receptors in primary fibroblasts has an antifibrotic effect and leads to a decrease in TGF- β production, increased collagen production, and increased metalloproteinase activity [143, 144].

The TLR signaling pathway during the reactive response to viruses acts as a blocker of fibroproliferation, so TLR3 signaling deficiency can cause an inadequate lung response to viral pathogens and expose them to chronic cycles of damage and repair considered the basis of IPF pathology [144].

5.5 Toll-interactin protein (TOLLIP)

Toll-interactin protein (TOLLIP) is a protein whose expression in the lungs has been observed in type II alveolar cells, macrophages, and basal cells. This protein has a role in important signaling pathways associated with lung diseases, including IL-1 β , IL-13, TLR, and TGF- β [145].

It has been found that the rs111521887 and rs5743894 gene variants located in TOLLIP introns are associated with 40–50% reduced TOLLIP gene expression in the lungs and susceptibility to IPF [146]. Interestingly, the rs5743890_G allele is related to increased mortality in IPF, although it is associated with decreased IPF susceptibility, which suggests that the genetic basis is related to different clinical outcomes [39]. This indicates the heterogeneity and complexity of the pathogenesis of IPF [146]. TOLLIP is an important regulator of innate immune responses mediated by Toll-like receptors and the TGF- β 1 signaling pathway through TGF- β 1 receptor degradation [92]. It antagonizes the TGF- β signaling pathway by degrading the TGF- β 1 receptor [147]. This TLR inhibitory protein is potentially useful for detecting various responses to the treatment of IPF in different genotypes [148].

Decreased TOLLIP expression increases proinflammatory cytokines IL-6 and TNF production in macrophages after TLR stimulation [149]. These data suggest that TOLLIP expression may be protective by reducing the proinflammatory and profibrotic cascade [144].

5.6 Defensins

Defensins are small antimicrobial peptides mainly secreted by neutrophils and epithelial cells, which affect some gram-positive and gram-negative bacteria, as well as viruses [92]. Comparative analysis of gene expression from blood and lung tissue samples of patients with stable IPF and those with acute exacerbation of IPF revealed increased gene expression for alpha-defensins 3 and 4 in IPF with acute disease exacerbation [150].

Alpha-defensins are activated by MMP7, whose gene expression is also increased in the lungs of patients with IPF [22]. It has been found that serum levels of alpha defensin are higher in patients with IPF than in healthy subjects and are associated with the deterioration of the disease [150, 151].

5.7 S100A4

S100 calcium-binding protein A4 (S100A4, fibroblast-specific protein-1) belongs to the S100 family containing calcium-binding motifs. S100A4 promotes

lung fibrosis via proliferation and activation of fibroblasts and promotes the transition of fibroblasts to myofibroblasts [152].

Akiyama et al. [153] have shown the clinical significance of serum S100A4 in IPF patients. They revealed an independent association of higher S100A4 levels with a higher disease progression rate and a higher mortality rate, suggesting that S100A4 may be promising in the prognosis and management of IPF. The presence of higher levels of S100A4 in the serum of participants with IPF was linked with a significantly lower progression-free survival and higher 2-year mortality.

5.8 S100A8/A9

S100A8/A9 belongs to the S100 family of calcium-binding proteins derived from neutrophils and monocytes, which modulate the immune response [154]. In the pathogenesis of pulmonary fibrosis, the role of these proteins is based on the proliferation of fibroblasts, the influence on their differentiation, and the increase in collagen production by mentioned cells [155]. Concentrations of S100A8 and A9 are, as recent research results show, significantly higher in patients with acute disease exacerbation than in healthy patients, as well as in patients with confirmed IPF without acute exacerbation [156]. Patients with higher concentrations of these two biomarkers had a significantly poorer three-month survival rate, so S100A8 and S100A9 proved to be significant prognostic markers [156].

5.9 S100A12

S100A12 is a member of the S100 family of calcium-binding proteins that has a significant role in regulating inflammatory processes and immune response. Its proinflammatory activity includes chemotaxis and activation of the intracellular signaling cascade, leading to cytokine and oxidative stress production [157]. In a study with a relatively large number of patients with IPF, serum concentrations of S100A12 in IPF were high and correlated with poor disease prognosis [158].

5.10 Anti-heat shock protein-72 antibodies (AHSP-72)

HSP production is regulated by various stress effects on cells, as well as their damage. They are located on the cell surface and have a role in transmitting information and modulation of the immune response [159]. Various autoantibodies to HSP have been found in patients with autoimmune diseases. What singled out HSP and autoantibodies to these proteins as potential biomarkers in IPF is, inter alia, the fact that cell cultures have been found to have the ability to activate monocytes and increase IL-8 production by these cells [158, 160]. IL-8, as a pro-inflammatory chemokine, further acts as a chemoattractant on neutrophils and activates them [161]. This interleukin is considered one of the major mediators in the pathogenesis of IPF, and its higher serum concentrations and BAL of these patients are associated with more extensive pulmonary fibrosis [162].

The results of a study conducted by Mills et al. indicate that IPF patients did not show a significant increase in serum antiHSP-72 antibodies compared to healthy subjects, nor did the concentration of the identical immunoglobulins differ between IPF and other interstitial lung diseases. However, in the bronchoalveolar lavage, an increase in the concentration of total antibodies (classes G, A, and M), but not of class G itself, is associated with a better disease outcome, i.e., it was observed in patients with slower disease progression [163]. These results contrast with the data from the previous study, which showed that the increase in the concentration of autoantibodies to HSP-70 in IPF patients was associated with a poor disease

outcome [164]. This discrepancy in the conclusions can be justified by applying different methods, i.e., the antigens used to isolate antibodies and the non-homogeneous groups in the research of Kahloon et al. in terms of age, gender and ethnicity. It is undeniable that these proteins and autoantibodies directed at them have their place in the pathogenesis of IPF, but further research is needed to elucidate the role and potential use of these biomarkers in pulmonary fibrosis.

5.11 YKL-40

YKL-40 is a glycoprotein, a member of the chitinase and chitin-like protein family, expressed in many tissues, especially those characterized by high metabolic activity [165]. The exact biological role of YKL40 is not fully known, but it is involved in various pathophysiological processes as an inflammatory glycoprotein, including cell proliferation, migration, and tissue remodeling [166].

YKL-40 is mainly expressed in alveolar epithelial cells and macrophages, and its values are elevated in the serum and lungs of patients with IPF [165]. In addition, high concentrations of YKL-40 are detected in other diseases accompanied by high levels of fibrosis, such as liver cirrhosis, Crohn's disease, and systemic sclerosis [165]. Elevated levels of YKL-40 in serum and bronchoalveolar lavage are associated with a higher risk of death in patients with IPF, although there is a weak correlation between these concentrations [104]. Also, YKL-40 values are inversely related to lung function in asthma, sarcoidosis, and IPF [165]. YKL-40 is not a marker specific for IPF, although the cut-off value of 79 ng/ml is mentioned in the literature and associated with a poorer prognosis [105]. Compared to the short-term prognostic markers SP-D and CCL18, YKL-40 has the highest predictive value 3–4 years after diagnosis, so a potential combination of these markers could allow a better assessment of survival [165].

5.12 Vimentin/anti-vimentin antibodies

Vimentin is a cytoskeleton protein in cells of mesenchymal origin which is considered responsible for increased cell invasiveness so that one can assume its importance in fibroblast invasion into the so-called fibrous foci in the lungs of IPF patients [167]. This filament is essential to the process of wound healing, so its overexpression results in increased cell invasiveness and excessive scar tissue formation [167, 168].

Immunochemical staining of tissue samples from IPF patients showed that vimentin was significantly more expressed in the cells at the periphery of the fibrous focus than in the center. In the same study, it was found that in the fasting state, as an inducer of the autophagy process, fibroblasts originating from IPF patients expressed vimentin more than control group fibroblasts, while the process of autophagy was lacking [169].

The defect of the autophagy process has already been associated with the development of idiopathic pulmonary fibrosis, where there is no removal of parts of the extracellular matrix by their implementation in autophagosomes and the destruction of these products after fusion with lysosomes [170, 171].

The antiangiogenic, as well as the antitumor agent WFA (withaferin A), can bind to vimentin, covalently modify it, and cause its aggregation [172]. Treatment of IPF fibroblasts with this agent increased the number of autophagosomes in these cells, i.e., it stimulated autophagy. In addition, the expression of vimentin and type I collagen were reduced, and the inhibition of vimentin reduced the invasiveness of fibroblasts [169]. All these facts confirm the role of vimentin in pulmonary fibrosis and its importance in the progression of the disease.

Various cells involved in the development of pulmonary fibrosis secrete vimentin under the influence of TGF- β 1 [173]. This secreted cytoskeletal protein was found in significantly higher IPF patients than in the healthy, control group [174]. Over-expression of otherwise immunologically inert molecules leads to their higher immunogenicity [175]. This is confirmed by the results of a 2017 study that proved anti-vimentin autoantibodies in IPF patients in a much higher concentration than is the case with other lung diseases and healthy subjects. Patients with poorer clinical and poor disease outcomes had higher circulating concentrations of anti-vimentin antibodies features [174].

5.13 T-lymphocytes

As mentioned above, the central event in the development of IPF is an excessive reaction to repeated damage to alveolar epithelial cells with the formation of scar tissue that replaces the functional one [176]. Pulmonary fibrosis was considered a non-immune disease, but more and more evidence speak in favor of the role of the immune system in initiating the onset of fibrotic changes, as well as in the progression of fibrosis.

Regulatory T-lymphocytes are CD4⁺ T-cells that participate in immunosuppression and prevent the development of an immune response to the body's antigens (autotolerance) [177]. These cells can produce various cytokines, including IL-10 and TGF- β 1, and therefore may have the potential to both suppress and promote the onset of fibrotic changes [148].

Activation of these T-lymphocytes increases the expression of semaphorin seven, which has a chemotactic effect on macrophages, stimulates the production of proinflammatory cytokines, and regulates collagen production by fibrocytes [178]. Increased expression of semaphorin seven on regulatory T cells has been found in IPF [179].

The cell population of Th2 lymphocytes (T-helper cells) and their product IL-13, which have long been known to have a role in allergic diseases and the pathogenesis of asthma, are now also associated with the development of IPF. Namely, this interleukin affects the extracellular matrix production and induces tissue fibrosis, which has been shown in animal models, where increased expression of IL-13 had profibrotic effects [180]. Studies show an increased concentration of this cytokine in the blood of patients with IPF and the correlation of these concentrations with disease progression [181]. These claims are consistent with the results of studies performed on mice lacking the IL-13 gene in which the induction of pulmonary fibrosis by bleomycin was inhibited [182].

5.14 Soluble receptor for advanced glycosylated end products (sRAGE)

The soluble receptor for advanced glycation end-products (sRAGE) acts as a decoy for capturing advanced glycation end-products (AGEs) and inhibits the activation of the oxidative stress and apoptotic pathways. The study of Manichaikul et al. [183] found that adults with IPF have lower sRAGE levels. They were associated with greater disease severity and a higher death rate or lung transplant at one year compared with healthy controls. Additionally, lower plasma sRAGE levels in patients with IPF and other ILDs when compared with healthy controls Lower sRAGE levels were associated with disease severity. In their study, Cabrera Cesar et al. [184] provide evidence, for the first time, for the possible use of AGE as a differential diagnostic biomarker to distinguish between IPF and connective tissue disease-associated interstitial lung disease (CTD-ILD). The role of RAGE in human and experimental models of IPF did not fully understand [185].

Machahua et al. [186] evaluated the AGEs, and sRAGE levels in serum as a potential biomarker in IPF, demonstrate that the increase of AGE/sRAGE ratio is higher in IPF. AGE/sRAGE increase correlates with respiratory functional progression (FVC and DLCO values); changes in serum AGEs and sRAGE correlated with % change of FVC, DLCO, and TLC during the follow-up.

No difference in AGE or RAGE expression was observed in lungs with non-specific interstitial pneumonia compared to that in the controls. Levels of circulating AGEs also increased significantly in the lungs of patients with IPF compared to those with NSIP and normal control [187].

6. Markers of endothelial damage

Aberrant angiogenesis is implicated in the pathogenesis of pulmonary fibrosis, and mediators of this process are VEGF, endothelin 1, interleukin 8.

6.1 Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor-A (VEGF-A) is the predominantly expressed member of the VEGF family and is often denoted as VEGF. It is a tyrosine kinase glycoprotein and is one of the most potent factors that stimulate angiogenesis. VEGF is elevated in IPF compared with healthy controls [137, 188].

Barratt et al. [189] report that the levels of VEGF-A165b protein were found to be dramatically elevated in the lung tissue of patients with IPF, is produced mostly by the alveolar epithelium but also by macrophages, lymphocytes, and fibroblasts.

Ando et al. reported reduced VEGF-A in the BALF of IPF patients compared to controls [190]. VEGF-A levels in peripheral blood are associated with the severity and progression of IPF [191]. Enhanced expression of VEGF-A is correlated with increased alveolar-capillary density in non-fibrotic regions of IPF lungs [192].

Nintedanib, therapeutics for IPF, acts by targeting VEGF receptor signaling, slows IPF progression, but the utility of VEGF as a marker of treatment success is not determined [193, 194].

6.2 Endothelin 1 (ET-1)

Endothelin-1 (ET-1) is a vasoactive peptide that plays a central role in lung fibrosis. ET-1 drives fibroblast activation, proliferation, differentiation into myofibroblast - processes that lead to excessive collagen deposition [195]. Barlo et al. [196] revealed that ET-1 in serum was significantly increased in IPF patients compared with healthy control subjects until it was significantly decreased in bronchoalveolar lavage fluid (BALF).

6.3 Interleukin-8 (IL-8)

Interleukin-8 (IL-8) is produced by phagocytes when exposed to inflammatory stimuli and promotes angiogenesis [191]. IL-8 levels were significantly higher in IPF exacerbated patients, and an increase in IL-8 by one pg/ml increases the odds of death by 6.7% in IPF patients [197]. Schupp et al. [198] found significantly higher levels of IL-8 in BAL samples from IPF-AE patients compared to stable IPF patients. Xaubet et al. [199] found that the percentage of IL-8-positive bronchoalveolar lavage macrophages was significantly higher in areas of IPF lung with extensive fibrosis defined by HRCT scans compared with BALF from healthy volunteers.

7. Multimarker approach

The literature supports the concept of combining multiple markers and/or clinical parameters in clinical decision support. Biomarker panels consisting of two or more suspected biomarkers may potentially indicate a higher likelihood of IPF than any single biomarker, more effectively differentiate IPF patients from healthy volunteers and patients with other pulmonary diseases, define prognosis at the time of diagnosis, identify responses to therapy.

For example, the improved predictive value of the combination of biomarkers SP-A and SP-D in IPF was observed [200]. Rosas et al. [86] found that the combination of serum MMP1 and MMP7 levels distinguish IPF from other chronic lung diseases more than either protein on its own. Also, the combination of five proteins (MMP-7, MMP-1, MMP-8, Insulin-like Growth Factor Binding Protein 1 (IGFBP1) and tumor necrosis factor receptor superfamily, member 1a (TNFRSF1A)) could distinguish with high sensitivity and specificity IPF patients from normal controls. White et al. [63] showed that a combined serum biomarker panel combining SP-D, MMP-7, and osteopontin differentiated IPF patients from other types of ILD (except for rheumatoid arthritis-associated ILD) more readily than each biomarker, and this biomarker index may improve diagnostic confidence in IPF. Hamai et al. [201] found that a combination of MMP-7 and KL-6 potentially support the diagnosis of IPF and might improve survival prediction in patients with IPF. Recently published study Xue et al. [202], found that KL-6, CCL3, and CXCL13 significantly improves the diagnosis of idiopathic interstitial pneumonia. IPF patients with a high level of SP-D but low KL-6 in their serum had a better prognosis [203]. A panel of mi-RNAs including miR-302c, miR-423, miR-210, miR-376C, and miR-185 has been shown to be associated with disease severity, differentiating fast from slow IPF progressors [204].

The next step was to examine the combination of clinical parameters and molecular biomarkers to achieve more accurate results regarding the prognosis of IPF. Kinder et al. [84] reported on a significant improvement in their prediction model of 1-year mortality in surgical lung biopsy-proven IPF, when serum levels of SP-A and SP-D were added to the clinical predictors of mortality alone [205]. Richards et al. [206] evaluated a panel of 92 proteins in a retrospective derivation cohort of IPF patients and tested significant findings in an independent validation cohort of IPF patients, and identified five biomarkers (MMP-7, intercellular adhesion molecule-1, Interleukin-8, vascular cell adhesion protein -1, S100A12) associated with disease progression or mortality. Combining clinical parameters and plasma protein concentrations (gender, FVC%, DLCO%, MMP-7), they constructed peripheral blood risk index-PCMI, distinguishing high and low mortality risk subgroups in the derivation was accurately predictive of mortality in the validation cohort. Song et al. [65] found that the predictive model of survival includes biomarkers (MMP7, SPA, KL6) and clinical variables (FVC%, DLCO%, age, change in FVC at six months) is better than the model based on clinical parameters.

Herazo-Maya et al. [207] have recently identified a 52-gene signature in peripheral blood mononuclear cells of patients with IPF, and further validated in six different cohorts of patients with IPF. They developed a SAMS (Scoring Algorithm for Molecular Subphenotypes) risk scoring system based on the 52-gene signature. Applying SAMS, low risk and high-risk groups of IPF patients with significant differences in outcome (mortality or transplant-free survival). This 52-gene signature could be valuable in predicting response to therapy.

In testing the idea that a combination of clinical and biological parameters can improve IPF patients' outcomes prediction, Adegunsoye et al. [208] derived a

clinical-molecular risk (CMR) score (CA-125, MMP7, YKL-40, OPN, age, and percent predicted FVC) for treatment exposed patients. They found that a clinical-molecular signature of IPF transplant-free survival may provide a reliable predictor of outcome risk in anti-fibrotic treated patients. This risk score may help identify individuals at risk of poor outcomes despite antifibrotic initiation and open the discussion of the application of CMS risk score before initiation of antifibrotic therapy to identify patients warranting closer clinical monitoring or earlier lung transplant referral [209].

8. Conclusions

Within the last decade, a broad range of molecular biomarkers for IPF has been reported. Until now, despite a large number of publications about IPF biomarkers, their use in routine is not recommended in international clinical practice yet. The successful translation of molecular biomarkers into clinical practice requires validation in large, multi-center, prospective studies with long-term, longitudinal follow-up, standardization of assays, serial measurements of biomarkers, and interventional trials that show changes related to clinical IPF state.

However, most data about IPF biomarkers originate from small-sized, single-center studies of the retrospective design, cross-sectional with measurements at a single time-point, and often in Asiatic cohorts of patients where their use is more common. This raises questions about the generalizability of the results obtained in Asiatic cohorts as well as about the determination of an optimal cut-off. Their accuracy should also be confirmed in non/Asiatic, Caucasian cohorts to routinely apply them in the management of IPF.

Furthermore, diagnostic criteria for IPF have recently changed, and most of the studies published before did not systematically use HRCT or histology. However, using these stringent criteria, confident data regarding biomarkers value could be obtained. Also, the gold standard for measuring disease activity is missing.

The validation of useful and accurate diagnostic markers could reduce uncertainty and the use of the invasive procedure. Inter-assay disagreement can represent a confounding factor in the interpretation of test results in different studies, and the definition of an optimal cut-off is very important.

Finally, as already touched on in the chapter, investigators are resorting to panels of multiple biomarkers to differentiate IPF patients more effectively from healthy volunteers or patients with other pulmonary diseases. The use of a biomarker index composed of multiple biomarkers already studied separately, with the aim of improving diagnostic accuracy in distinguishing IPF from other ILDs or healthy controls, is promising.

There is evidence of extremely strong genetic association in IPF. Recent advances in genetic sequencing and bioinformatics have made it much easier to detect genetic variants rapidly. It seems that in the near future, we will be able to analyze genetic markers to gain prognostic information for IPF patients or help screen at-risk patients with a familial history that do not exhibit signs or symptoms of IPF.

The utilization of high-throughput sequencing to detect microbial and/or viral genetic material in bronchoalveolar lavage fluid or lung tissue samples has amplified the ability to identify and quantify specific microbial and viral populations [210].

Use of liquid biopsy, which allows the isolation of circulating cell-free DNA from blood, could be very important in the discrimination of patients affected by IPF from those with other ILDs [211].

Discovery, validation, and implementation of clinically useful molecular biomarkers discovered through omics (genomics, epigenomics, transcriptomics, proteomics, and metabolomics) will facilitate precision medicine in IPF [212–214].

Soon, we expect the results of many clinical trials evaluating as primary or secondary outcomes known and new biomarkers that will convince clinicians of the value of using biomarkers at multiple stages of the diagnosis and personalized management of IPF.

Conflict of interest

The authors declare no conflict of interest.

Author details

Sanja Stankovic^{1,2*}, Mihailo Stjepanovic^{1,3} and Milika Asanin^{1,3}

1 University Clinical Center of Serbia, Belgrade, Serbia

2 Faculty of Medical Sciences, University of Kragujevac, Serbia

3 Medical Faculty, University of Belgrade, Serbia

*Address all correspondence to: sanjast2013@gmail.com

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M, et al. Idiopathic pulmonary fibrosis. *Nat Rev Dis Primer* 2017;3:17074. doi: org/10.1038/nrdp.2017.74.
- [2] Olson AL, Gifford AH, Inase N, Fernández Pérez ER, Suda T. The epidemiology of idiopathic pulmonary fibrosis and interstitial lung diseases at risk of a progressive-fibrosing phenotype. *Eur Respir Rev.* 2018 ;27(150):180077. doi: 10.1183/16000617.0077-2018.
- [3] Duchemann B, Annesi-Maesano I, Jacobe de Naurois C, Sanyal S, Brillet PY, Brauner M, et al. Prevalence and incidence of interstitial lung diseases in a multi-ethnic county of Greater Paris. *Eur Respir J.* 2017;50(2):1602419. doi: 10.1183/13993003.02419-2016.
- [4] Wuyts WA, Dahlqvist C, Slabbynck H, Schlessner M, Gusbin N, Compere C, et al. Baseline clinical characteristics, comorbidities and prescribed medication in a real-world population of patients with idiopathic pulmonary fibrosis: the PROOF registry. *BMJ Open Respir Res.* 2018;5(1):e000331. doi: 10.1136/bmjresp-2018-000331.
- [5] Kulkarni T, Duncan SR. Acute exacerbation of idiopathic pulmonary fibrosis: who to treat, how to treat. *Curr Pulmonol Rep.* 2019 ;8(4):123-130. doi: 10.1007/s13665-019-00238-7.
- [6] Sgalla G, Iovene B, Calvello M, Ori M, Varone F, Richeldi L. Idiopathic pulmonary fibrosis: pathogenesis and management. *Respir Res.* 2018;19(1):32. doi: 10.1186/s12931-018-0730-2.
- [7] Moua T, Lee AS, Ryu JH. Comparing effectiveness of prognostic tests in idiopathic pulmonary fibrosis. *Expert Rev Respir Med.* 2019 ;13(10):993-1004. doi: 10.1080/17476348.2019.1656069.
- [8] Ley B, Collard HR, King TE Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2011;183(4):431-40. doi: 10.1164/rccm.201006-0894CI.
- [9] Alfaro TM, Robalo Cordeiro C. Comorbidity in idiopathic pulmonary fibrosis - what can biomarkers tell us? *Ther Adv Respir Dis.* 2020;14:1753466620910092. doi: 10.1177/1753466620910092.
- [10] Mori Y, Kondoh Y. What parameters can be used to identify early idiopathic pulmonary fibrosis? *Respir Investig.* 2021;59(1):53-65. doi: 10.1016/j.resinv.2020.10.008.
- [11] Kishaba T. Evaluation and management of idiopathic pulmonary fibrosis. *Respir Investig.* 2019;57(4):300-311. doi: 10.1016/j.resinv.2019.02.003.
- [12] Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, et al; American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Society. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med.* 2018;198(5):e44-e68. doi: 10.1164/rccm.201807-1255ST.
- [13] Wells AU, Desai SR, Rubens MB, Goh NS, Cramer D, Nicholson AG, et al. Idiopathic pulmonary fibrosis: a composite physiologic index derived from disease extent observed by computed tomography. *Am J Respir Crit Care Med.* 2003 ;167(7):962-9. doi: 10.1164/rccm.2111053.
- [14] Ley B, Ryerson CJ, Vittinghoff E, Ryu JH, Tomassetti S, Lee JS, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. *Ann Intern Med.* 2012 ;156(10):684-91. doi:

10.7326/0003-4819-156-10-201205150-00004.

[15] Nathan SD, Albera C, Bradford WZ, Costabel U, Glaspole I, Glassberg MK, et al. Effect of pirfenidone on mortality: pooled analyses and meta-analyses of clinical trials in idiopathic pulmonary fibrosis. *Lancet Respir Med.* 2017 ;5(1):33-41. doi: 10.1016/S2213-2600(16)30326-5.

[16] van den Blink B, Wijsenbeek MS, Hoogsteden HC. Serum biomarkers in idiopathic pulmonary fibrosis. *Pulm Pharmacol Ther.* 2010;23(6):515-20. doi: 10.1016/j.pupt.2010.08.001.

[17] Behr J, Günther A, Bonella F, Dinkel J, Fink L, Geiser T, et al. S2K Guideline for Diagnosis of Idiopathic Pulmonary Fibrosis. *Respiration.* 2021;100(3):238-271. doi: 10.1159/000512315.

[18] American Thoracic Society; European Respiratory Society. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med.* 2002 ;165(2):277-304. doi: 10.1164/ajrccm.165.2.ats01.

[19] Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al.; ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med.* 2011;183(6):788-824. doi: 10.1164/rccm.2009-040GL.

[20] Prasse A, Müller-Quernheim J. Non-invasive biomarkers in pulmonary

fibrosis. *Respirology.* 2009;14(6):788-95. doi: 10.1111/j.1440-1843.2009.01600.x.

[21] Ray P, Le Manach Y, Riou B, Houle TT. Statistical evaluation of a biomarker. *Anesthesiology.* 2010;112(4):1023-40. doi: 10.1097/ALN.0b013e3181d47604.

[22] Ley B, Brown KK, Collard HR. Molecular biomarkers in idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol.* 2014;307(9):L681-91. doi: 10.1152/ajplung.00014.2014.

[23] Jee AS, Sahhar J, Youssef P, Bleasel J, Adelstein S, Nguyen M, Corte TJ. Review: Serum biomarkers in idiopathic pulmonary fibrosis and systemic sclerosis associated interstitial lung disease - frontiers and horizons. *Pharmacol Ther.* 2019;202:40-52. doi: 10.1016/j.pharmthera.2019.05.014.

[24] Strimbu K, Tavel JA. What are biomarkers? *Curr Opin HIV AIDS.* 2010;5(6):463-6. doi: 10.1097/COH.0b013e32833ed177.

[25] Ou FS, Michiels S, Shyr Y, Adjei AA, Oberg AL. Biomarker Discovery and Validation: Statistical Considerations. *J Thorac Oncol.* 2021;16(4):537-545. doi: 10.1016/j.jtho.2021.01.1616.

[26] Panteghini M. Cardiac: is this biomarker ready for the prime time? *Scand J Clin Lab Invest Suppl.* 2010;242:66-72. doi: 10.3109/00365513.2010.493394.

[27] Hayton C, Terrington D, Wilson AM, Chaudhuri N, Leonard C, Fowler SJ. Breath biomarkers in idiopathic pulmonary fibrosis: a systematic review. *Respir Res.* 2019;20(1):7. doi: 10.1186/s12931-019-0971-8.].

[28] Chiba H, Otsuka M, Takahashi H. Significance of molecular biomarkers in idiopathic pulmonary fibrosis: a mini

- review. *Respir Investig*. 2018;56:384-91. doi.org/10.1016/j.resinv.2018.06.001.
- [29] Horimasu Y, Hattori N, Ishikawa N, Kawase S, Tanaka S, Yoshioka K, et al. Different MUC1 gene polymorphisms in German and Japanese ethnicities affect serum KL-6 levels. *Respir Med*. 2012;106(12):1756-64. doi: 10.1016/j.rmed.2012.09.001.
- [30] Kokosi MA, Margaritopoulos GA, Wells AU. Personalised medicine in interstitial lung diseases: Number 6 in the Series "Personalised medicine in respiratory diseases" Edited by Renaud Louis and Nicolas Roche. *Eur Respir Rev*. 2018;27(148):170117. doi: 10.1183/16000617.0117-2017.
- [31] d'Alessandro M, Bergantini L, Cameli P, Vietri L, Lanzarone N, Alonzi V, et al. Krebs von den Lungen-6 as a biomarker for disease severity assessment in interstitial lung disease: a comprehensive review. *Biomark Med*. 2020;14(8):665-674. doi: 10.2217/bmm-2019-0545.
- [32] Sokai A, Tanizawa K, Handa T, Kanatani K, Kubo T, Ikezoe K, et al. Importance of serial changes in biomarkers in idiopathic pulmonary fibrosis. *ERJ Open Res*. 2017;3(3):00019-2016. doi: 10.1183/23120541.00019-2016.
- [33] Wakamatsu K, Nagata N, Kumazoe H, Oda K, Ishimoto H, Yoshimi M, et al. Prognostic value of serial serum KL-6 measurements in patients with idiopathic pulmonary fibrosis. *Respir Investig*. 2017;55(1):16-23. doi: 10.1016/j.resinv.2016.09.003.
- [34] Bennett D, Salvini M, Fui A, Cillis G, Cameli P, Mazzei MA, et al. Calgranulin B and KL-6 in bronchoalveolar lavage of patients with IPF and NSIP. *Inflammation*. 2019;42(2):463-470. doi: 10.1007/s10753-018-00955-2.
- [35] Qiu M, Chen Y, Ye Q. Risk factors for acute exacerbation of idiopathic pulmonary fibrosis: A systematic review and meta-analysis. *Clin Respir J*. 2018;12(3):1084-1092. doi: 10.1111/crj.12631.
- [36] Elhai M, Avouac J, Allanore Y. Circulating lung biomarkers in idiopathic lung fibrosis and interstitial lung diseases associated with connective tissue diseases: Where do we stand? *Semin Arthritis Rheum*. 2020;50(3):480-491. doi: 10.1016/j.semarthrit.2020.01.006.
- [37] Aloisio E, Braga F, Puricelli C, Panteghini M. Prognostic role of Krebs von den Lungen-6 (KL-6) measurement in idiopathic pulmonary fibrosis: a systematic review and meta-analysis. *Clin Chem Lab Med*. 2021;59(8):1400-1408. doi: 10.1515/cclm-2021-0199.
- [38] Ballester B, Milara J, Cortijo J. Mucins as a new frontier in pulmonary fibrosis. *J Clin Med*. 2019;8(9):1447. doi: 10.3390/jcm8091447.
- [39] Campo I, Zorzetto M, Bonella F. Facts and promises on lung biomarkers in interstitial lung diseases. *Expert Rev Respir Med*. 2015;9(4):437-57. doi: 10.1586/17476348.2015.1062367.
- [40] Kim KC. Role of epithelial mucins during airway infection. *Pulm Pharmacol Ther*. 2012;25(6):415-9. doi: 10.1016/j.pupt.2011.12.003.
- [41] Seibold MA, Wise AL, Speer MC, Steele MP, Brown KK, Loyd JE, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1503-12. doi: 10.1056/NEJMoa1013660.
- [42] Boucher RC. Idiopathic pulmonary fibrosis-a sticky business. *N Engl J Med*. 2011;364(16):1560-1. doi: 10.1056/NEJMe1014191.

- [43] Seibold MA, Wise AL, Speer MC, Steele MP, Brown KK, Loyd JE, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1503-12. doi: 10.1056/NEJMoa1013660.
- [44] Kropski JA, Blackwell TS, Loyd JE. The genetic basis of idiopathic pulmonary fibrosis. *Eur Respir J*. 2015;45(6):1717-27. doi: 10.1183/09031936.00163814.
- [45] Zhu QQ, Zhang XL, Zhang SM, Tang SW, Min HY, Yi L, Xu B, Song Y. Association between the MUC5B promoter polymorphism rs35705950 and idiopathic pulmonary fibrosis: A meta-analysis and trial sequential analysis in Caucasian and Asian populations. *Medicine (Baltimore)*. 2015 ;94(43):e1901. doi: 10.1097/MD.0000000000001901.
- [46] Nakano Y, Yang IV, Walts AD, Watson AM, Helling BA, Fletcher AA, et al. MUC5B promoter variant rs35705950 affects MUC5B expression in the distal airways in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2016;193(4):464-6. doi: 10.1164/rccm.201509-1872LE.
- [47] Helling BA, Gerber AN, Kadiyala V, Sasse SK, Pedersen BS, Sparks L, et al. Regulation of MUC5B expression in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2017;57(1):91-99. doi: 10.1165/rcmb.2017-0046OC.
- [48] Peljto AL, Zhang Y, Fingerlin TE, Ma SF, Garcia JG, Richards TJ, et al. Association between the MUC5B promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. *JAMA*. 2013;309(21):2232-9. doi: 10.1001/jama.2013.5827.
- [49] d'Alessandro M, Bergantini L, Torricelli E, Cameli P, Lavorini F, Pieroni M, et al. Systematic review and meta-analysis of oncomarkers in IPF patients and serial changes of oncomarkers in a prospective Italian real-life case series. *Cancers (Basel)*. 2021;13(3):539. doi: 10.3390/cancers13030539.
- [50] Rusanov V, Kramer MR, Raviv Y, Medalion B, Guber A, Shitrit D. The significance of elevated tumor markers among patients with idiopathic pulmonary fibrosis before and after lung transplantation. *Chest*. 2012;141(4):1047-1054. doi: 10.1378/chest.11-0284.
- [51] Scarà S, Bottoni P, Scatena R. CA 19-9: Biochemical and clinical aspects. *Adv Exp Med Biol*. 2015;867:247-60. doi: 10.1007/978-94-017-7215-0_15.
- [52] Fahim A, Crooks MG, Wilmot R, Campbell AP, Morice AH, Hart SP. Serum carcinoembryonic antigen correlates with severity of idiopathic pulmonary fibrosis. *Respirology*. 2012;17(8):1247-52. doi: 10.1111/j.1440-1843.2012.02231.x.
- [53] Yin BW, Lloyd KO. Molecular cloning of the CA125 ovarian cancer antigen: identification as a new mucin, MUC16. *J Biol Chem*. 2001;276(29):27371-5. doi: 10.1074/jbc.M103554200.
- [54] Maher TM, Oballa E, Simpson JK, Porte J, Habgood A, Fahy WA, et al. An epithelial biomarker signature for idiopathic pulmonary fibrosis: an analysis from the multicentre PROFILE cohort study. *Lancet Respir Med*. 2017;5(12):946-955. doi: 10.1016/S2213-2600(17)30430-7.
- [55] Balestro E, Castelli G, Bernardinello N, Cocconcelli E, Biondini D, Fracasso F, et al. CA 19-9 serum levels in patients with end-stage idiopathic pulmonary fibrosis (IPF) and other interstitial lung diseases (ILDs): Correlation with functional decline. *Chron Respir Dis*. 2020;17:1479973120958428. doi: 10.1177/1479973120958428.

- [56] Mason RJ, Greene K, Voelker DR. Surfactant protein A and surfactant protein D in health and disease. *Am J Physiol*. 1998;275(1):L1-13. doi: 10.1152/ajplung.1998.275.1.L1.
- [57] Whitsett JA, Wert SE, Weaver TE. Alveolar surfactant homeostasis and the pathogenesis of pulmonary disease. *Annu Rev Med*. 2010;61:105-19. doi: 10.1146/annurev.med.60.041807.123500.
- [58] Greene KE, King TE Jr, Kuroki Y, Bucher-Bartelson B, Hunninghake GW, Newman LS, et al. Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis. *Eur Respir J*. 2002;19(3):439-46. doi: 10.1183/09031936.02.00081102.
- [59] Maitra M, Wang Y, Gerard RD, Mendelson CR, Garcia CK. Surfactant protein A2 mutations associated with pulmonary fibrosis lead to protein instability and endoplasmic reticulum stress. *J Biol Chem*. 2010;285(29):22103-13. doi: 10.1074/jbc.M110.121467.
- [60] Bridges JP, Xu Y, Na CL, Wong HR, Weaver TE. Adaptation and increased susceptibility to infection associated with constitutive expression of misfolded SP-C. *J Cell Biol*. 2006;172(3):395-407. doi: 10.1083/jcb.200508016.
- [61] Lawson WE, Cheng DS, Degryse AL, Tanjore H, Polosukhin VV, Xu XC, et al. Endoplasmic reticulum stress enhances fibrotic remodeling in the lungs. *Proc Natl Acad Sci U S A*. 2011;108(26):10562-7. doi: 10.1073/pnas.1107559108.
- [62] Collard HR, Calfee CS, Wolters PJ, Song JW, Hong SB, Brady S, et al. Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol*. 2010;299(1):L3-7. doi: 10.1152/ajplung.90637.2008.
- [63] White ES, Xia M, Murray S, Dyal R, Flaherty CM, Flaherty KR, et al. Plasma surfactant protein-D, matrix metalloproteinase-7, and osteopontin index distinguishes idiopathic pulmonary fibrosis from other idiopathic interstitial pneumonias. *Am J Respir Crit Care Med*. 2016;194(10):1242-1251. doi: 10.1164/rccm.201505-0862OC.
- [64] Wang K, Ju Q, Cao J, Tang W, Zhang J. Impact of serum SP-A and SP-D levels on comparison and prognosis of idiopathic pulmonary fibrosis: A systematic review and meta-analysis. *Medicine (Baltimore)*. 2017 Jun;96(23):e7083. doi: 10.1097/MD.0000000000007083.
- [65] Song JW, Do KH, Jang SJ, Colby TV, Han S, Kim DS. Blood biomarkers MMP-7 and SP-A: predictors of outcome in idiopathic pulmonary fibrosis. *Chest*. 2013;143(5):1422-1429. doi: 10.1378/chest.11-2735.
- [66] Brasch F, Johnen G, Winn-Brasch A, Guttentag SH, Schmiedl A, Kapp N, et al. Surfactant protein B in type II pneumocytes and intra-alveolar surfactant forms of human lungs. *Am J Respir Cell Mol Biol*. 2004;30(4):449-58. doi: 10.1165/rcmb.2003-0262OC.
- [67] Guttentag SH, Beers MF, Bieler BM, Ballard PL. Surfactant protein B processing in human fetal lung. *Am J Physiol*. 1998;275(3):L559-66. doi: 10.1152/ajplung.1998.275.3.L559.
- [68] Kahn N, Rossler AK, Hornemann K, Muley T, Grünig E, Schmidt W, et al. C-proSP-B: A possible biomarker for pulmonary diseases? *Respiration*. 2018;96(2):117-126. doi: 10.1159/000488245.
- [69] Doyle IR, Nicholas TE, Bersten AD. Partitioning lung and plasma proteins: circulating surfactant proteins as biomarkers of alveolocapillary permeability. *Clin Exp Pharmacol*

Physiol. 1999;26(3):185-97. doi: 10.1046/j.1440-1681.1999.03015.x.

[70] Almunashiri S, Zhu Y, Han Y, Wang X, Somanath PR, Zhang D. Club Cell Secreted Protein CC16: potential applications in prognosis and therapy for pulmonary diseases. *J Clin Med*. 2020;9(12):4039. doi: 10.3390/jcm9124039.

[71] Rokicki W, Rokicki M, Wojtacha J, Dżeljjli A. The role and importance of club cells (Clara cells) in the pathogenesis of some respiratory diseases. *Kardiochir Torakochirurgia Pol*. 2016;13(1):26-30. doi: 10.5114/kitp.2016.58961.

[72] Buendía-Roldán I, Ruiz V, Sierra P, Montes E, Ramírez R, Vega A, et al. Increased Expression of CC16 in Patients with Idiopathic Pulmonary Fibrosis. *PLoS One*. 2016;11(12):e0168552. doi: 10.1371/journal.pone.0168552.

[73] Lee HW, Blasco MA, Gottlieb GJ, Horner JW 2nd, Greider CW, DePinho RA. Essential role of mouse telomerase in highly proliferative organs. *Nature*. 1998;392(6676):569-74. doi: 10.1038/33345.

[74] Zvereva MI, Shcherbakova DM, Dontsova OA. Telomerase: structure, functions, and activity regulation. *Biochemistry (Mosc)*. 2010;75(13):1563-83. doi: 10.1134/s0006297910130055.

[75] Courtwright AM, El-Chemaly S. Telomeres in interstitial lung disease: The short and the long of It. *Ann Am Thorac Soc*. 2019;16(2):175-181. doi: 10.1513/AnnalsATS.201808-508CME.

[76] Cronkhite JT, Xing C, Raghu G, Chin KM, Torres F, Rosenblatt RL, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2008;178(7):729-37. doi: 10.1164/rccm.200804-550OC.

[77] Stuart BD, Lee JS, Kozlitina J, Noth I, Devine MS, Glazer CS, et al. Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. *Lancet Respir Med*. 2014;2(7):557-65. doi: 10.1016/S2213-2600(14)70124-9.

[78] Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, Vulto I, Xie M, Qi X, Tudor RM, Phillips JA 3rd, Lansdorp PM, Loyd JE, Armanios MY. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proc Natl Acad Sci U S A*. 2008;105(35):13051-6. doi: 10.1073/pnas.0804280105.

[79] Campbell ID, Humphries MJ. Integrin structure, activation, and interactions. *Cold Spring Harb Perspect Biol*. 2011;3(3):a004994. doi: 10.1101/cshperspect.a004994.

[80] Tatler AL, Jenkins G. TGF- β activation and lung fibrosis. *Proc Am Thorac Soc*. 2012;9(3):130-6. doi: 10.1513/pats.201201-003AW.

[81] Horan GS, Wood S, Ona V, Li DJ, Lukashev ME, Weinreb PH, et al. Partial inhibition of integrin $\alpha(v)$ β_6 prevents pulmonary fibrosis without exacerbating inflammation. *Am J Respir Crit Care Med*. 2008;177(1):56-65. doi: 10.1164/rccm.200706-805OC.

[82] Saini G, Porte J, Weinreb PH, Violette SM, Wallace WA, McKeever TM, et al. $\alpha v \beta_6$ integrin may be a potential prognostic biomarker in interstitial lung disease. *Eur Respir J*. 2015;46(2):486-94. doi: 10.1183/09031936.00210414.

[83] Mahalanobish S, Saha S, Dutta S, Sil PC. Matrix metalloproteinase: An upcoming therapeutic approach for idiopathic pulmonary fibrosis. *Pharmacol Res*. 2020;152:104591. doi: 10.1016/j.phrs.2019.104591.

- [84] Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res*. 2003;92(8):827-39. doi: 10.1161/01.RES.0000070112.80711.3D.
- [85] Craig VJ, Zhang L, Hagood JS, Owen CA. Matrix metalloproteinases as therapeutic targets for idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2015;53(5):585-600. doi: 10.1165/rcmb.2015-0020TR.
- [86] Rosas IO, Richards TJ, Konishi K, Zhang Y, Gibson K, Lokshin AE, et al. MMP1 and MMP7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. *PLoS Med*. 2008;5(4):e93. doi: 10.1371/journal.pmed.0050093.
- [87] Pardo A, Gibson K, Cisneros J, Richards TJ, Yang Y, Becerril C, et al. Up-regulation and profibrotic role of osteopontin in human idiopathic pulmonary fibrosis. *PLoS Med*. 2005;2(9):e251. doi: 10.1371/journal.pmed.0020251.
- [88] Richards TJ, Park C, Chen Y, Gibson KF, Peter Di Y, Pardo A, et al. Allele-specific transactivation of matrix metalloproteinase 7 by FOXA2 and correlation with plasma levels in idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol*. 2012;302(8):L746-54. doi: 10.1152/ajplung.00319.2011.
- [89] Zuo F, Kaminski N, Eugui E, Allard J, Yakhini Z, Ben-Dor A, et al. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci U S A*. 2002;99(9):6292-7. doi: 10.1073/pnas.092134099.
- [90] Morais A, Beltrão M, Sokhatska O, Costa D, Melo N, Mota P, et al. Serum metalloproteinases 1 and 7 in the diagnosis of idiopathic pulmonary fibrosis and other interstitial pneumonias. *Respir Med*. 2015;109(8):1063-8. doi: 10.1016/j.rmed.2015.06.003.
- [91] Bauer Y, White ES, de Bernard S, Cornelisse P, Leconte I, Morganti A, et al. MMP-7 is a predictive biomarker of disease progression in patients with idiopathic pulmonary fibrosis. *ERJ Open Res*. 2017;3(1):00074-2016. doi: 10.1183/23120541.00074-2016.
- [92] Tzouveleakis A, Herazo-Maya J, Sakamoto K, Bouros D. Biomarkers in the evaluation and management of idiopathic pulmonary fibrosis. *Curr Top Med Chem*. 2016;16(14):1587-98. doi: 10.2174/15680266166666150930120959.
- [93] Kadota J, Mizunoe S, Mito K, Mukae H, Yoshioka S, Kawakami K, et al. High plasma concentrations of osteopontin in patients with interstitial pneumonia. *Respir Med*. 2005;99(1):111-7. doi: 10.1016/j.rmed.2004.04.018.
- [94] Goyal M, Jaswal S, Garg K, Gupta S, Dey S, Dutta K. Diagnostic role of osteopontin in interstitial lung disease. *J Health Sci Med Res* 2021;39(3):181-189 doi: 10.31584/jhsmr.2021780.
- [95] Gui X, Qiu X, Xie M, Tian Y, Min C, Huang M, et al. Prognostic value of serum osteopontin in acute exacerbation of idiopathic pulmonary fibrosis. *Biomed Res Int*. 2020;2020:3424208. doi: 10.1155/2020/3424208.
- [96] Foster MW, Morrison LD, Todd JL, Snyder LD, Thompson JW, Soderblom EJ, et al. Quantitative proteomics of bronchoalveolar lavage fluid in idiopathic pulmonary fibrosis. *J Proteome Res*. 2015;14(2):1238-49. doi: 10.1021/pr501149m.
- [97] Yoshihara T, Nanri Y, Nunomura S, Yamaguchi Y, Feghali-Bostwick C, Ajito K, et al. Periostin plays a critical role in the cell cycle in lung fibroblasts.

Respir Res. 2020;21(1):38. doi: 10.1186/s12931-020-1299-0.

[98] Ohta S, Okamoto M, Fujimoto K, Sakamoto N, Takahashi K, Yamamoto H, et al; Consortium for Development of Diagnostics for Pulmonary Fibrosis Patients (CoDD-PF). The usefulness of monomeric periostin as a biomarker for idiopathic pulmonary fibrosis. *PLoS One*. 2017;12(3):e0174547. doi: 10.1371/journal.pone.0174547.

[99] Okamoto M, Izuhara K, Ohta S, Ono J, Hoshino T. Ability of periostin as a new biomarker of idiopathic pulmonary fibrosis. *Adv Exp Med Biol*. 2019;1132:79-87. doi: 10.1007/978-981-13-6657-4_9.

[100] Naik PK, Bozyk PD, Bentley JK, Popova AP, Birch CM, Wilke CA, et al.; COMET Investigators. Periostin promotes fibrosis and predicts progression in patients with idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol*. 2012;303(12):L1046-56. doi: 10.1152/ajplung.00139.2012.

[101] Chen L, Li S, Li W. LOX/LOXL in pulmonary fibrosis: potential therapeutic targets. *J Drug Target*. 2019;27(7):790-796. doi: 10.1080/1061186X.2018.1550649.

[102] Chien JW, Richards TJ, Gibson KF, Zhang Y, Lindell KO, Shao L, et al. Serum lysyl oxidase-like 2 levels and idiopathic pulmonary fibrosis disease progression. *Eur Respir J*. 2014;43(5):1430-8. doi: 10.1183/09031936.00141013.

[103] Annunziata M, Granata R, Ghigo E. The IGF system. *Acta Diabetol*. 2011;48(1):1-9. doi: 10.1007/s00592-010-0227-z.

[104] Le Roith D. Seminars in medicine of the Beth Israel Deaconess Medical Center. Insulin-like growth factors. *N Engl J Med*. 1997;336(9):633-40. doi: 10.1056/NEJM199702273360907.

[105] Guiot J, Bondue B, Henket M, Corhay JL, Louis R. Raised serum levels of IGFBP-1 and IGFBP-2 in idiopathic pulmonary fibrosis. *BMC Pulm Med*. 2016;16(1):86. doi: 10.1186/s12890-016-0249-6.

[106] Kelley KM, Oh Y, Gargosky SE, Gucev Z, Matsumoto T, Hwa V, et al. Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. *Int J Biochem Cell Biol*. 1996;28(6):619-37. doi: 10.1016/1357-2725(96)00005-2.

[107] Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev*. 1995;16(1):3-34. doi: 10.1210/edrv-16-1-3.

[108] Russo VC, Azar WJ, Yau SW, Sabin MA, Werther GA. IGFBP-2: The dark horse in metabolism and cancer. *Cytokine Growth Factor Rev*. 2015;26(3):329-46. doi: 10.1016/j.cytogfr.2014.12.001.

[109] Jaffar J, Unger S, Corte TJ, Keller M, Wolters PJ, Richeldi L, et al. Fibulin-1 predicts disease progression in patients with idiopathic pulmonary fibrosis. *Chest*. 2014;146(4):1055-1063. doi: 10.1378/chest.13-2688.

[110] Ge Q, Chen L, Jaffar J, Argraves WS, Twal WO, Hansbro P, et al. Fibulin1C peptide induces cell attachment and extracellular matrix deposition in lung fibroblasts. *Sci Rep*. 2015;5:9496. doi: 10.1038/srep09496.

[111] Liu G, Cooley MA, Jarnicki AG, Hsu AC, Nair PM, Haw TJ, et al. Fibulin-1 regulates the pathogenesis of tissue remodeling in respiratory diseases. *JCI Insight*. 2016;1(9):e86380. doi: 10.1172/jci.insight.86380.

[112] Magnini D, Montemurro G, Iovene B, Tagliaboschi L, Gerardi RE, Lo Greco E, et al. Idiopathic pulmonary fibrosis: molecular endotypes of fibrosis

stratifying existing and emerging therapies. *Respiration*. 2017;93(6):379-395. doi: 10.1159/000475780.

[113] Hynes RO. The extracellular matrix: not just pretty fibrils. *Science*. 2009;326(5957):1216-9. doi: 10.1126/science.1176009.

[114] Leeming DJ, Sand JM, Nielsen MJ, Genovese F, Martinez FJ, Hogaboam CM, et al. Serological investigation of the collagen degradation profile of patients with chronic obstructive pulmonary disease or idiopathic pulmonary fibrosis. *Biomark Insights*. 2012;7:119-26. doi: 10.4137/BMI.S9415.

[115] Organ LA, Duggan AR, Oballa E, Taggart SC, Simpson JK, Kang'ombe AR, et al. Biomarkers of collagen synthesis predict progression in the PROFILE idiopathic pulmonary fibrosis cohort. *Respir Res*. 2019;20(1):148. doi: 10.1186/s12931-019-1118-7.

[116] Jenkins RG, Simpson JK, Saini G, Bentley JH, Russell AM, Braybrooke R, et al. Longitudinal change in collagen degradation biomarkers in idiopathic pulmonary fibrosis: an analysis from the prospective, multicentre PROFILE study. *Lancet Respir Med*. 2015;3(6):462-72. doi: 10.1016/S2213-2600(15)00048-X.

[117] Kakugawa T, Yokota S, Ishimatsu Y, Hayashi T, Nakashima S, Hara S, et al. Serum heat shock protein 47 levels are elevated in acute exacerbation of idiopathic pulmonary fibrosis. *Cell Stress Chaperones*. 2013;18(5):581-90. doi: 10.1007/s12192-013-0411-5.

[118] Yokota S, Kubota H, Matsuoka Y, Naitoh M, Hirata D, Minota S, et al. Prevalence of HSP47 antigen and autoantibodies to HSP47 in the sera of patients with mixed connective tissue disease. *Biochem Biophys Res Commun*. 2003;303(2):413-8. doi: 10.1016/S0006-291x(03)00352-8.

[119] Nakayama S, Mukae H, Sakamoto N, Kakugawa T, Yoshioka S, Soda H, et al. Pirfenidone inhibits the expression of HSP47 in TGF-beta1-stimulated human lung fibroblasts. *Life Sci*. 2008;82(3-4):210-7. doi: 10.1016/j.lfs.2007.11.003.

[120] King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet*. 2011;378(9807):1949-61. doi: 10.1016/S0140-6736(11)60052-4.

[121] Roy SG, Nozaki Y, Phan SH. Regulation of alpha-smooth muscle actin gene expression in myofibroblast differentiation from rat lung fibroblasts. *Int J Biochem Cell Biol*. 2001;33(7):723-34. doi: 10.1016/S1357-2725(01)00041-3.

[122] Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM, et al. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. *Am J Pathol*. 2005;166(5):1321-32. doi: 10.1016/S0002-9440(10)62351-6.

[123] Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, et al. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest*. 2004;114(3):438-46. doi: 10.1172/JCI20997.

[124] Heukels P, van Hulst JAC, van Nimwegen M, Boorsma CE, Melgert BN, van den Toorn LM, et al. Fibrocytes are increased in lung and peripheral blood of patients with idiopathic pulmonary fibrosis. *Respir Res*. 2018;19(1):90. doi: 10.1186/s12931-018-0798-8.

[125] Moeller A, Gilpin SE, Ask K, Cox G, Cook D, Gauldie J, et al. Circulating fibrocytes are an indicator of poor prognosis in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2009;179(7):588-94. doi: 10.1164/rccm.200810-1534OC.

- [126] Prasse A, Pechkovsky DV, Toews GB, Schäfer M, Eggeling S, Ludwig C, et al. CCL18 as an indicator of pulmonary fibrotic activity in idiopathic interstitial pneumonias and systemic sclerosis. *Arthritis Rheum.* 2007;56(5):1685-93. doi: 10.1002/art.22559.
- [127] Prasse A, Probst C, Bargagli E, Zissel G, Toews GB, Flaherty KR, et al. Serum CC-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2009;179(8):717-23. doi: 10.1164/rccm.200808-1201OC.
- [128] Hieshima K, Imai T, Baba M, Shoudai K, Ishizuka K, Nakagawa T, et al. A novel human CC chemokine PARC that is most homologous to macrophage-inflammatory protein-1 alpha/LD78 alpha and chemotactic for T lymphocytes, but not for monocytes. *J Immunol.* 1997;159(3):1140-9. PMID: 9233607.
- [129] Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat Rev Immunol.* 2004;4(8):583-94. doi: 10.1038/nri1412.
- [130] Saito Y, Azuma A, Matsuda K, Kamio K, Abe S, Gemma A. Pirfenidone exerts a suppressive effect on CCL18 expression in U937-derived macrophages partly by inhibiting STAT6 phosphorylation. *Immunopharmacol Immunotoxicol.* 2016;38(6):464-471. doi: 10.1080/08923973.2016.1247852.
- [131] Neighbors M, Cabanski CR, Ramalingam TR, Sheng XR, Tew GW, Gu C, et al. Prognostic and predictive biomarkers for patients with idiopathic pulmonary fibrosis treated with pirfenidone: post-hoc assessment of the CAPACITY and ASCEND trials. *Lancet Respir Med.* 2018;6(8):615-626. doi: 10.1016/S2213-2600(18)30185-1.
- [132] Baran CP, Opalek JM, McMaken S, Newland CA, O'Brien JM Jr, Hunter MG, et al. Important roles for macrophage colony-stimulating factor, CC chemokine ligand 2, and mononuclear phagocytes in the pathogenesis of pulmonary fibrosis. *Am J Respir Crit Care Med.* 2007;176(1):78-89. doi:10.1164/rccm.200609-1279OC.
- [133] Moore BB, Murray L, Das A, Wilke CA, Herrygers AB, Toews GB. The role of CCL12 in the recruitment of fibrocytes and lung fibrosis. *Am J Respir Cell Mol Biol.* 2006;35(2):175-81. doi:10.1165/rcmb.2005-0239OC.
- [134] Suga M, Iyonaga K, Ichiyasu H, Saita N, Yamasaki H, Ando M. Clinical significance of MCP-1 levels in BALF and serum in patients with interstitial lung diseases. *Eur Respir J.* 1999;14(2):376-82. doi:10.1034/j.1399-3003.1999.14b23.x
- [135] Gui X, Qiu X, Tian Y, Xie M, Li H, Gaoet Y et al. Prognostic value of IFN- γ , sCD163, CCL2 and CXCL10 involved in acute exacerbation of idiopathic pulmonary fibrosis. *Int Immunopharmacol.* 2019;70:208-15. doi:10.1016/j.intimp.2019.02.039
- [136] Xue J, Kass DJ, Bon J, Vuga L, Tan J, Csizmadia E, et al. Plasma B lymphocyte stimulator and B cell differentiation in idiopathic pulmonary fibrosis patients. *J Immunol.* 2013;191(5):2089-95. doi: 10.4049/jimmunol.1203476.
- [137] Vuga LJ, Tedrow JR, Pandit KV, Tan J, Kass DJ, Xue J, et al. C-X-C motif chemokine 13 (CXCL13) is a prognostic biomarker of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2014;189(8):966-74. doi: 10.1164/rccm.201309-1592OC.
- [138] O'Dwyer DN, Armstrong ME, Kooblall M, Donnelly SC. Targeting defective Toll-like receptor-3 function and idiopathic pulmonary fibrosis. *Expert Opin Ther Targets.* 2015;19(4):507-14. doi: 10.1517/14728222.2014.988706.

- [139] O'Dwyer DN, Armstrong ME, Trujillo G, Cooke G, Keane MP, Fallon PG, et al. The Toll-like receptor 3 L412F polymorphism and disease progression in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2013;188(12):1442-50. doi: 10.1164/rccm.201304-0760OC.
- [140] Hambly N, Shimbori C, Kolb M. Molecular classification of idiopathic pulmonary fibrosis: personalized medicine, genetics and biomarkers. *Respirology*. 2015;20(7):1010-22. doi: 10.1111/resp.12569.
- [141] Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol*. 2010;11(5):373-84. doi: 10.1038/ni.1863.
- [142] Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature*. 2001;413(6857):732-8. doi: 10.1038/35099560.
- [143] Fang F, Ooka K, Sun X, Shah R, Bhattacharyya S, Wei J, et al. A synthetic TLR3 ligand mitigates profibrotic fibroblast responses by inducing autocrine IFN signaling. *J Immunol*. 2013;191(6):2956-66. doi: 10.4049/jimmunol.1300376.
- [144] Michalski JE, Schwartz DA. Genetic Risk Factors for Idiopathic Pulmonary Fibrosis: Insights into Immunopathogenesis. *J Inflamm Res*. 2021;13:1305-1318. doi: 10.2147/JIR.S280958.
- [145] Li X, Kim SE, Chen TY, Wang J, Yang X, Tabib T, et al. Toll interacting protein protects bronchial epithelial cells from bleomycin-induced apoptosis. *FASEB J*. 2020;34(8):9884-9898. doi: 10.1096/fj.201902636RR.
- [146] Noth I, Zhang Y, Ma SF, Flores C, Barber M, Huang Y, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. *Lancet Respir Med*. 2013;1(4):309-317. doi: 10.1016/S2213-2600(13)70045-6.
- [147] Zhu L, Wang L, Luo X, Zhang Y, Ding Q, Jiang X, et al. Tollip, an intracellular trafficking protein, is a novel modulator of the transforming growth factor- β signaling pathway. *J Biol Chem*. 2012;287(47):39653-63. doi: 10.1074/jbc.M112.388009.
- [148] Inchingolo R, Varone F, Sgalla G, Richeldi L. Existing and emerging biomarkers for disease progression in idiopathic pulmonary fibrosis. *Expert Rev Respir Med*. 2019;13(1):39-51. doi: 10.1080/17476348.2019.1553620.
- [149] Didierlaurent A, Brissoni B, Velin D, Aebi N, Tardivel A, Käslin E, et al. Tollip regulates proinflammatory responses to interleukin-1 and lipopolysaccharide. *Mol Cell Biol*. 2006;26(3):735-42. doi: 10.1128/MCB.26.3.735-742.2006.
- [150] Konishi K, Gibson KF, Lindell KO, Richards TJ, Zhang Y, Dhir R, et al. Gene expression profiles of acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2009;180(2):167-75. doi: 10.1164/rccm.200810-1596OC.
- [151] Mukae H, Iiboshi H, Nakazato M, Hiratsuka T, Tokojima M, Abe K, et al. Raised plasma concentrations of alpha-defensins in patients with idiopathic pulmonary fibrosis. *Thorax*. 2002;57(7):623-8. doi: 10.1136/thorax.57.7.623.
- [152] Zhang W, Ohno S, Steer B, Klee S, Staab-Weijnitz CA, Wagner D, et al. S100a4 is secreted by alternatively activated alveolar macrophages and promotes activation of lung fibroblasts in pulmonary fibrosis. *Front Immunol*. 2018 ;9:1216. doi:10.3389/fimmu.2018.01216.

- [153] Akiyama N, Hozumi H, Isayama T, Okada J, Sugiura K, Yasui H, et al. Clinical significance of serum S100 calcium-binding protein A4 in idiopathic pulmonary fibrosis. *Respirology*. 2020;25(7):743-749. doi: 10.1111/resp.13707
- [154] Pruenster M, Vogl T, Roth J, Sperandio M. S100A8/A9: From basic science to clinical application. *Pharmacol Ther*. 2016;167:120-31. doi:10.1016/j.pharmthera.2016.07.015.
- [155] Araki K, Kinoshita R, Tomonobu N, Gohara Y, Tomida S, Takahashi Y et al. The heterodimer S100A8/A9 is a potent therapeutic target for idiopathic pulmonary fibrosis. *J Mol Med (Berl)*. 2021;99(1):131-45. doi:10.1007/s00109-020-02001-x
- [156] Tanaka K, Enomoto N, Hozumi H, Isayama T, Naoi H, Aono Y et al. Serum S100A8 and S100A9 as prognostic biomarkers in acute exacerbation of idiopathic pulmonary fibrosis [published online ahead of print, 2021 Jun 18]. *Respir Investig*. 2021;S2212-5345(21)00089-7. doi:10.1016/j.resinv.2021.05.008.
- [157] Tzouvelekis A, Herazo-Maya JD, Ryu C, Chu JH, Zhang Y, Gibson KF, et al. S100A12 as a marker of worse cardiac output and mortality in pulmonary hypertension. *Respirology*. 2018;23(8):771-779. doi: 10.1111/resp.13302.
- [158] Richards TJ, Kaminski N, Baribaud F, Flavin S, Brodmerkel C, Horowitz D, et al. Peripheral blood proteins predict mortality in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2012;185(1):67-76. doi: 10.1164/rccm.201101-0058OC.
- [159] Calderwood SK, Mambula SS, Gray PJ Jr, Theriault JR. Extracellular heat shock proteins in cell signaling. *FEBS Lett*. 2007;581(19):3689-94. doi: 10.1016/j.febslet.2007.04.044.
- [160] Purcell AW, Todd A, Kinoshita G, Lynch TA, Keech CL, Gething MJ, et al. Association of stress proteins with autoantigens: a possible mechanism for triggering autoimmunity? *Clin Exp Immunol*. 2003;132(2):193-200. doi: 10.1046/j.1365-2249.2003.02153.x.
- [161] Fichtner F, Koslowski R, Augstein A, Hempel U, Röhlecke C, Kasper M. Bleomycin induces IL-8 and ICAM-1 expression in microvascular pulmonary endothelial cells. *Exp Toxicol Pathol*. 2004;55(6):497-503. doi: 10.1078/0940-2993-00345.
- [162] Carré PC, Mortenson RL, King TE Jr, Noble PW, Sable CL, Riches DW. Increased expression of the interleukin-8 gene by alveolar macrophages in idiopathic pulmonary fibrosis. A potential mechanism for the recruitment and activation of neutrophils in lung fibrosis. *J Clin Invest*. 1991;88(6):1802-10. doi: 10.1172/JCI115501.
- [163] Mills R, Mathur A, Nicol LM, Walker JJ, Przybylski AA, Mackinnon AC, et al. Intrapulmonary autoantibodies to HSP72 are associated with improved outcomes in IPF. *J Immunol Res*. 2019;2019:1845128. doi: 10.1155/2019/1845128.
- [164] Kahloon RA, Xue J, Bhargava A, Csizmadia E, Otterbein L, Kass DJ, et al. Patients with idiopathic pulmonary fibrosis with antibodies to heat shock protein 70 have poor prognoses. *Am J Respir Crit Care Med*. 2013;187(7):768-75. doi: 10.1164/rccm.201203-0506OC.
- [165] Korthagen NM, van Moorsel CH, Barlo NP, Ruven HJ, Kruit A, Heron M, et al. Serum and BALF YKL-40 levels are predictors of survival in idiopathic pulmonary fibrosis. *Respir Med*. 2011;105(1):106-13. doi: 10.1016/j.rmed.2010.09.012.
- [166] Tong X, Ma Y, Liu T, Li Z, Liu S, Wu G, et al. Can YKL-40 be used as a

biomarker for interstitial lung disease?: A systematic review and meta-analysis. *Medicine (Baltimore)*. 2021;100(17):e25631. doi: 10.1097/MD.00000000000025631.

[167] Li H, Chang L, Du WW, Gupta S, Khorshidi A, Sefton M, et al. Anti-microRNA-378a enhances wound healing process by upregulating integrin beta-3 and vimentin. *Mol Ther*. 2014;22(10):1839-50. doi: 10.1038/mt.2014.115.

[168] Eckes B, Colucci-Guyon E, Smola H, Nodder S, Babinet C, Krieg T, et al. Impaired wound healing in embryonic and adult mice lacking vimentin. *J Cell Sci*. 2000;113 (Pt 13):2455-62. PMID: 10852824.

[169] Surolia R, Li FJ, Wang Z, Li H, Dsouza K, Thomas V, et al. Vimentin intermediate filament assembly regulates fibroblast invasion in fibrogenic lung injury. *JCI Insight*. 2019;4(7):e123253. doi: 10.1172/jci.insight.123253.

[170] Araya J, Kojima J, Takasaka N, Ito S, Fujii S, Hara H, et al. Insufficient autophagy in idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol*. 2013;304(1):L56-69. doi: 10.1152/ajplung.00213.2012.

[171] O'Dwyer DN, Ashley SL, Moore BB. Influences of innate immunity, autophagy, and fibroblast activation in the pathogenesis of lung fibrosis. *Am J Physiol Lung Cell Mol Physiol*. 2016;311(3):L590-601. doi: 10.1152/ajplung.00221.2016.

[172] Bargagna-Mohan P, Hamza A, Kim YE, Khuan Abby Ho Y, Mor-Vaknin N, Wendschlag N, et al. The tumor inhibitor and antiangiogenic agent withaferin A targets the intermediate filament protein vimentin. *Chem Biol*. 2007;14(6):623-34. doi: 10.1016/j.chembiol.2007.04.010.

[173] Meng X, Ezzati P, Wilkins JA. Requirement of podocalyxin in TGF-beta induced epithelial mesenchymal transition. *PLoS One*. 2011;6(4):e18715. doi: 10.1371/journal.pone.0018715.

[174] Li FJ, Surolia R, Li H, Wang Z, Kulkarni T, Liu G, et al. Autoimmunity to vimentin is associated with outcomes of patients with idiopathic pulmonary fibrosis. *J Immunol*. 2017;199(5):1596-1605. doi: 10.4049/jimmunol.1700473.

[175] Kurts C, Sutherland RM, Davey G, Li M, Lew AM, Blanas E, et al. CD8 T cell ignorance or tolerance to islet antigens depends on antigen dose. *Proc Natl Acad Sci U S A*. 1999;96(22):12703-7. doi: 10.1073/pnas.96.22.12703.

[176] Moore MW, Herzog EL. Regulatory T Cells in idiopathic pulmonary fibrosis: Too much of a good thing? *Am J Pathol*. 2016;186(8):1978-1981. doi: 10.1016/j.ajpath.2016.06.002.

[177] Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol*. 2012;30:531-64. doi: 10.1146/annurev.immunol.25.022106.141623.

[178] Holmes S, Downs AM, Fosberry A, Hayes PD, Michalovich D, Murdoch P, et al. Sema7A is a potent monocyte stimulator. *Scand J Immunol*. 2002;56(3):270-5. doi: 10.1046/j.1365-3083.2002.01129.x.

[179] Kang HR, Lee CG, Homer RJ, Elias JA. Semaphorin 7A plays a critical role in TGF-beta1-induced pulmonary fibrosis. *J Exp Med*. 2007;204(5):1083-93. doi: 10.1084/jem.20061273.

[180] Kolodsick JE, Toews GB, Jakubzick C, Hogaboam C, Moore TA, McKenzie A, et al. Protection from fluorescein isothiocyanate-induced fibrosis in IL-13-deficient, but not IL-4-deficient, mice results from impaired collagen synthesis by fibroblasts. *J Immunol*.

2004;172(7):4068-76. doi: 10.4049/jimmunol.172.7.4068.

[181] Murray LA, Zhang H, Oak SR, Coelho AL, Herath A, Flaherty KR, et al. Targeting interleukin-13 with tralokinumab attenuates lung fibrosis and epithelial damage in a humanized SCID idiopathic pulmonary fibrosis model. *Am J Respir Cell Mol Biol*. 2014;50(5):985-94. doi: 10.1165/rcmb.2013-0342OC.

[182] Liu T, Jin H, Ullenbruch M, Hu B, Hashimoto N, Moore B, et al. Regulation of found in inflammatory zone 1 expression in bleomycin-induced lung fibrosis: role of IL-4/IL-13 and mediation via STAT-6. *J Immunol*. 2004;173(5):3425-31. doi: 10.4049/jimmunol.173.5.3425.

[183] Manichaikul A, Sun L, Borczuk AC, Onengut-Gumuscu S, Farber EA, Mathai SK, et al. Plasma soluble receptor for advanced glycation end products in idiopathic pulmonary fibrosis. *Ann Am Thorac Soc*. 2017;14(5):628-635. doi: 10.1513/AnnalsATS.201606-485OC

[184] Cabrera Cesar E, Lopez-Lopez L, Lara E, Hidalgo-San Juan MV, Parrado Romero C, et al. Serum biomarkers in differential diagnosis of idiopathic pulmonary fibrosis and connective tissue disease-associated interstitial lung disease. *J Clin Med*. 2021;10(14):3167. doi: 10.3390/jcm10143167.

[185] Perkins TN, Oury TD. The perplexing role of RAGE in pulmonary fibrosis: causality or casualty? *Ther Adv Respir Dis*. 2021;15:17534666211016071. doi: 10.1177/17534666211016071.

[186] Machahua C, Montes-Worboys A, Planas-Cerezales L, Buendia-Flores R, Molina-Molina M, Vicens-Zygmunt V. Serum AGE/RAGEs as potential biomarker in idiopathic pulmonary fibrosis. *Respir Res*. 2018;19(1):215. doi: 10.1186/s12931-018-0924-7.

[187] Kyung SY, Byun KH, Yoon JY, Kim YJ, Lee SP, Park JW, et al. Advanced glycation end-products and receptor for advanced glycation end-products expression in patients with idiopathic pulmonary fibrosis and NSIP. *Int J Clin Exp Pathol*. 2013;7(1):221-8. PMID: 24427342; PMCID: PMC3885476.

[188] Smadja DM, Nunes H, Juvin K, Bertil S, Valeyre D, Gaussem P, et al. Increase in both angiogenic and angiostatic mediators in patients with idiopathic pulmonary fibrosis. *Pathol Biol (Paris)*. 2014 ;62(6):391-4. doi: 10.1016/j.patbio.2014.07.006.

[189] Barratt SL, Blythe T, Jarrett C, Ourradi K, Shelley-Fraser G, Day MJ, et al. Differential expression of VEGF-Axxx isoforms is critical for development of pulmonary fibrosis. *Am J Respir Crit Care Med* 2017;196:479-493.

[190] Ando M, Miyazaki E, Ito T, Hiroshige S, Nureki SI, Ueno T, et al. Significance of serum vascular endothelial growth factor level in patients with idiopathic pulmonary fibrosis. *Lung*. 2010;188(3):247-52. doi: 10.1007/s00408-009-9223-x.

[191] Simler NR, Brenchley PE, Horrocks AW, Greaves SM, Hasleton PS, Egan JJ. Angiogenic cytokines in patients with idiopathic interstitial pneumonia. *Thorax*. 2004;59(7):581-5. doi: 10.1136/thx.2003.009860.

[192] Ebina M, Shimizukawa M, Shibata N, Kimura Y, Suzuki T, Endo M, et al. Heterogeneous increase in CD34-positive alveolar capillaries in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2004;169(11):1203-8. doi: 10.1164/rccm.200308-1111OC.

[193] Wollin L, Wex E, Pautsch A, Schnapp G, Hostettler KE, Stowasser S, Kolb M. Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. *Eur Respir J* 2015;45:1434-1445.

- [194] Flynn M, Baker S, Kass DJ. Idiopathic pulmonary fibrosis biomarkers: Clinical utility and a way of understanding disease pathogenesis. *Current Biomarker Findings* 2015; 5:21-33.
- [195] Ross B, D'Orléans-Juste P, Giaid A. Potential role of endothelin-1 in pulmonary fibrosis: from the bench to the clinic. *Am J Respir Cell Mol Biol*. 2010;42(1):16-20. doi: 10.1165/rcmb.2009-0175TR.
- [196] Barlo NP, van Moorsel CH, Kazemier KM, van den Bosch JM, Grutters JC. Potential role of endothelin-1 in pulmonary fibrosis: from the bench to the clinic. *Am J Respir Cell Mol Biol*. 2010;42(5):633. doi: 10.1165/rcmb.2009-0410OC.
- [197] Papiris SA, Tomos IP, Karakatsani A, Spathis A, Korbila I, Analitis A, et al. High levels of IL-6 and IL-8 characterize early-on idiopathic pulmonary fibrosis acute exacerbations. *Cytokine*. 2018;102:168-172. doi: 10.1016/j.cyto.2017.08.019.
- [198] Schupp JC, Binder H, Jäger B, Cillis G, Zissel G, Müller-Quernheim J, et al. Macrophage activation in acute exacerbation of idiopathic pulmonary fibrosis. *PLoS One*. 2015;10(1):e0116775. doi: 10.1371/journal.pone.0116775.
- [199] Xaubet A, Agustí C, Luburich P, Barberá JA, Carrión M, Ayuso MC, et al. Interleukin-8 expression in bronchoalveolar lavage cells in the evaluation of alveolitis in idiopathic pulmonary fibrosis. *Respir Med*. 1998;92(2):338-44. doi: 10.1016/s0954-6111(98)90118-4.
- [200] Takahashi H, Fujishima T, Koba H, Murakami S, Kurokawa K, Shibuya Y, et al. Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. *Am J Respir Crit Care Med*. 2000 ;162(3 Pt 1):1109-14. doi: 10.1164/ajrccm.162.3.9910080.
- [201] Hamai K, Iwamoto H, Ishikawa N, Horimasu Y, Masuda T, Miyamoto S, et al. Comparative Study of Circulating MMP-7, CCL18, KL-6, SP-A, and SP-D as Disease Markers of Idiopathic Pulmonary Fibrosis. *Dis Markers*. 2016;2016:4759040. doi: 10.1155/2016/4759040.
- [202] Xue M, Guo Z, Cai C, Sun B, Wang H. Evaluation of the diagnostic efficacies of serological markers KL-6, SP-A, SP-D, CCL2, and CXCL13 in idiopathic interstitial pneumonia. *Respiration*. 2019;98(6):534-545. doi: 10.1159/000503689.
- [203] Hisata S, Kimura Y, Shibata N, Ono S, Kobayashi T, Chiba S, et al. Normal range of KL-6/MUC1 independent of elevated SP-D indicates a better prognosis in the patients with honeycombing on high-resolution computed tomography. *Pulm Med*. 2011;2011:806014. doi: 10.1155/2011/806014.
- [204] Oak SR, Murray L, Herath A, Sleeman M, Anderson I, Joshi AD, et al. A micro RNA processing defect in rapidly progressing idiopathic pulmonary fibrosis. *PLoS One*. 2011;6(6):e21253. doi: 10.1371/journal.pone.0021253.
- [205] Kinder BW, Brown KK, McCormack FX, Ix JH, Kervitsky A, Schwarz MI, et al. Serum surfactant protein-A is a strong predictor of early mortality in idiopathic pulmonary fibrosis. *Chest*. 2009;135(6):1557-1563. doi: 10.1378/chest.08-2209.
- [206] Richards TJ, Kaminski N, Baribaud F, Flavin S, Brodmerkel C, Horowitz D, et al. Peripheral blood proteins predict mortality in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2012 ;185(1):67-76. doi: 10.1164/rccm.201101-0058OC.

[207] Herazo-Maya JD, Sun J, Molyneaux PL, Li Q, Villalba JA, Tzouvelekis A, et al. Validation of a 52-gene risk profile for outcome prediction in patients with idiopathic pulmonary fibrosis: an international, multicentre, cohort study. *Lancet Respir Med.* 2017;5(11):857-868. doi: 10.1016/S2213-2600(17)30349-1.

medicine for interstitial lung disease. *Clin Chest Med.* 2021;42(2):357-364. doi: 10.1016/j.ccm.2021.03.011.

[208] Adegunsoye A, Alqalyoobi S, Linderholm A, Bowman WS, Lee CT, Pugashetti JV, et al. Circulating plasma biomarkers of survival in antifibrotic-treated patients with idiopathic pulmonary fibrosis. *Chest.* 2020;158(4):1526-1534. doi: 10.1016/j.chest.2020.04.066.

[209] Swaminathan AC, Todd JL. That Was Then, This Is Now: A Fresh Look at Idiopathic pulmonary fibrosis biomarkers in the antifibrotic era. *Chest.* 2020;158(4):1321-1322. doi: 10.1016/j.chest.2020.05.564.

[210] Ntoliou P, Tzilas V, Bouros E, Avdoula E, Karakasiliotis I, Bouros D, Steiropoulos P. The role of microbiome and virome in idiopathic pulmonary fibrosis. *Biomedicines.* 2021;9(4):442. doi: 10.3390/biomedicines9040442.

[211] Pallante P, Malapelle U, Nacchio M, Sgariglia R, Galati D, Capitelli L, et al. Liquid biopsy is a promising tool for genetic testing in idiopathic pulmonary fibrosis. *Diagnostics (Basel).* 2021;11(7):1202. doi: 10.3390/diagnostics11071202.

[212] Yang IV, Schwartz DA. Epigenetics of idiopathic pulmonary fibrosis. *Transl Res.* 2015;165(1):48-60. doi: 10.1016/j.trsl.2014.03.011.

[213] Khan T, Dasgupta S, Ghosh N, Chaudhury K. Proteomics in idiopathic pulmonary fibrosis: the quest for biomarkers. *Mol Omics.* 2021;17(1):43-58. doi: 10.1039/d0mo00108b.

[214] Newton CA, Herzog EL. Molecular markers and the promise of precision