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A Comprehensive Clinical Guide

Second Edition

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Keith Meyer would like to dedicate this book to his wife, Emily Auerbach; to his three children, David, Beth, and Melanie; to his three wonderful granddaughters, Maddy, Eliza, and Emmy; to his father-in-law, Bob Auerbach, and mother-in-law, Wanda Auerbach, who have both provided sage advice concerning his career as a physician scientist; to George and Julie Mosher for their extremely generous support of his academic endeavors; to his mentors and colleagues; and, especially, to his patients, who have trusted his guidance and taught him so much about coping with lung disease.

Steve Nathan would like to dedicate this book to his family, especially his wife Romy and two sons, Jack and Max. (Guys, this is what I do when I am waiting at the baseball fields in between your games ☺) He would also like to dedicate this book to his colleagues who have supported and fostered his career, as well as to his patients who continue to inspire and challenge him.

Preface

The first edition of this book was published in 2014, but many new developments in the field concerning our understanding of pathogenesis, advances in diagnosis, and new therapies to treat patients with idiopathic pulmonary fibrosis (IPF) have transpired in just the past few years. This second edition of the book is intended to provide readers with an up-to-date and comprehensive understanding of this enigmatic lung disease that continues to elude more effective pharmacologic therapies. Despite the two approved drugs that are variably available around the world, further therapeutics to arrest or reverse the fibrotic process remains an urgent yet unmet need.

Chapter 1 reviews the evolution of definitions and classification systems for the interstitial lung diseases and the subset of idiopathic interstitial pneumonias (IIPs) of which IPF is the most commonly encountered and diagnosed disorder. As we learn more about the etiology, pathogenesis, and underlying genetic and epigenetic phenomena that are associated with various forms of interstitial lung disease (ILD), current classification systems will undoubtedly change considerably over the coming decades. The incidence and prevalence of IPF has been estimated by a number of investigators over the past two decades, and tools to determine disease demographics and identify risk factors for developing IPF have improved over time. Drs. Michael Mohning, Jeffrey Swigris, and Amy Olson comprehensively review and provide up-to-date knowledge concerning the epidemiology and natural history of IPF and the many factors that have been implicated as increasing the risk for developing IPF in Chap. 2.

Imaging of the thorax with high-resolution computed tomography (HRCT) and examining lung tissue specimens for histopathological manifestations of IPF are key procedures in diagnosing IPF and differentiating IPF from other forms of fibrosing ILD. Drs. Amir Lagstein and Jeffrey Myers provide a review of the pathologic features of IPF in Chap. 3 and identify key features that help differentiate IPF from other mimics of the disease that may have similar clinical presentations and characteristics. Next up in Chap. 4, Drs. Jonathan Chung and Jeffrey Kanne update the current role of radiologic imaging of ILD and discuss the key role that HRCT plays in the diagnosis of IPF. Pulmonary function testing (PFT) plays an important role in characterizing disease status, providing a prognosis, making clinical decisions

concerning disease management, and assessing responses to therapies. In Chap. 5 Drs. Francesco Bonella, Fabiano di Marco, and Paolo Spagnolo review the various components of such testing in the clinical practice setting and in clinical trials assessing the efficacy of novel agents for treating IPF.

In the later 1900s, immune-mediated inflammation was thought to be a defining feature of the disease. However, as more has been learned through the turn of the century and beyond, the role of inflammation has been questioned, and a new pathogenetic paradigm has emerged. The newer concept that currently prevails recognizes the dominant role of epithelial cell injury, aberrant wound healing responses, and fibrosing tissue responses. Additionally, the notion that IPF may respond to immunomodulatory/anti-inflammatory therapies has been dampened not only by a lack of clinical response to a variety of such agents but also the potential harms that such treatments can cause. Drs. Marcus Butler and Michael Keane provide a review of the literature that has investigated the role of inflammation in the pathogenesis of IPF in Chap. 6. While the impact and significance of innate and adaptive immune responses in IPF have been difficult to tease out, there is renewed interest in the role of various aspects of immune-mediated inflammation including macrophages, autoimmunity, chemokines, vascular remodeling, and altered host defense mechanisms in IPF. Dr. Nathan Sandbo discusses the meat of the matter regarding wound healing responses and tissue fibrosis in the IPF lung in Chap. 7 as he reviews the plethora of literature from the past two decades that enables a better understanding of the interactions of alveolar epithelial cells, fibroblasts/myofibroblasts, and other cell types with the lung matrix in IPF. Included within this section is the potentially key contribution of tissue stiffness and mechanical forces in initiating and promoting the fibroproliferative response.

The fields of genetics and genomics have literally exploded over the past two decades, and various studies of gene variants and epigenetic gene regulation have yielded important information that may, at least in part, explain disease risk and disease behavior in IPF. Drs. Traci Adams and Christine Garcia review the many studies that have detected a variety of gene variants in kindreds of patients with familial pulmonary fibrosis (FPF), sporadic IPF, and other fibrosing IIPs in Chap. 8. Drs. Gabriel Ibarra, Jose Herazo-Maya, and Naftali Kaminski complement Chap. 8 with their cogent and comprehensive discussion of the evolving knowledge of genomics in fibrosing ILD in Chap. 9. A variety of novel techniques are now available to evaluate differential gene expression, such as genome-scale transcript profiling, and such methods may help phenotype IPF variants, identify and validate useful biomarkers, provide key prognostic information, and differentiate IPF from other forms of fibrosing ILD. Clearly, the study of gene variants and epigenetic gene regulation hold the promise of detecting and characterizing the molecular phenomena that underpin disease risk and pathogenesis of IPF and other fibrosing ILDs.

Many potentially useful biomarkers of IPF have been identified by examining lung tissue, bronchoalveolar lavage fluid, and peripheral blood from patients with IPF. These are discussed by Drs. Shweta Sood, Tonya Russell, and Adrian Shifren in Chap. 10. While there is a critical need for biomarkers that are useful in differential diagnosis, assessing prognosis, and monitoring disease course, a single

biomarker or combination of multiple biomarkers has yet to be validated as being sufficiently reliable for use in clinical settings or in trials evaluating new therapies. However, biomarker surrogates may become a reality in the near future. Another important issue is identifying and characterizing phenotypes that exist within the broad spectrum of patients with IPF, and a fair degree of disease heterogeneity in terms of manifestations and disease behavior over time complicates such an endeavor. Drs. Christopher King, Shambhu Aryal, and Steven Nathan discuss current views concerning IPF phenotypes as well as the various comorbidities and complications that can arise in patients with IPF in Chap. 11.

Making a confident diagnosis of IPF requires clinicians to obtain and integrate all clinical data, serologic studies, HRCT imaging, and histopathologic specimens (if needed) to determine whether criteria are met that are consistent with a diagnosis of IPF. Drs. Jamie Sheth, Anish Wadhwa, and Kevin Flaherty provide an excellent review of the key aspects of IPF diagnosis along with a diagnostic algorithm for evaluating patients with suspected IPF in Chap. 12. Because attaining an accurate and confident diagnosis of IPF can be challenging even at centers with extensive experience in diagnosing IPF, a multidisciplinary approach is likely to provide maximal diagnostic confidence.

Many clinical trials evaluating novel pharmacologic therapies for IPF have been conducted over the past two decades, but the majority of candidate drugs did not demonstrate a favorable impact on the disease. However, the antifibrotic agents, pirfenidone and nintedanib, were found to significantly slow disease progression and are now available for clinical use. Drs. Andrea Smargiassi, Giuliana Pasciuto, Emanuele Conte, Mariarita Andreani, Roberta Marra, and Luca Richeldi provide a comprehensive review of randomized clinical trials that have been completed or are currently in progress in Chap. 13.

Chapters 14, 15, 16, and 17 address additional aspects that clinicians should be aware of when diagnosing and managing patients with IPF. The coeditors tackle Chap. 14 wherein we discuss other forms of fibrosing ILD that can mimic IPF in their clinical presentation, imaging characteristics, and histopathological appearance. Chronic hypersensitivity pneumonitis is especially difficult to differentiate from IPF, and antifibrotic therapies are not currently indicated for fibrosing ILDs other than IPF. An abnormal degree of gastroesophageal reflux (GER) is highly prevalent in patients with IPF, and a considerable body of literature supports a role for GER with microaspiration of refluxed gastric secretions as a potential trigger and/or driver of lung injury and fibrosis in IPF. Dr. Joyce Lee discusses various studies that support gastroesophageal reflux disease (GERD) as an important comorbidity to diagnose and manage in Chap. 15. Other important components in the comprehensive management of IPF patients are the implementation of pulmonary rehabilitation and prescribing supplemental oxygen to facilitate symptom relief and help maintain the quality of life. Drs. Catherine Wittman and Jeffrey Swigris discuss the integral role of these interventions and provide guidance for their use in IPF patients in Chap. 16. Despite optimal care many patients with IPF will suffer a devastating decline in lung function with a high probability of death should they develop an acute exacerbation of IPF (AEIPF). Drs. Joyce Lee and Harold Collard

present a case of AEIPF in Chap. 17 and discuss current concepts of AEIPF pathogenesis, diagnostic criteria, prognosis, and management.

For patients with progressive disease and evolving respiratory insufficiency, lung transplantation may be the only treatment option that can provide an opportunity to have a second chance at normalizing lung function and restoring the quality of life. Unfortunately, however, only a minority of patients will be able to meet the criteria that allow them to become candidates for lung transplantation. Drs. Daniela Lamas and David Lederer discuss the role of lung transplantation in the treatment of patients with IPF in Chap. 18 and review criteria and timing for referral and listing as well as outcomes and potential complications once patients receive a lung transplant.

Many clinical trials have been performed over the past two decades with a variety of novel agents that had shown promise in preclinical investigations and early-phase studies in human volunteers. However, only pirfenidone and nintedanib have demonstrated significant efficacy as pharmacological therapies, and both have been variably approved around the world for clinical use in IPF patients. Considerable ongoing research has provided new insights into IPF pathogenesis and identified a variety of agents that may benefit patients. However, detecting a significant treatment response has become more challenging since the majority of patients enrolled in clinical trials will be on background therapy with either of the two approved agents, which are now perceived as standard of care for patients with IPF. Drs. Paolo Spagnolo, Elisabetta Cocconcelli, and Vincent Cottin discuss the challenges that researchers face when attempting to demonstrate a significant effect of novel therapies on the clinical course of IPF in Chap. 19. They highlight that the choice of endpoints may prove to be critical for detecting a treatment response. In addition, enrichment strategies for patients at higher risk of disease progression, identifying reliable biomarkers as surrogate endpoints, and using composite endpoints may all help to improve trial efficiency.

Finally, Drs. Matt Craig, Neil Aggarwal, and James Kiley provide an excellent review of basic research that has been performed to date on the pathogenesis of IPF in Chap. 20. Their chapter reviews current knowledge of the salient features of the interplay of alveolar epithelial cells, myofibroblasts, extracellular matrix, and immune activation in the development and progression of IPF. They also cover emerging knowledge concerning the role of various genes and environmental influences in disease risk and pathogenesis. Finally, they provide a roadmap for the future of clinical trials to identify novel therapies for IPF.

We trust that the second edition of this book will update and improve our readers' knowledge of the various aspects of the disease that we recognize as IPF. We hope that it serves as an inspiration to engage in and/or support meaningful basic and clinical research in the ongoing quest to identify therapies that can successfully stop disease progression and even restore lung function without resorting to lung transplantation. The fact that IPF is the number one indication for lung transplantation is a composite measure of its prevalence and lack of sufficiently effective medications. Both coeditors are in the lung transplant "business," and while it is a lofty goal to achieve a successful transplant with long-term survival and restored quality of life,

perhaps we can be put of this “business” in the not too distant future with the advent of earlier diagnosis and more effective therapies for this devastating disease. Finally but very importantly, we are extremely indebted to the authors who graciously contributed their time, energy, and passion in providing chapters for this book. As is the hope for combination therapy, we believe that the synergy of these chapters will render this book an indispensable resource for anyone with an interest in IPF.

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Chapter 1
Classification and Nomenclature of Interstitial Lung Disease

Keith C. Meyer

Introduction

The term idiopathic pulmonary fibrosis (IPF) was initially used by clinicians and radiologists in the mid-1900s to refer to fibrosing pneumonitis of unknown cause. IPF was one of a number of terminologies coined over the span of the twentieth century by leaders in the field as a diagnosis for patients whose lungs showed interstitial patterns on plain chest radiographs that could not be explained by entities such as congestive heart failure. In the 1930s Hamman and Rich described four patients with rapidly progressive respiratory worsening due to diffuse alveolar wall thickening of unidentifiable cause, and the term "Hamman-Rich syndrome" came into use as a diagnosis for patients with evidence of either acute, subacute, or even chronic onset ILD with features of lung fibrosis [1]. As knowledge of interstitial disorders increased, diffuse pulmonary fibrosis was recognized as frequently associated with forms of connective tissue disease (CTD) such as rheumatoid arthritis (RA) or scleroderma, exposure to inhaled inorganic or organic agents, and pneumotoxic reactions to drugs. However, no explanations or plausible associations could be identified for many cases of ILD, but because these disorders were thought to generally occur as a consequence of alveolar wall inflammation ("alveolitis"), terms such as diffuse fibrosing alveolitis, chronic idiopathic interstitial fibrosis, or IPF were used as diagnostic terms to designate fibrosing ILD of unknown etiology. Scadding coined the term, diffuse fibrosing alveolitis, to indicate the presence of widespread fibrotic change beyond the level of terminal bronchioles on histopathologic tissue specimens. He subdivided various entities according to known or as yet unrecognized

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associations and patterns of fibrosis [2]. Additionally, Liebow and Carrington proposed a classification system for differentiating forms of chronic idiopathic interstitial pneumonia based on histopathologic changes [3, 4]. One of the five major entities they described was termed "usual" interstitial pneumonia (UIP), and the Hamman-Rich syndrome was perceived as an acute form of UIP. Although the histopathology-based classification systems that were proposed by Scadding or Liebow and Carrington (Table 1.1) had many similarities but recognized significant variations among a number of entities, clinicians tended to overlook these differences and used the terms cryptogenic fibrosing alveolitis (Europe) or IPF (United States) as a diagnosis for what was perceived as idiopathic chronic fibrosing ILD [5].

Table 1.1 Evolution of terminology and classification for interstitial lung disorders

Diffuse fibrosing alveolitis [2]
Known etiology (e.g., inhaled substances, infections)
Defined histopathology with unknown etiology
Systemic diseases with similar histopathology
Limited to lung (cryptogenic fibrosing alveolitis)
Desquamative changes only
Fibrosis with architectural distortion (known or unknown etiology)
Idiopathic interstitial pneumonias [3, 4]
Usual interstitial pneumonia (UIP)
Desquamative interstitial pneumonia (DIP)
Bronchiolocentric interstitial pneumonia (BIP)
Giant cell interstitial pneumonia (GIP)
Lymphoid interstitial pneumonia (LIP)
Idiopathic pulmonary fibrosis/idiopathic interstitial pneumonias [6]
Acute interstitial pneumonia (AIP)
Usual interstitial pneumonia (UIP)
Desquamative interstitial pneumonia (DIP)
Respiratory bronchiolitis with interstitial lung disease (RBILD)
Non-specific interstitial pneumonia (NSIP)
Interstitial lung diseases/idiopathic interstitial pneumonias ([7, 8]; ATS/ERS Statements in 2002 and 2013)
Diffuse parenchymal lung disease (DPLD) of known cause (e.g., asbestosis)
Granulomatous DPLD (e.g., sarcoidosis)
Other DPLD (e.g., PLCH, PAP)
Idiopathic interstitial pneumonias
Idiopathic pulmonary fibrosis (i.e., idiopathic UIP)
Idiopathic non-specific interstitial pneumonia (NSIP)
Respiratory bronchiolitis with interstitial lung disease (RBILD)
Desquamative interstitial pneumonia (DIP)
Cryptogenic organizing pneumonia (COP)
Idiopathic lymphoid interstitial pneumonia (LIP)
Idiopathic pleuropulmonary fibroelastosis (PPFE)
Undifferentiated idiopathic interstitial pneumonia (UIIP)

Katzenstein and Myers [6] reexamined earlier classification systems and added the new entities of non-specific interstitial pneumonia (NSIP) and respiratory bronchiolitis-associated ILD (RBILD) while retaining and/or revising Liebow's entities of usual interstitial pneumonia (UIP) and desquamative interstitial pneumonia (DIP). Additionally, they coined the term acute interstitial pneumonia (AIP) as a replacement for the "Hamman-Rich syndrome" term. Their scheme recognized giant cell interstitial pneumonia (GIP) as caused by hard-metal exposure, and lymphoid interstitial pneumonia (LIP) was recognized as a lymphoproliferative disorder. Bronchiolitis interstitial pneumonia (BIP) was recognized as an intraluminal (not interstitial) process that could take the form of organizing pneumonia (aka bronchiolitis obliterans organizing pneumonia [BOOP]). The proposed histopathologic pattern-based classification system was also correlated with clinical features and natural history of specific disorders.

These evolving classification and diagnostic schemes combined with the advent of high-resolution computed tomography (HRCT) imaging of the lung and novel approaches to the histopathologic examination of diseased lung tissue facilitated the identification of disorders with distinct clinical, radiologic, and histopathologic characteristics that allowed different forms of ILD to be recognized as unique diagnoses [7–11]. As the ILD classification system evolved, it became clear that the term IPF had to be redefined, and it was transformed from a relatively non-specific diagnosis to current usage as a diagnostic term for patients with a usual interstitial pneumonia (UIP) pattern on histopathology (or definite UIP pattern radiologically in the absence of a surgical lung biopsy). A diagnosis of IPF can only be made in the context of a consistent clinical presentation and the lack of an alternative explanation for the presence of UIP such as connective tissue disease (CTD) or chronic hypersensitivity pneumonitis (HP) [12]. Indeed, one must beware of these and other mimics of IPF that have a UIP histopathology and UIP HRCT pattern when making a diagnosis of IPF.

Current Approaches to the Classification of ILD

Over 200 forms of ILD comprise the pantheon of disorders that are now recognized as relatively distinct ILD entities (Table 1.2). A consensus classification system, which was recently updated and forged by expert opinion while using a multidisciplinary approach to combine clinical characteristics with HRCT and histopathologic patterns, recognized four major categories of diffuse parenchymal lung disease (DPLD) and focused especially on the category of idiopathic interstitial pneumonias (IIPs) [7, 8]. One can also approach the classification of ILD by focusing specifically on etiologies, clinical presentation and findings on physical examination, HRCT imaging patterns, and/or histopathologic characteristics. Differentiating factors include acute (e.g., acute interstitial pneumonia [AIP]) versus chronic onset (e.g., IPF/UIP), disorders that tend to be more responsive to anti-inflammatory/immunomodulatory therapies, (e.g., sarcoidosis, hypersensitivity pneumonitis

Table 1.2 A comprehensive classification scheme for interstitial lung disease (diffuse parenchymal lung disease)

1. Primary disease-related
Sarcoidosis
Pulmonary Langerhans cell histiocytosis (PLCH)
Lymphangioleiomyomatosis (LAM)
Eosinophilic lung disease-related (e.g., eosinophilic pneumonia)
Chronic aspiration
Pulmonary alveolar proteinosis (PAP)
2. Idiopathic interstitial pneumonia
Idiopathic pulmonary fibrosis (i.e., idiopathic usual interstitial pneumonia)
Non-specific interstitial pneumonia (NSIP)
Desquamative interstitial pneumonia (DIP)
Respiratory bronchiolitis-associated interstitial lung disease (RBILD)
Acute interstitial pneumonia (AIP)
Cryptogenic organizing pneumonia (COP)
Lymphocytic interstitial pneumonia (LIP)
Pleuro-parenchymal fibroelastosis (PPFE)
Non-classifiable interstitial pneumonia (NCIP)
3. Inherited lung disease
Familial interstitial pneumonia (FIP)
Hermansky-Pudlak syndrome (HPS)
Others (e.g., metabolic storage diseases)
4. Connective tissue disease-associated
Rheumatoid arthritis
Systemic sclerosis (scleroderma)
Anti-synthetase syndromes
Sjögren syndrome
Systemic lupus erythematosus
Ankylosing spondylitis
5. Inhalational exposure-related (occupational or environmental)
Hypersensitivity pneumonitis (organic antigen inhalation)
Acute/subacute
Chronic fibrosing
Inorganic dust/fiber/fume-related
Pneumoconiosis (e.g., asbestosis, silicosis, hard metal lung disease)
Others (e.g., berylliosis, chronic beryllium disease, ILD induced by gaseous phase agents)
6. Iatrogenic
Drug-induced
Radiation pneumonitis/fibrosis
7. Miscellaneous disorders
Interstitial pneumonia with autoimmune features (IPAF)
Diffuse alveolar hemorrhage (e.g., Goodpasture syndrome)
Idiopathic diffuse alveolar hemorrhage
Acute fibrinous and organizing pneumonia (AFOP)

Table 1.2 (continued)

Bronchiolocentric pattern of interstitial pneumonia
Amyloidosis
Diffuse alveolar damage (idiopathic, subacute onset)
Chronic lung allograft dysfunction (restrictive allograft syndrome)
ILD associated with inflammatory bowel disease
ILD associated with hepatic disease (e.g., primary biliary cirrhosis, viral hepatitis)
Mimics of ILD (e.g., infection or malignancy-associated)

[HP], CTD-associated ILD, interstitial pneumonia with autoimmune features (IPAF), cryptogenic organizing pneumonia [COP]) versus those unlikely to respond to such therapy (e.g., UIP, asbestosis, silicosis). Additional differentiating characteristics include disorders that may remit with appropriate therapy but have a propensity to relapse when such therapy is tapered or withdrawn (e.g., COP), disorders linked to ambient/environmental/occupational exposures (e.g., pneumoconioses, HP), lung-limited disorders (e.g., IIPs) versus those linked to extrapulmonary disease processes (e.g., sarcoidosis, CTD-associated ILD), iatrogenic disorders caused by therapeutic interventions for pulmonary or non-pulmonary disorders (e.g., ILD due to drug reactions or radiation therapy), or disorders that are clearly caused by inherited gene variants (e.g., Hermansky-Pudlak syndrome).

Because there can be considerable overlap in characteristics of various forms of ILD, making a definitive diagnosis can be quite challenging. However, various findings that can help to narrow the differential diagnosis, such as velcro-like crackles on chest auscultation, are usually detected in patients with IPF, although such auscultatory findings may also be present with other forms of ILD when advanced fibrosis is present. Other examples include finding a UIP radiologic and/or histopathologic pattern, which can be seen not only in IPF but also other ILD such as CTD-associated ILD, chronic HP, asbestosis, or drug reactions, or detecting a significant lymphocytosis in bronchoalveolar lavage fluid (BALF), which essentially rules out IPF but implicates other entities such as sarcoidosis, acute HP, or cellular NSIP. However, some ILD presentations do not adequately satisfy criteria that allow a definitive diagnosis to be assigned, and the terms undifferentiated ILD or undifferentiated IIP may be needed when a diagnosis cannot be confidently made. One must, nonetheless, put all the data together to navigate through various levels of potential overlap among characteristics of specific entities to arrive at a consensus clinical-radiologic-pathologic diagnosis that is most consistent with the specific disease at hand.

Many forms of ILD including IPF have recently been linked to specific inherited gene mutations and polymorphisms, and an evolving understanding of genomics has also identified various epigenetic mechanisms that are associated with disease pathogenesis [13–17]. Telomere dysfunction (e.g., TERT or TERC gene variants), MUC5B gene polymorphisms, and a variety of single nucleotide polymorphisms for genes such as TOLLIP or TLR3 have been associated with both disease risk and disease behavior for patients with IPF. Ongoing studies are likely to discover many

other genetic factors that are associated with ILD diagnoses and identify specific genotype-phenotype relationships that modulate the natural history of disease and/or interact with environmental risk factors to increase the risk of developing a specific ILD entity. The terms, familial pulmonary fibrosis or familial interstitial pneumonia, have been used as a diagnostic term for patients when ILD has been linked to an inherited genetic variant that is found in multiple family members who develop an interstitial disorder. Although the interstitial disorder is usually UIP or UIP-like radiologically and/or on lung histopathology, variations in histopathologic patterns and disease characteristics can be found among family members with the same predisposing gene variant [18]. As useful biomarkers of disease and genetic/genomic characteristics of ILD are identified and validated, classification systems are likely to change and evolve as new discoveries further our understanding of disease processes and relationships.

Idiopathic Interstitial Pneumonias

IPF is the most common form of IIP that is encountered in clinical medicine. For an IPF diagnosis to be made, the criteria of a consistent clinical presentation, the presence of a confident UIP pattern on HRCT imaging, exclusion of other potential diagnoses, and, if a lung tissue biopsy is needed, a UIP pattern on lung biopsy specimens (if the HRCT does not adequately identify a typical UIP pattern) must be present [12]. However, a definitive diagnosis may not be forthcoming despite obtaining HRCT imaging and an adequately sampled lung biopsy specimen, and a multidisciplinary discussion may be required to facilitate inter-observer agreement and reach a consensus diagnosis of a specific form of IIP versus other possibilities such as chronic HP [19–22]. An important confounder in making an IPF diagnosis is that other disease entities (e.g., CTD-ILD, chronic HP) can present with a HRCT UIP pattern and even have a histopathologic UIP or UIP-like pattern. A search for evidence of CTD is essential, as UIP, NSIP, AIP, or DIP patterns can be seen when patients have CTD-associated ILD [23] or IPAF (when criteria for a specific CTD diagnosis are not adequately met) [24]. When clinical and laboratory data are obtained that suggest a diagnosis of CTD but criteria for a specific CTD are not met and the only finding is a positive antinuclear antibody or rheumatoid factor without any other criteria for a diagnosis of CTD or IPAF, a diagnosis of IIP (e.g., IPF) can still be assigned and maintained. Some of these patients may, however, develop criteria for a diagnosis of CTD as their disease evolves over time, and the diagnosis can be revised if such occurs.

The updated statement on multidisciplinary classification of the IIPs [8] added the category of undifferentiated IIP for cases that appear to be consistent with an IIP diagnosis but do not adequately satisfy criteria for diagnosis of a specific form of IIP. Circumstances in which a final diagnosis cannot be reached include (1) a lack of adequate clinical, radiologic, or pathologic data that allow a specific diagnosis to be rendered or (2) major discordance among clinical, radiologic, and pathologic

findings that preclude reaching a specific diagnosis. Additionally, an accurate diagnosis can be obscured if previous therapies (e.g., corticosteroids) alter subsequent radiologic imaging characteristics or histologic findings that are obtained at a later point in time when a patient undergoes a diagnostic evaluation for suspected IIP.

Differentiating IPF from Non-IPF ILD

A systematic approach [25] is required to accurately diagnose IPF and differentiate IPF from other specific forms of ILD. Although the majority of patients present with new onset of symptoms such as dyspnea on exertion, cough, and/or fatigue, patients may be in earlier stages of disease and be asymptomatic or relatively asymptomatic with interstitial abnormalities as an incidental finding on thoracic imaging that is obtained for other indications. A careful and comprehensive interview is a key first step and should include whether there is a history of medication/drug exposures (e.g., amiodarone, nitrofurantoin, methotrexate), occupational or environmental exposures, or a history of CTD; such information may provide important clues to an ultimate diagnosis. Advanced age and to a lesser extent male gender and prior smoking history increase the likelihood of IPF as an ultimate diagnosis. The presence of "velcro-like" crackles upon auscultation of the lower lung regions or the finding of diffuse digital clubbing are also quite suggestive of a diagnosis of IPF, but other physical examination findings may suggest a non-IPF diagnosis. Laboratory testing (pulmonary function testing, CTD serologies, other testing as appropriate) combined with the history, physical examination, and routine chest radiographic imaging (posteroanterior and lateral view x-rays) may provide adequate information to establish a reasonably confident ILD diagnosis. However, additional diagnostic testing is usually needed, and a non-contrast HRCT that is performed at full inspiration with both supine and prone positioning as well as expiratory views can provide essential diagnostic information. If a definite radiologic pattern of UIP (subpleural and basilar predominant changes, reticular pattern, honeycomb change with or without traction bronchiectasis, and absence of features that are inconsistent with a UIP pattern) is present on HRCT, a confident diagnosis of IPF can be made if clinical features do not suggest the presence of a non-IPF ILD diagnosis, such as a CTD that presents with lung involvement and a UIP pattern on HRCT.

If a confident ILD diagnosis cannot be made by combining findings from a patient's clinical presentation (comprehensive medical history, physical examination) combined with HRCT imaging results, invasive testing should be considered to secure a diagnosis. Bronchoscopy is a relatively safe procedure, and bronchoalveolar lavage (BAL) and/or endoscopic lung biopsies may provide very useful information that can allow a reasonably confident diagnosis to be made when combined with other clinical data and HRCT imaging. However, bronchoscopy is often perceived as unlikely to aid in securing a diagnosis, especially if a form of IIP is strongly suspected. Progression to a more invasive, non-bronchoscopic type of lung biopsy without performing bronchoscopy may be reasonable and may also be

required if a bronchoscopy does not provide useful diagnostic findings. Furthermore, current analytic techniques for BAL fluid/cells or TBLB tissue are unlikely to provide useful diagnostic information if a patient has IPF. Bronchoscopic lung cryobiopsy (BLC), which can retrieve much larger tissue specimens than endoscopic transbronchial biopsies, is being increasingly used as an alternative to surgical lung biopsy (SLB) [26, 27]. However, the accuracy, safety, and utility of BLC remains to be determined [28]. Obtaining a SLB, which is usually performed via a video-assisted thoracoscopic surgery (VATS) approach, remains the procedure of choice at most centers if other diagnostic testing does not establish a confident diagnosis and BLC is not an option. Patients with significant comorbidities may be at high risk for serious complications and, therefore, may not be good candidates for SLB. Patients should also thoroughly understand the potential risks and benefits when SLB is considered. Importantly, multidisciplinary discussions among clinicians, radiologists, and pathologists (especially if lung tissue biopsies are obtained) should be held to attain an ultimate, "best fit" diagnosis [20].

Summary

The definition and implementation of the term IPF as a diagnostic entity has changed since it was first applied to cases of pulmonary fibrosis of unknown cause. IPF is now used as a diagnostic term for idiopathic UIP, and its diagnosis is based upon consistent clinical, radiologic, and, if needed, histopathologic data that are consistent with the presence of UIP. It is incumbent on clinicians to rule out other possible diagnoses that can have a UIP or UIP-like histopathology and mimic IPF. Such mimics of IPF include cases of CTD-ILD and chronic HP and may be difficult to diagnose. Multidisciplinary discussions are very helpful in making a confident diagnosis of IPF (other forms of IIP and non-IIP ILD) and should be utilized whenever possible. Nonetheless, some cases of ILD or probable IIP may remain unclassifiable, even after adequate lung tissue has been sampled via a surgical lung biopsy. Although the causes of IPF remain elusive, it is now firmly linked to genetic and epigenetic variants as risk factors for developing the disease. The term, familial interstitial pneumonia (FIP), is generally used when multiple cases occur within families with a specific gene variant. As our understanding of the genetic and molecular underpinnings of IPF and other forms of ILD advance, classification systems and terminology for specific entities will undoubtedly change in the future.

References

1. Hamman L, Rich AR. Fulminating diffuse interstitial fibrosis of the lungs. Trans Am Clin Climatol Assoc. 1935;51:154–63.
2. Scadding JG. Diffuse pulmonary alveolar fibrosis. Thorax. 1974;29:271–81.

3. Liebow AA, Carrington DB. The interstitial pneumonias. In: Simon M, Potchen EJ, LeMay M, editors. Frontiers of pulmonary radiology. New York: Grune & Stratton; 1969. p. 102–41.
4. Liebow AA. Definition and classification of interstitial pneumonias in human pathology. Prog Respir Res. 1975;8:1–33.
5. Meyer KC. Idiopathic pulmonary fibrosis: a historical perspective. In: Meyer KC, Nathan SD, editors. Idiopathic pulmonary fibrosis: a comprehensive clinical guide. New York: Humana Press, Springer; 2014. p. 1–8.
6. Katzenstein ALA, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. Am J Respir Crit Care Med. 1998;157:1301–15.
7. 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;165:277–304.
8. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013;188(6):733–48.
9. Gomez AD, King TE Jr. Classification of diffuse parenchymal lung disease. In: Costabel U, du Bois RM, Egan JJ, editors. Diffuse parenchymal lung disease, progress in respiratory research, vol. 36. Basel: Karger; 2007. p. 2–10.
10. Collard HR, King TE Jr. Diffuse lung disease: classification and evaluation. In: Baughman RP, du Bois RM, editors. Diffuse lung disease: a practical approach. 2nd ed. New York: Springer; 2012. p. 85–100.
11. Leslie KO. My approach to interstitial lung disease using clinical, radiological and histopathological patterns. J Clin Pathol. 2009;62:387–401.
12. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183:788–824.
13. Devine MS, Garcia CK. Genetic interstitial lung disease. Clin Chest Med. 2012;33:95–110.
14. Mathai SK, Schwartz DA, Warg LA. Genetic susceptibility and pulmonary fibrosis. Curr Opin Pulm Med. 2014;20:429–35.
15. Chu SG, El-Chemaly S, Rosas IO. Genetics and idiopathic interstitial pneumonias. Semin Respir Crit Care Med. 2016;37:321–30.
16. Mathai SK, Yang IV, Schwarz MI, et al. Incorporating genetics into the identification and treatment of idiopathic pulmonary fibrosis. BMC Med. 2015;13:191.
17. Yang IV, Schwartz DA. Epigenetics of idiopathic pulmonary fibrosis. Transl Res. 2015;165:48–60.
18. Newton CA, Batra K, Torrealba J, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. Eur Respir J. 2016;48(6):1710–20.
19. Flaherty KR, King TE Jr, Raghu G, et al. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? Am J Respir Crit Care Med. 2004;170:904–10.
20. Walsh SL, Wells AU, Desai SR, et al. Multicentre evaluation of multidisciplinary team meeting agreement on diagnosis in diffuse parenchymal lung disease: a case-cohort study. Lancet Respir Med. 2016;4:557–65.
21. Meyer KC. Multidisciplinary discussions and interstitial lung disease diagnosis: how useful is a meeting of the minds? Lancet Respir Med. 2016;4:529–31.
22. Jo HE, Glaspole IN, Levin KC, et al. Clinical impact of the interstitial lung disease multidisciplinary service. Respirology. 2016;21(8):1438–44.
23. Swigris JJ, Brown KK, Flaherty KR. The idiopathic interstitial pneumonias and connective tissue disease-associated interstitial lung disease. Curr Rheumatol Rev. 2010;6:91–8.
24. Fischer A, Antoniou KM, Brown KK, ERS/ATS Task Force on Undifferentiated Forms of CTD-ILD, et al. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. Eur Respir J. 2015;46:976–87.

25. Meyer KC. Diagnosis and management of interstitial lung disease. Translat Respir Med. 2014;2:4. https://doi.org/10.1186/2213-0802-2-4. eCollection 2014.
26. Ravaglia C, Bonifazi M, Wells AU, et al. Safety and diagnostic yield of transbronchial lung cryobiopsy in diffuse parenchymal lung diseases: a comparative study versus video-assisted thoracoscopic lung biopsy and a systematic review of the literature. Respiration. 2016;91(3):215–27.
27. Sharp C, McCabe M, Adamali H, et al. Use of transbronchial cryobiopsy in the diagnosis of interstitial lung disease-a systematic review and cost analysis. QJM. 2017;110(4):207–14.
28. Patel NM, Borczuk AC, Lederer DJ. Cryobiopsy in the diagnosis of interstitial lung disease. A step forward or back? Am J Respir Crit Care Med. 2016;193:707–9.

Chapter 2
Idiopathic Pulmonary Fibrosis: The Epidemiology and Natural History of Disease

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Introduction

Idiopathic pulmonary fibrosis (IPF) has been classically described as a disease that progresses in a "relentless and often insidious manner," with median survival estimates of 2–3 years from the time of diagnosis [1, 2]. However, research over the past two decades has improved our understanding of the natural history of IPF. Although some patients experience steadily progressive respiratory decline, it is now recognized that the clinical course for others is marked by rapid progression and/or acute episodes of worsening that not infrequently result in death. At the group level, clinical factors associated with an increased risk of mortality have been identified, but predicting the course of disease in an individual patient is challenging, if not impossible. Whether differences in the clinical course result from varying phenotypes of IPF or from other factors (e.g., differences in the type, degree, or intensity of environmental exposures or ethnic and racial differences) is unclear [2, 3]. While certain investigators were generating research that refined understanding of how IPF behaves over time, others were performing epidemiologic studies that better defined the societal burden of IPF and identified environmental exposures associated with an increased risk for developing the disease. In this chapter, we review recently acquired epidemiologic data on IPF and describe the variable natural history of a disease that continues to confound clinicians and researchers alike.

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The Epidemiology of IPF

Background

Investigators have used epidemiologic studies to determine the societal burden of IPF and to identify possible exposures and risk factors (predominantly through case-control studies) for disease development. These studies have revealed that IPF is not as rare as it was once believed to be, underscoring the need for more resources to advance research for this devastating condition. Results from additional epidemiologic studies have identified specific risk factors for IPF, providing insight into possible pathobiologic mechanisms for disease. Hopefully, these studies will prove useful as investigators search for approaches to limit disease occurrence [4].

Prior to the 1990s, factors that kept investigators from conducting large-scale epidemiologic studies in IPF included the supposed rarity of disease, the evolving (changing) case definition of IPF, and the lack of a specific International Classification of Diseases (ICD) diagnostic code. Since then, three developments have changed the landscape of epidemiologic research in IPF: (1) the ninth revision of the ICD coding (ICD-9) system (which for the first time assigned a diagnostic code for IPF and occurred at the end of the 1970s), (2) large population databases (including death certificate data and healthcare claims data), and (3) both regional and multicenter collaborative efforts to determine both the extent of and risk factors for disease.

Prevalence, Incidence, and Secular Trends

Prevalence is a ratio defined as the number of persons with a disease at a specific point in time divided by the total population at that time. Incidence is a rate, defined as the number of new cases (that have developed over a given period of time) divided by the number of persons at risk for developing disease over that period of time.

Coultas and colleagues performed the first regional epidemiologic investigation in the United States to determine the prevalence and incidence of interstitial lung disease (ILD) [5]. Using multiple case-finding methods (including primary care and pulmonary physician's records, histopathology reports, hospital discharge diagnoses, death certificates, and autopsy reports), these investigators established a population-based ILD registry in Bernalillo County, New Mexico – a county with a population of nearly one-half million at the time of this study. Based on data from 1988 to 1993, the overall prevalence of IPF was 20.2 cases per 100,000 men and 13.2 cases per 100,000 women. When these data were stratified by age and gender, the prevalence of IPF increased with increasing age and was higher for men than for women in each age strata (Table 2.1). The incidence of IPF was 10.7 per 100,000 persons/year in men and 7.4 per 100,000 persons/year in women. Again, when stratified by age and gender, the incidence of IPF generally increased with increasing age and was typically higher for men than for women (Table 2.2).

Table 2.1 The prevalence of IPF by age strata and gender in Bernalillo County, New Mexico, from 1988 to 1993 [5] compared to a healthcare claims processing system of a large US health plan from 1996 to 2000 using the broad case definition [6] (see text)

Idiopathic pulmonary fibrosis (prevalence, per 100,000 persons)				
	1988–1993		1996–2000	
Age strata (years)	Men	Women	Men	Women
35–44	2.7	–	4.9	12.7
45–54	8.7	8.1	22.3	22.6
55–64	28.4	5.0	62.8	50.9
65–74	104.6	72.3	148.5	106.7
≥75	174.7	73.2	276.9	192.1

Adapted from Table 4 in [5] and Fig. 1 in [6]

Table 2.2 The incidence of IPF by age strata and gender from Bernalillo County, New Mexico, from 1988 to 1993 [5] compared to a healthcare claims processing system of a large US health plan from 1996 to 2000 using the broad case definition [6] (see text)

Idiopathic pulmonary fibrosis (incidence, per 100,000 persons/year)				
	1988–1993		1996–2000	
Age strata (years)	Men	Women	Men	Women
35–44	4.0	–	1.1	5.4
45–54	2.2	4.0	11.4	10.9
55–64	14.2	10.0	35.1	22.6
65–74	48.6	21.1	49.1	36.0
≥75	101.9	57.0	97.6	62.2

Adapted from Table 5 in [5] and Fig. 2 in [6]

Raghu and colleagues determined the prevalence and incidence of IPF from 1996 to 2000 using data from a large US healthcare plan's claims system [6]. Using a broad definition for IPF (age >18 years, one or more medical encounters coded for IPF, and no medical encounters after that IPF encounter with a diagnosis code for any other type of ILD), these investigators estimated the prevalence and annual incidence of the disease to be 42.7 and 16.3 per 100,000 people, respectively. A narrow case definition (broad definition plus at least one medical encounter with a procedure code for a surgical lung biopsy, transbronchial biopsy, or computed tomography [CT] of the thorax) yielded a prevalence and annual incidence of 14.0 per 100,000 people and 6.8 per 100,000 people, respectively. In their dataset both prevalence and incidence increased with increasing age, and rates were higher in men than women (see Tables 2.1 and 2.2). Results from these two studies suggest that rates have increased over time; however, their limitations constrain these studies as only being hypothesis-generating.

Fernández-Pérez and colleagues performed a population-based, historical cohort study in Olmsted County, Minnesota, of patients evaluated at their center between 1997 and 2005. They had three aims for their study: (1) determine the prevalence and incidence of IPF, (2) determine if incidence changed over time, and (3) predict

the future burden of disease [7]. For 2005, using narrow case-finding criteria (usual interstitial pneumonia [UIP] pattern on surgical lung biopsy or definite UIP pattern on high-resolution CT [HRCT]), the age- and sex-adjusted prevalence (for people over the age of 50 years) was 27.9 cases per 100,000 persons (95% CI = 10.4–45.4); using broad case-finding criteria (UIP pattern on surgical lung biopsy or definite *or possible* UIP pattern on HRCT), it was 63 cases per 100,000 persons (95% CI = 36.4–89.6). Over the 9 years of this study, the age- and sex-adjusted incidence (for those over the age of 50) was 8.8 cases per 100,000 person-years (95% CI = 5.3–12.4) and 17.4 cases per 100,000 person-years (95% CI = 12.4–22.4) for the narrow and broad case-finding criteria, respectively. In contrast to the incidence rates reported by Coultas and Raghu [5, 6], results here suggest significantly decreasing incidence rates over the last 3 years of the study to 6.0 or 11.0 per 100,000 person-years using the narrow or broad case-finding criteria, respectively ($p < 0.001$). Despite the estimated declining incidence, given the aging US population, these investigators projected that the annual number of new cases will continue to rise with between 12,000 and 21,000 new IPF cases diagnosed annually by the year 2050. However, several limitations including the small total number of incident IPF cases (only 47 based on the broad case criteria) detract from the confidence that these results accurately reflect national trends.

In a second large-scale epidemiologic study, Raghu and colleagues [8] determined the annual incidence and prevalence of IPF in a 5% random sample of Medicare beneficiaries during the years 2001–2011. Using the ICD-9 codes 516.3 for IPF and 515 for post-inflammatory pulmonary fibrosis, the authors found the incidence of IPF to be stable over the time period at 93.7 cases per 100,000 person-years (95% CI = 91.9–95.4). However, it was notable that the annual cumulative prevalence increased dramatically from 202.2 cases per 100,000 persons in 2001 to 494.2 cases per 100,000 persons in 2011. To possibly account for the increasing cumulative prevalence in spite of the stable incidence rates, the investigators found that cases diagnosed in 2007 had longer survival times (4 years vs. 3.3 years) than those diagnosed earlier in the years that were evaluated. Because this study specifically examined patients 65 years or older (Medicare beneficiaries), a follow-up study by Raghu and colleagues [9] was performed to assess the incidence and prevalence in a younger population for comparison. A large patient claims database covering more than 89 million people aged 18–64 was examined, and it was found that the annual incidence decreased from 7.9 cases per 100,000 person-years in 2005 to 5.8 cases per 100,000 person-years in 2010. However, the cumulative prevalence was again found to have increased from 13.4 cases per 100,000 persons in 2005 to 18.2 cases per 100,000 persons in 2010.

Because of the concern that the use of electronic databases to determine incidence and prevalence of IPF may provide inaccurate data when case validation is not performed, Esposito and colleagues [10] developed algorithms using the HealthCore Integrated Research Database to identify IPF cases. Positive predictive values (PPVs) for their algorithms were determined after cases were adjudicated. Using a broad definition algorithm (an ICD-9 code-based algorithm similar to those used in prior studies), the PPV was found to be only 44.4%, suggesting that overes-

timation had occurred in prior studies. After correcting for the PPV of the algorithm, the authors determined the incidence of IPF to be 14.6 per 100,000 person-years with a prevalence of 58.7 per 100,000 persons.

Large-scale epidemiologic studies from the United Kingdom also suggest an increase in the incidence of IPF over time. Gribbin and colleagues [11] analyzed a large longitudinal general practice database in the United Kingdom from 1991 to 2003 and found that overall the incidence of IPF more than doubled during this time period. The overall crude incidence of IPF was 4.6 per 100,000 person-years, and the annual increase in the incidence of IPF was 11% (rate ratio 1.11; 95% CI = 1.09–1.13, $p < 0.0001$) after adjusting for sex, age, and geographic region. As in the studies described above, these investigators found the incidence of IPF was higher in men than women and increased with age (until >85 years of age). They could not determine if the trends observed were from increased case ascertainment due either to the expanding routine use of HRCT scanning or simply and increased awareness that perhaps emanated from globally visible consensus statements and multinational IPF drug trials.

Recently, Navaratnam and colleagues [12] extended the work of Gribbin and colleagues. Using the same longitudinal primary care database from the United Kingdom, these investigators determined the incidence of what they called the IPF clinical syndrome (IPF-CS) (defined by the diagnostic codes of idiopathic fibrosing alveolitis, Hamman-Rich syndrome, cryptogenic fibrosing alveolitis, diffuse pulmonary fibrosis, or idiopathic fibrosing alveolitis NOS but excluding connective tissue disease, extrinsic allergic alveolitis, asbestosis, pneumoconiosis, and sarcoidosis) from 2000 to 2008. The overall crude incidence of IPF-CS in their study was 7.44 per 100,000 person-years (nearly double the rate that Gribbin and colleagues reported for the prior decade); it was higher in men than women and generally increased with age. After adjusting for age, sex, and health authority, the incidence of IPF-CS increased by 5% annually from 2000 to 2008 (rate ratio 1.05, 95% CI = 1.03–1.06).

As highlighted in a recent systematic review by Hutchinson et al. [13], the majority of these data suggest the incidence of IPF is increasing worldwide. Because the disease is lethal within a relatively short period of time, mortality rates should mirror incidence rates, making mortality rate studies an additional, potentially rich source of data on these trends.

Mortality Rates and Secular Trends

Mortality rates for a condition are calculated as the number of deaths per year caused by the condition of interest, divided by the number of persons alive in the midyear population. Death certificate and census recording can provide data for such calculations. Because the validity of IPF death certificate data is largely unknown, studies using these data should be interpreted with caution. In the era of ICD-9 coding, when IPF (ICD-9 code 516.3) was coded on a death certificate, it was

generally accurate. However, because a significant proportion of decedents with IPF were coded as 515 (the code for post-inflammatory pulmonary fibrosis [PIPF]), IPF (whose ICD-9 code is 516.3) was typically under-recorded as the cause of death [14, 15]. In 1998 the ICD-10 coding system combined both IPF and PIPF into one diagnostic code (J84.1). Investigators have used this code in some studies (while making concerted efforts to exclude decedents with codes for known causes of ILD) in an attempt to capture a cohort most likely to have IPF. Other investigators have conducted similar studies and either intentionally or unintentionally included decedents with coexisting conditions associated with pulmonary fibrosis (e.g., connective tissue disease), leaving cohorts they labeled as having pulmonary fibrosis (PF) or IPF clinical syndrome (IPF-CS) [12, 16, 17]. Regardless of the term used, a great many decedents in these studies had IPF, and all of them almost certainly had progressive fibrotic lung disease that resulted in death.

In the first large-scale study of mortality rates from IPF, Johnston and colleagues examined ICD-9-coded death certificates from 1979 to 1988 and found that mortality rates from IPF (ICD-9 code 516.3) in England and Wales more than doubled over this time period [14]. Although more men than women died of IPF (60% of decedents) over the duration of the study period, mortality rates increased in both men and women (after standardization for age) and were greater among those of older age. Specifically, the mortality rate in those aged ≥75 years was eight times that of those aged 45–54. They identified higher mortality rates in the industrialized central areas of England and Wales, raising the possibility of occupational or environmental exposures as potential risk factors for the disease. Confirming and expanding the findings of Johnston and colleagues, Hubbard and colleagues examined ICD-9-coded death certificates and found that mortality rates from IPF rose in England, Wales, Scotland, Australia, and Canada from 1979 to 1992 [18].

Mannino and colleagues examined US death certificate data from 1979 to 1991 and found that age-adjusted mortality for pulmonary fibrosis (PF) increased 4.7% in men (from 48.6 deaths per million to 50.9 deaths per million) and 27.1% in women (from 21.4 deaths per million to 27.2 deaths per million). Again, PF-associated mortality increased with increasing age [16]. Higher mortality rates were identified in the West and Southeast, and lower mortality rates occurred in the Midwest and Northeast.

Using the same database as Mannino and colleagues, our group found that, from 1992 to 2003, PF-associated mortality rates increased 29.4% in men (from 49.7 deaths per million to 64.3 deaths per million) and increased 38.1% in women (from 42.3 deaths per million to 58.4 deaths per million) (Fig. 2.1). Mortality rates increased with advancing age and were consistently higher in men than in women; however, mortality rates increased at a faster pace in women than in men over this period of time [17].

Similar trends in mortality were recently reported in the United Kingdom; the overall age- and sex-adjusted mortality rate from IPF-CS from 2005 to 2008 was found to be 50.1 per million person-years. The overall annual increase in mortality was approximately 5% per year (RR = 1.05, 95% CI = 1.04–1.05) from 1968 to 2008, which equated to a sixfold increase in mortality over this study period [12].

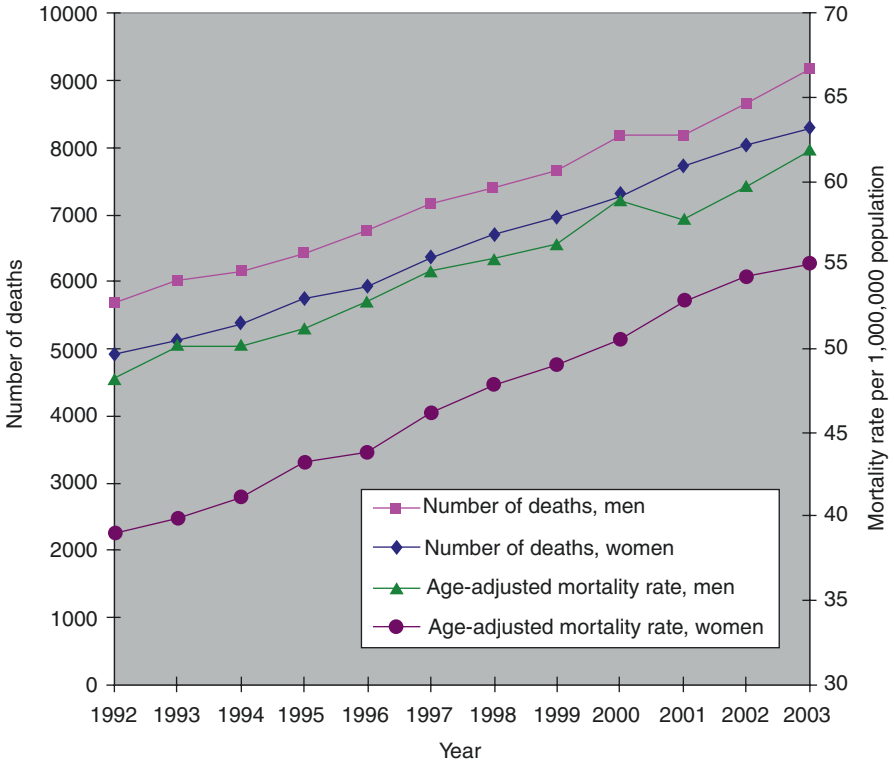

Fig. 2.1 Actual number of deaths per year (first y-axis) and age-adjusted mortality rates (second y-axis) in decedents with PF per 1,000,000 population from 1992 to 2003 in the United States. Mortality rates are standardized to the 2000 US Census Population. (Reprinted with permission of the American Thoracic Society. Copyright (C) 2012 American Thoracic Society. Olson et al. [17]. *Official journal of the American Thoracic Society*)

Hutchinson and colleagues also recently demonstrated a steadily increasing mortality rate in ten countries (including the United States and United Kingdom) using data collected between the years 1999 and 2012 [19]. These studies suggest mortality from IPF is increasing, and IPF is an important and growing public health concern, particularly in the aging population.

Risk Factors

Definitions and Limitations

Most studies of risk factors for IPF have been retrospective and subject to a number of limitations. Because the disease status and the exposure are assessed at the same time, a temporal relationship cannot be established. Furthermore,

systematic biases resulting from both exposure recall and diagnostic misclassification are possible. Recall bias exists when subjects recall past exposures differently than controls, and the net effect results in an exaggeration of risk [20]. Diagnostic misclassification bias arises when cases are incorrectly diagnosed with the disease or when controls have subclinical and undiagnosed disease. These scenarios have likely occurred in IPF, specifically in the time period before the routine use of HRCT scanning and the emergence of consensus statements on the classification of idiopathic interstitial pneumonias (IIPs) including IPF [1, 21]. The net effect of this type of error results in bias toward the null (a reduction in the strength of the association between exposure and disease). When identified, dose-response relationships strengthen the likelihood of a significant risk for the development of disease.

Genetic Risk Factors

Over the past decade, there have been many new and important studies evaluating genetic risk factors and susceptibility for IPF. These significant genetic risk factors are discussed separately in Chap. 8.

Cigarette Smoking

Cigarette smoking has been identified as a risk factor for IPF and for familial pulmonary fibrosis (FPF) in a number of case-control studies. In the United States, Baumgartner and colleagues performed an extensive analysis of the risk of IPF associated with smoking [22]. From 1989 to 1993, they compared 248 IPF patients at any of 16 referral centers to 491 controls matched for age, sex, and geography. They found that a history of ever smoking was associated with a 60% increase in risk for the development of IPF (OR = 1.6, 95% CI = 1.1–2.2). Additional analysis revealed that former smoking was associated with a 90% increased risk for the development of IPF (OR = 1.9, 95% CI = 1.3–2.9), whereas current smoking was not associated with an elevated risk (OR = 1.06, 95% CI = 0.6–1.8). A dose-response relationship was not identified; when compared to subjects with a less than 20 pack-year history, those who smoked 21–40 pack-years had an increased risk of IPF (OR = 2.26, 95% CI = 1.3–3.8), while those who smoked more than 40 pack-years did not (OR = 1.12, 95% CI = 0.7–1.9). However, among former smokers those who had recently stopped smoking possessed the highest risk for the development of IPF (for those who stopped smoking less than 2.5 years prior, OR = 3.5, 95% CI = 1.1–11.9; for those who stopped smoking 2.5–10 years prior, OR = 2.3, 95% CI = 1.3–4.2; for those who stopped smoking 10–25 years prior, OR = 1.9, 95% CI = 1.1–3.2; and for those who stopped smoking more than 25 years ago, OR = 1.3, 95% CI = 0.7–2.3). Similar to Baumgartner and colleagues, Miyake and colleagues compared 102 cases of IPF to 59 controls in Japan and found an increased risk of IPF only in those who smoked between 20 and 40 pack-years (OR = 3.23, 95% CI = 1.01–10.84) compared to never smokers [23].

Taskar and colleagues [24] conducted a meta-analysis that included the two investigations above [22, 23] plus three additional case-control studies from the United Kingdom [25, 26] and Japan [27]. Ever smoking was associated with a 58% increase in the risk for the development of IPF (OR = 1.58, 95% CI = 1.27–1.97). Given the high prevalence of smoking, these investigators determined that 49% of IPF cases could be prevented by entirely eliminating smoking within the population. The results from two other case-control studies from Mexico that were not included in the meta-analysis also suggest that smoking is a risk factor for IPF (OR adjusted = 3.2, 95% CI = 1.2–8.5 and OR adjusted = 2.5, 95% CI = 1.4–4.6) [28, 29]. An association between smoking and lung fibrosis has also been identified in FPF. Steele and colleagues compared 309 cases of FPF with 360 unaffected family members from 111 families and found that after adjustment for age and sex, ever smoking was associated with a greater than threefold increased odds of developing disease (OR = 3.6, 95% CI = 1.3–9.8) [30].

Occupational Exposures

Case-control studies have also found an association between a number of dusts and/or dusty environments and the development of IPF.

Metal Dusts

In a meta-analysis of five case-control studies published between 1990 and 2005, investigators found a significant association between metal dust exposure and the development of IPF (OR = 2.44, 95% CI = 1.74–3.40) [23–27, 31]. Baumgartner and colleagues identified a dose-response relationship between metal dust exposure and IPF. For subjects with less than 5 years of metal dust exposure, no association was identified (OR = 1.4, 95% CI = 0.4–4.9); however, for those with more than 5 years of metal dust exposure, the risk for the development of IPF was elevated more than twofold (OR = 2.2, 95% CI = 1.1–4.7) [31].

Hubbard and colleagues analyzed data from the pension fund archives of a metal engineering company and identified more deaths within this cohort than would be expected from national mortality data [32]. For all decedents with IPF and available records, an increased risk of IPF associated with metal dust exposure was not found. However, there was a dose-response relationship for those with more than 10 years of exposure as well as an increased risk of IPF (OR = 1.71, 95% CI = 1.09–2.68).

Pinheiro and colleagues analyzed mortality data from 1999 to 2003 and found an increased proportionate mortality ratio (PMR) and mortality odds ratio (MOR) among decedents with ICD-10 for pulmonary fibrosis and whose records also contained a code for “metal mining” (PMR = 2.4, 95% CI = 1.3–4.0; MOR 2.2, 95% CI = 1.1–4.4) and “fabricated structural metal products” (PMR = 1.9, 95% CI = 1.1–3.1; MOR 1.7, 95% CI = 1.0–3.1) [33]. In contrast a recent study from Sweden did not identify an association between metal dust exposure and IPF among patients on oxygen therapy (OR = 0.8, 95% CI = 0.43–1.44) [34].

Wood Dust

Results from two of five case-control studies (one from the United Kingdom and one from Japan) plus a meta-analysis of these studies suggest an association between wood dust exposure and IPF (summary OR= 1.94, 95% CI = 1.34–2.81) [23–26, 31, 35]. Discrepancies in results between individual studies may result from differences in the type of wood exposure. In a case-control study, investigators in Sweden found an association between both birch (OR = 2.4, 95% CI = 1.18–4.92) and hardwood dust (OR = 2.5, 95% CI = 1.06–5.89) exposure and IPF, but an association with fir dust (OR = 1.4, 95% CI = 0.82–2.52) was not identified [34].

Agriculture (Farming and Livestock)

Both farming and livestock exposures have been linked to an increased risk of IPF. In each of two case-control studies (one from the United States and one from Japan), investigators found a significant association between farming or residing in an agricultural region and IPF (summary OR = 1.65, 95% CI = 1.20–2.26) [24, 27, 31]. Exposure to agricultural chemicals was also associated with an increased risk of IPF in the Japanese study (OR = 3.32, 95% CI = 1.22–9.05) [27].

Results from two case-control studies (one from the United States and one from the United Kingdom) suggest an association between livestock and IPF (summary OR = 2.17, 95% CI = 1.28–3.68) [24, 25, 31]. In the US study, investigators observed a dose-response relationship between exposure to livestock and IPF; no association was identified for subjects with less than 5 years of exposure (OR = 2.1, 95% CI = 0.7–6.1), but subjects with more than 5 years of exposure to livestock had a greater than threefold increased risk for IPF (OR = 3.3, 95% CI = 1.3–8.3) [31].

Sand, Stone, and Silica

Results from a meta-analysis of four studies with contrasting results show a significant association between IPF and exposure to stone, sand, and silica dusts (summary OR = 1.97, 95% CI = 1.09–3.55) [23–25, 31, 35].

Miscellaneous Exposures

Baumgartner and colleagues found an association between IPF and hairdressing (OR = 4.4, 95% CI = 1.2–16.3) or raising birds (OR = 4.7, 95% CI = 4.7, 95% 1.6–14.1) after adjusting for age and cigarette smoking [31]. The latter association raises the possibility that some patients with chronic hypersensitivity pneumonitis might have been inadvertently diagnosed as having IPF. Residing in an urban or polluted area is another risk factor for IPF that had emerged from a case-control study in Japan (OR = 3.33, 95% CI = 1.26–8.79) [27], and a cluster of IPF cases was recently identified in dental personnel in Virginia, raising the possibility of occupational exposure in dental work as a potential risk [36].

The Natural History of IPF

Background

IPF has historically been described as a disease marked by inexorable progression [1, 2]. For patients with steadily progressive disease (i.e., moderately worsening lung function with each passing year), symptoms of breathlessness typically precede the diagnosis of IPF by 1–3 years [37–39], and median survival ranges from 2 to 3 years from the time of diagnosis [1, 2, 37–40]. However, careful inspection of results reveals significant heterogeneity in survival rates within cohorts [1, 41, 42]. Over the past few years, investigators have drilled deeply into their datasets in an attempt to better understand this heterogeneity. Although some of the heterogeneity may result from differences in disease severity at the time of diagnosis, it has become clear to the ILD field that there are actually different IPF phenotypes that can be defined by disease behavior over time (Fig. 2.2). For example, in every IPF study, a subgroup of long-term survivors is identified, a significant minority of IPF patients will suffer one or more acute exacerbations of IPF, and investigators are finding more and more patients with subclinical disease. What drives the phenotypic expression is unknown, but current theory

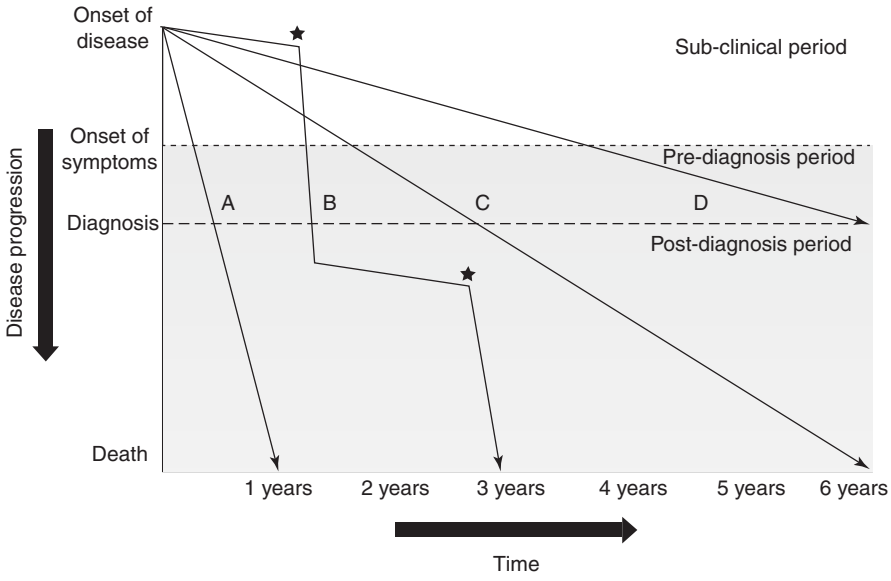

Fig. 2.2 Schematic representation of potential clinical courses of IPF. The y-axis represents disease progression from the onset of disease with a likely subclinical/asymptomatic period, which is followed by a period of symptoms that precede a formal diagnosis and then followed by the period of diagnosis through death with the x-axis representing time. As noted in the text, disease progression may be accelerated (A), relatively stable (C, D), or alternate between periods of relative stability marked by acute worsening (stars) (B). (Reprinted with permission of the American Thoracic Society. Copyright (C) 2012 American Thoracic Society. Ley et al. [43]. *Official journal of the American Thoracic Society*)

holds that it results from complex interactions involving the age and genetic makeup of the host and environmental exposures.

Predicting Survival

Nathan and colleagues examined data from their center collected over the previous decade and found that the median survival for 357 IPF patients was 45.9 months (3.8 years) from the time of their initial pulmonary function test. When stratified by disease severity, patients with percent predicted forced vital capacity (FVC%) ≥70%, 55–69%, or <55% had median survival values of 55.6 months (4.6 years), 38.7 months (3.2 years), and 27.4 months (2.3 years), respectively [42].

In addition to FVC, a number of other individual clinical, radiographic, physiologic, and pathologic variables as well as various biomarkers correlate with survival [43]. Several investigators have generated prognostic models that incorporate combinations of these variables collected at the time of diagnosis [38, 39]. For example, King and colleagues used data from 183 patients with biopsy-proven IPF and found that survival was dependent on a combination of age, smoking status, clubbing, extent of interstitial abnormalities, findings suggesting the presence of pulmonary hypertension on chest radiograph, total lung capacity (TLC), and abnormal gas exchange during maximal exercise [39]. Based on this model (with these clinical, radiological, and physiological [CRP] determinants), 5-year survival ranged from 89% in patients with lower scores to <1% in patients with higher CRP scores. Although this model and other similar modeling [44, 45] have revealed that differences in survival depend on baseline characteristics, none have been formally externally validated, and each model has limited ability to predict disease behavior in an individual patient.

Collard and colleagues determined that after adjustment for baseline values, 6- and 12-month change in any of a number of variables including dyspnea score, TLC, FVC, partial pressure of arterial oxygen, peripheral oxyhemoglobin saturation, and alveolar-arterial oxygen gradient predicted survival time [41]. As with baseline predictors, these seem to perform well at the group level [46, 47] but may not be predictive at the patient level. Furthermore, while these prediction models may provide some utility in mortality and respiratory hospitalization prediction, they perform very poorly in predicting risk of disease progression [48].

Rate of Decline in FVC

Data from the placebo arms of several therapeutic trials reveal that the annual decline in absolute FVC ranges from 0.15 to 0.22 L [49–56] (Table 2.3). Given the inclusion criteria (which typically seek to identify patients with earlier/milder disease) and exclusion criteria (which typically exclude patients with significant

comorbid conditions) used in these clinical trials [42], these estimates of disease progression as reflected by a decline in FVC are unlikely to apply to the general population of IPF patients.

The Underlying Cause of Death

The underlying cause of death (UCD) for the majority of patients with IPF is respiratory failure [16, 17, 43, 57]. Panos and colleagues reviewed a series of cases with mortality data published from 1964 to 1983 and found that among 326 deaths respiratory failure was the UCD in 38.7% of the decedents [57]. Using US death certificate data from 1979 to 1991, Mannino and colleagues found that in patients with pulmonary fibrosis, the UCD was the disease itself in 50% of decedents [16]. Our group extended the work of Mannino and colleagues by examining US death certificate data from 1992 to 2003 and found that pulmonary fibrosis was the UCD in 60% of

Table 2.3 Recent randomized, placebo-controlled trials in which the absolute decline in forced vital capacity (FVC) for the placebo group was reported over the study period [49–56]

Study	Drug	Baseline FVC, L (FVC%)	Absolute decline in FVC, L	Time of assessment	Annual rate of decline in FVC, L/year
ASCEND(King 2014) [106]	Pirfenidone	NR (68.6%)	−0.28/year	52 weeks	−0.28/year
INPULSIS-1 (Richeldi 2014) [107]	Nintedanib	2.85 (80.5%)	−0.24/year	52 weeks	−0.24/year
INPULSIS-2 (Richeldi 2014) [107]	Nintedanib	2.62 (78.1%)	−0.21/year	52 weeks	−0.21/year
TOMORROW (Richeldi 2011)	Nintedanib	2.70 (77.6%)	−0.19	52 weeks	−0.19/year
BUILD-3 (King 2011)	Bosentan	2.66 (73.1%)	−0.18	52 weeks	−0.18/year
Imatinib (Daniels 2010)	Imatinib	2.54 (65.5%)	−0.14	48 weeks	−0.15/year
Shionogi, (Taniguchi 2010)	Pirfenidone	2.47[a] (79.1%)[a]	−0.16[a]	52 weeks	−0.16/year[a]
Etanercept (Raghu 2008)	Etanercept	NR (63.0%)	−0.20	48 weeks	−0.22/year
Shionogi (Azuma 2005)	Pirfenidone	NR (78.4)[a]	−0.13[a]	36 weeks	−0.19/year[a]
IFIGENIA, (Demedts 2005)	NAC	2.36[a] (66.6%)[a]	−0.19[a]	52 weeks	−0.19/year[a]
GIPF-001 (Raghu 2004)	Interferon gamma-1b	NR (64.1%)	−0.16	48 weeks	−0.17/year

In those studies that were less than 52 weeks in duration, the annual rate of decline was determined from available data by assuming a constant rate of decline
Abbreviation: *NR* not reported
[a]Studies actually reported vital capacity (VC)

decedents with IPF [17]. Among IPF subjects in therapeutic trials, the UCD is a respiratory cause in nearly 80% [43, 51, 53, 58]. Taken together, these data reveal that over the past 50 years, the proportion of patients with IPF who are dying from (rather than with) the disease has grown, and these trends may reflect advances in diagnostic accuracy. However, another potential explanation is that effective therapies for some of the more common comorbid conditions (e.g., cardiovascular disease) result in patients being more likely to die from IPF rather than other treatable conditions (Table 2.4).

Apart from lung disease progression, UCDs in patients with IPF include coronary artery disease (CAD), pulmonary embolism, and lung cancer. While the proportion dying from cardiovascular disease has declined over time (see Table 2.4), patients with IPF appear to be at greater risk for CAD than patients with chronic obstructive pulmonary disease (COPD) (or other respiratory diseases requiring transplantation) [59–61] or matched people in the background population [62–64]. Thromboembolic disease and pulmonary embolism occur more often in patients with IPF than those with COPD and lung cancer or in people in the background population [63, 65, 66]. Furthermore, IPF decedents with a code for thromboembolic disease on their death certificates died younger (74.3 vs. 77.4 years in females [$p < 0.0001$] and 72.0 vs. 74.4 years in males [$p < 0.0001$]) than IPF decedents without codes for thromboembolic disease [65]. Compared with the background population, the risk for lung cancer is significantly elevated in patients with IPF, and this risk appears to be independent of smoking history [67, 68]; however, its overall effect on survival in this population remains unknown [69].

Phenotypic Subgroups

Long-Term Survivors

In studies conducted prior to the development of the current IIP classification system [16], nearly 30% of subjects with IPF were alive at 10 years from diagnosis

Table 2.4 The underlying cause of death in patients with idiopathic pulmonary fibrosis (see text) [16, 17, 57, 65]

Underlying cause of death / Study	Respiratory Pulmonary fibrosis	Respiratory Pneumonia	Respiratory COPD	Respiratory PE	Lung cancer	Cardio-vascular disease	Other
Panos (1964–1983)	39%	2.8%	NR	3.4%	10.4%	27.0%	14.1%
Mannino (1979–1991)	50.0%	NR	22.6%	NR	4.8%	22.6%	NR
Olson/ Sprunger [17] (1992–2003)/ (1998–2007) [65]	60.0%	2.4%	NR	1.74% [65]	2.9%	9.6%	23.4%

Abbreviation: *NR* not reported

[70, 71]. In retrospect it has been assumed that these long-term survivors had diseases other than IPF (e.g., non-specific interstitial pneumonia [NSIP]). However, using the ATS/ERJ criteria for the diagnosis of IPF [1] and cumulative data from the previous decade, Nathan and colleagues found that approximately one-quarter of their IPF patients (n = 357) survived more than 5 years from the time of diagnosis, and survival time was not necessarily associated with baseline FVC [42].

Rapid Progression from Diagnosis

Some patients with IPF follow a rapidly progressive clinical course from the onset (see Fig. 2.2). Selman and colleagues compared IPF patients with ≤6 months of symptoms (rapid progressors) to those with symptoms for ≥24 months (slow progressors) prior to first presentation. They found that despite the absence of differences between groups in baseline age, physiology, or gas exchange parameters, rapid progressors had a significantly increased risk of death when compared with slow progressors (HR = 9.0; 95% CI = 4.48–18.3) and were more likely to be male (OR = 6.5; 95% CI = 1.4–29.5) and either former or current smokers (OR = 3.04; 95% CI = 1.1–8.3) [72]. Additionally, the authors found a distinctive gene expression pattern in rapid progressors that was marked by overexpression of genes involved in morphogenesis, oxidative stress, and migration and proliferation of fibroblasts and smooth muscle cells.

Boon and colleagues examined gene expression profiles in surgical lung biopsy specimens and identified 134 transcripts that sufficiently distinguished relatively stable disease from progressive IPF [73]. They commented that similar to human cancers, genes related to cell proliferation, migration, invasion, and morphology were overrepresented in subjects with progressive disease. These findings highlight the heterogeneity of IPF at the transcriptional level and probably partly explain the varying clinical courses among patients with disease.

Stable Disease Followed by Accelerated Disease

Some IPF patients follow a relatively stable or mildly progressive course for months to years, and then their disease accelerates. Using data from the placebo arm of a large therapeutic trial, Martinez and his co-investigators observed that among patients who survived to the end of the 72-week study (78.6%), the mean FVC% decreased from 64.5 ± 11.1 to 61 ± 14.1, the mean DLCO% decreased from 37.8 ± 11.1% to 37.0 ± 19.9%, and there was little worsening in dyspnea [58]. However, among 36 subjects who died (21.4%), death was IPF-related in 32 patients (89%) and the result of disease progression in 20 patients (56%). Of those deaths resulting from progressive IPF, 47% were acute (deterioration over 4 weeks or less), and 50% were subacute (progression over weeks to months), thus demonstrating that disease progression accelerates prior to death in some patients.

Acute Exacerbations of IPF

In Japan it has been recognized for over 30 years that some patients with IPF experience acute respiratory decline [74, 75], but this was thought to be a rare phenomenon in Western countries until recently [76]. However, sudden respiratory decline in a previously stable patient is now a well-recognized phenomenon that can affect IPF patients around the world. When these events appear to be idiopathic, they have been termed acute exacerbations (AEx) of IPF and are associated with significant morbidity and mortality [77].

To help unify research efforts, Collard and colleagues proposed the following definition for AEx: (1) a previous or concurrent diagnosis of IPF, (2) unexplained development of dyspnea or worsening within 30 days, (3) high-resolution computed tomography (HRCT) with new bilateral ground-glass abnormality and/or consolidation superimposed on a background pattern consistent with IPF, (4) no evidence of pulmonary infection by endotracheal aspirate or bronchoalveolar lavage (BAL), and (5) exclusion of alternative causes including left heart failure, pulmonary embolism, and identifiable causes of acute lung injury [77].

Since these criteria were proposed, two retrospective analyses have better defined the incidence of risk factors for AEx and mortality from these events. Kondoh and colleagues retrospectively studied 74 patients with IPF and observed that the 1-year, 2-year, and 3-year incidence of AEx was 8.6% (95% CI = 1.7–12.6%), 12.6% (95% CI = 4.5–20.0%), and 23.9% (95% CI = 12.9–33.5%), respectively [78]. In a multivariate analysis, they found that a decline of 10% in FVC at 6 months, a higher BMI, and greater dyspnea at baseline were significant risk factors for AEx. The survival time in subjects with an AEx was significantly shorter (median 26.4 months) compared to those without an AEx (median 52.8 months). Song and colleagues reviewed records of 461 patients with IPF with a median follow-up time of 22.9 months and observed that 96 patients (20.8%) had either a definite (using Collard's criteria) or suspected AEx [79] and 17 of these patients (17.7%) experienced multiple episodes of AEx. The 1-, 2-, and 3-year incidences (excluding patients who presented concurrently with a new diagnosis of IPF while having an AEx event) were 11.6%, 16.3%, and 18.2%, respectively. A multivariate analysis showed that a lower FVC% and never smoking were significant risk factors for an AEx, and AEx events were associated with poor outcomes: 50% of patients died during hospitalization for the AEx, 90% of those who required mechanical ventilation died, and 60% of patients died within 90 days. For those who lived past 90 days, the median survival was 15.5 months as compared with 60.6 months for those without an AEx ($p < 0.001$). Clearly, AEx are not as rare as once believed and are associated with poor survival.

Additional data from recent prospective therapeutic trials have reported AEx frequencies ranging from 1.7% over 96 weeks to 14.2% over 36 weeks [49–52, 54, 56, 58, 80–83] (Table 2.5). Differences in baseline patient populations, diagnostic criteria used, and case-finding methods likely account for some of the variability in reported frequency of AEx. These discordant data confirm that additional research regarding AEx of IPF is needed.

Table 2.5 Recent randomized, placebo-controlled trials in which the incidence or percentage of patients with acute decompensation and/or an acute exacerbation was reported [49–52, 54, 58, 80–83]. Definitions from the study for acute exacerbation, acute worsening, or acute decompensation are given below

Study	Drug	Placebo cohort (*n*)	FVC (L or % predicted)	Definition	Incidence or percentage reported	Study period
IMPULSIS-1 (Richeldi 2014) [107]	Nintedanib	204	2.85 (80.5%)	Acute exacerbation[a]	5.4%	52 weeks
IMPULSIS-2 (Richeldi 2014) [107]	Nintedanib	219	2.62 (78.1%)	Acute exacerbation[a]	9.6%	52 weeks
ACE-IPF (IPFnet 2012)	Warfarin	73	58.7%	Acute exacerbation[a]	2.7%	28 weeks (mean follow-up)
TOMORROW (Richeldi 2011)	Nintedanib	87	2.70 L	Acute exacerbation[b]	15.7 per 100 patient-years	52 weeks
BUILD-3 (King 2011)	Bosentan	209	2.66 L	Acute exacerbation[c]	2.9%	80 weeks (mean study duration)
STEP-IPF (IPFnet 2010)	Sildenafil	91	58.7%	Acute exacerbation[d]	4.4%	12 weeks
STEP-IPF (IPFnet 2010)	Sildenafil	91	58.7%	Acute exacerbation[d]	7.7%	24 weeks (last 12 weeks on therapy)
Imatinib (Daniels 2010)	Imatinib	60	2.54 L	Acute worsening[e]	1.7%	96 weeks
Shionogi (Taniguchi 2010)	Pirfenidone	104	2.47 L	Acute exacerbation[f]	3.8%	52 weeks
INSPIRE (King 2009)	INF-γ	275	73.1%	Acute decompensation[g]	8.7%	77 weeks (mean study duration)
INSPIRE (King 2009)	INF-γ	275	73.1%	Acute exacerbation[g]	5.4%	77 weeks (mean study duration)
BUILD-1 (King 2008)	Bosentan	83	69.5%	Acute decompensation[h]	3.6%	54 weeks (mean study duration)

(continued)

Table 2.5 (continued)

Study	Drug	Placebo cohort (*n*)	FVC (L or % predicted)	Definition	Incidence or percentage reported	Study period
Shionogi, (Azuma 2005)	Pirfenidone	35	78.4%	Acute exacerbation[i]	14.2%	36 weeks
GIPF-001, (Raghu 2004; Martinez 2005)	Interferon gamma-1b	168	64.1%	Death from either progression of IPF or acute respiratory distress syndrome after a period of decompensation lasting <4 weeks	4.8%	76 weeks (median observation period)

[a]Acute exacerbation was determined via adjudication as part of the study

[b]Acute exacerbation definition: Progression of dyspnea over several days to 4 weeks, new parenchymal ground-glass abnormalities on x-ray or HRCT, and a decrease in $PaO_2 \geq 10$ mmHg or increase in alveolar-arterial oxygen gradient, within a 1-month period that could not be otherwise explained

[c]Acute exacerbation definition: Unexplained rapid deterioration of condition within 4 weeks with increasing dyspnea requiring hospitalization and O_2 supplementation

[d]Acute exacerbation definition: (1) Unexplained worsening of dyspnea or cough within 30 days, triggering medical care with no clinical suspicion or overt evidence of cardiac event, pulmonary embolism, deep venous thrombosis to explain worsening of dyspnea, or pneumothorax; (2) one of the following radiologic or physiologic findings: (a) new ground-glass opacity or consolidation on CT scan or new alveolar opacities on chest x-ray or (b) decline of ≥5% in resting room air SpO_2 from last recorded level or decline of ≥8 mmHg in resting room air PaO_2 from last recorded level; and (3) no clinical or microbiologic evidence of infection

[e]Acute worsening was not otherwise specified

[f]Acute exacerbation definition: Worsening clinical features within 1 month including progression of dyspnea, new radiographic/HRCT ground-glass abnormalities without pneumothorax or pleural effusion, a decrease in PaO_2 by 10 mmHg or more, and exclusion of obvious causes including infection, cancer, pulmonary thromboembolism, malignancy, or congestive heart failure

[g]Acute respiratory decompensation: Evidence of all of the following must be present within a 4-week period: worsening PaO_2 or new or significant increase in the use of supplemental oxygen, clinically significant worsening of dyspnea, and new or worsening radiographic abnormalities on chest radiograph or HRCT. Acute exacerbation = Evidence of all of the following must be present within a 4-week period: worsening PaO_2 at rest (≥8 mmHg drop from most recent pre-worsening value), clinically significant worsening of dyspnea, new ground-glass opacities on HRCT, and all other causes, such as cardiac, thromboembolic, aspiration, or infectious processes, have been excluded

[h]Acute decompensation definition: Unexplained rapid deterioration over 4 weeks with increased dyspnea requiring hospitalization and oxygen supplementations of ≥5 L/min to maintain a resting oxygen saturation by blood gas of ≥90% or $PaO_2 \geq 55$ mmHg (sea level) or $PaO_2 \geq 50$ mmHg (above 1400 m)

[i]Acute exacerbation definition: Worsening clinical features within 1 month with progression of dyspnea over a few days to less than 5 weeks, new radiographic/HRCT parenchymal abnormalities without pneumothorax or pleural effusion, a decrease in PaO_2 by 10 mmHg or more, and exclusion of apparent infection by absence of *Aspergillus* and pneumococcus antibodies in blood, urine for *Legionella pneumophilia*, and sputum cultures

Subclinical Disease

Based largely on studies of family members of patients with familial FPF, it is apparent that asymptomatic/subclinical disease precedes the development of symptomatic IPF. Some asymptomatic relatives from FPF kindreds have evidence of alveolar inflammation on bronchoalveolar lavage [84] or evidence of pulmonary fibrosis (with a usual interstitial pneumonia [UIP] pattern of injury) on either imaging or on the basis of a surgical lung biopsy [30, 85].

Among 417 unaffected (by self-report) family members from 111 families with FPF, 28 (6.7%) had possible disease (based on chest radiographs), and 33 persons (7.9%) had either probable (based on HRCT abnormalities) or definite (based on either surgical lung biopsy or autopsy evidence of an IIP) disease [30]. Rosas and colleagues evaluated 143 asymptomatic subjects from 18 kindreds with FPF and found that 31 subjects (22%) had HRCT changes (including increased septal lines, peribronchovascular thickening, reticulation, and ground-glass opacities) consistent with interstitial lung disease (ILD) [85]. When compared with affected family members, those with HRCT evidence of ILD but without symptoms were younger (46 years vs. 67 years, $p < 0.001$). These findings suggest that progression of asymptomatic to symptomatic disease may occur over a period of decades; however, the proportion of people who will progress, over what time frame progression occurs, and which variables predict progression remain unknown.

In 1982 Bitterman and colleagues assessed 17 clinically unaffected family members of three families with FPF and found that 8 (47%) had evidence of alveolar inflammation on BAL studies [84]. Two of these patients were reassessed 27 years later; one had developed symptomatic IPF, and the other was asymptomatic but did have evidence of early IPF on HRCT, suggesting that there may be a latency period of two to three decades in some cases from early asymptomatic alveolar inflammation to overt fibrotic disease [86].

Additional evidence suggesting that subclinical disease precedes symptomatic clinical disease is found in reports of acute exacerbations in the subclinical period. Case reports and series have described patients without known ILD who present with acute respiratory failure (clinical adult respiratory distress syndrome [ARDS]) and histopathologic findings of diffuse alveolar damage (DAD) superimposed on a UIP pattern, which is the same pattern observed in AEx of IPF [79, 87–89].

Patients with subclinical IPF and lung cancer who undergo surgical lobectomy appear to be at an increased risk of AEx. In a review of 1148 patients with lung cancer who underwent thoracotomy, investigators found 15 patients who developed postoperative ARDS. Eleven (73%) of these patients had both interstitial abnormalities on preoperative CT and a UIP pattern in resected lung tissue. The risk of postoperative ARDS was significantly higher in those with evidence of subclinical IPF on CT imaging (8.8%) compared to those without ILD (0.4%) ($p < 0.001$) [90]. Fukushima and colleagues found subpleural fibrosis in 127 of 776 patients (16.4%) who underwent lobectomy for lung cancer. Three patients progressed acutely following surgery, and another seven progressed to classic IPF over a period of 5 years [91].

Araya and colleagues reviewed 14 autopsy cases of idiopathic DAD (acute interstitial pneumonia [AIP]) and found that 50% of cases also had evidence of subpleural fibrosis, suggesting that some cases of AIP may in fact be the result of an AEx of subclinical IPF [92].

With the increasing use of HRCT, a new category of subclinical ILD has been defined. Interstitial lung abnormalities (ILAs) have recently been investigated in a number of large cohort studies [93, 94]. In the Framingham Heart Study and the AGES-Reykjavik study, it was determined that 7% of participants had ILAs present on CT imaging. Furthermore, in the COPDGene study, ILAs were present in 8% of participants, whereas 9% of participants in the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) study had ILAs present. Using these cohorts Putman and colleagues demonstrated that ILAs were associated with greater all-cause mortality, and in the AGES-Reykjavik cohort, ILAs were associated with greater mortality due to pulmonary fibrosis [94]. Notably, ILAs were associated with the MUC5B promoter polymorphism that has been associated with IPF [93]. Given the commonality of ILAs in these cohorts, it is clear that they do not universally lead to the development of IPF. However, it is possible that some ILAs may represent an early stage of disease, and detection could allow for early treatment.

Although subclinical disease is becoming increasingly recognized [95], many questions concerning the clinical significance of subclinical disease remain. Longitudinal studies are needed to determine risk factors for disease progression, the time period over which the transition from subclinical to clinically relevant disease occurs, and whether early interventions can improve outcomes.

Specific Clinical Phenotypes of Disease

Identifying specific clinical phenotypes of disease is paramount, because doing so may provide insight into the pathobiology of disease [96]. Patients with IPF and either disproportionate pulmonary hypertension or concurrent emphysema are believed by some experts to represent distinct clinical phenotypes of disease, and investigation of these concurrent processes has furthered our understanding of the heterogeneous clinical course.

IPF with Pulmonary Hypertension

The development of pulmonary hypertension in patients with IPF was once believed to be due to vascular obliteration from pulmonary fibrosis. However, in several studies investigators have not found a clear association between the severity of fibrosis and the presence or severity of pulmonary hypertension, suggesting that additional factors are involved [97–99]. Regardless of the underlying mechanisms that lead to the development of pulmonary hypertension, its presence negatively impacts survival [39, 98, 100, 101].

Combined Pulmonary Fibrosis and Emphysema

There is increasing recognition of the coexistence of pulmonary fibrosis and emphysema (a syndrome termed combined pulmonary fibrosis and emphysema [CPFE]) within individual patients. CPFE is characterized by relatively preserved static and forced lung volumes, a disproportionately reduced diffusing capacity, and a high prevalence of pulmonary hypertension [102, 103]. In patients with apparent IPF, concurrent evidence of emphysema on HRCT imaging ranges from 18.8% to 50.9%, and the median survival in such patients is estimated at 2.1–8.5 years [104]. It remains unclear if patients with CPFE have a worse survival compared to those with IPF alone. Mejía and colleagues suggested that the reduced survival among subjects with CPFE compared to IPF subjects was due to the presence of pulmonary hypertension in patients with CPFE [105].

Summary

Over the past two decades, results from multiple studies have advanced our understanding of the natural history of IPF. It has become evident that IPF, once thought to be a steadily progressive disease in all patients, may actually follow any number of different courses. This heterogeneity makes it impossible to confidently determine how the disease will behave over time in an individual patient. However, given this knowledge investigators may now embark on studies to explain this variability and tease out the pathobiologic mechanisms that drive it. Epidemiologic studies suggest that IPF should no longer be considered an orphan disease, especially considering that mortality rates are similar to those associated with some common malignancies. Case-control studies have revealed potential exposures for disease development, but these studies are subject to a number of potential biases. Maintaining the momentum of clinical research and propelling the field forward will require carefully planned, well-designed studies to further decipher disease heterogeneity, identify additional risk factor for disease development, and determine how to prevent and treat this devastating disease.

References

1. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med. 2000;161(2 Pt 1):646–64.
2. Raghu G, et al. 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.
3. Swigris JJ, et al. Ethnic and racial differences in the presence of idiopathic pulmonary fibrosis at death. Respir Med. 2012;106(4):588–93.

4. Gordis L. The epidemiologic approach to disease and intervention. In: Epidemiology. 3rd ed. Philadelphia: Elsevier Saunders; 2004. p. 1–14.
5. Coultas DB, et al. The epidemiology of interstitial lung diseases. Am J Respir Crit Care Med. 1994;150(4):967–72.
6. Raghu G, et al. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006;174(7):810–6.
7. Fernandez Perez ER, et al. Incidence, prevalence, and clinical course of idiopathic pulmonary fibrosis: a population-based study. Chest. 2010;137(1):129–37.
8. Raghu G, et al. Idiopathic pulmonary fibrosis in US Medicare beneficiaries aged 65 years and older: incidence, prevalence, and survival, 2001–11. Lancet Respir Med. 2014;2(7):566–72.
9. Raghu G, et al. Incidence and prevalence of idiopathic pulmonary fibrosis in US adults 18–64 years old. Eur Respir J. 2016;48(1):179–86.
10. Esposito DB, et al. Idiopathic pulmonary fibrosis in United States automated claims. Incidence, prevalence, and algorithm validation. Am J Respir Crit Care Med. 2015;192(10):1200–7.
11. Gribbin J, et al. Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. Thorax. 2006;61(11):980–5.
12. Navaratnam V, et al. The rising incidence of idiopathic pulmonary fibrosis in the UK. Thorax. 2011;66(6):462–7.
13. Hutchinson J, et al. Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. Eur Respir J. 2015;46(3):795–806.
14. Johnston I, et al. Rising mortality from cryptogenic fibrosing alveolitis. BMJ. 1990;301(6759):1017–21.
15. Coultas DB, Hughes MP. Accuracy of mortality data for interstitial lung diseases in New Mexico, USA. Thorax. 1996;51(7):717–20.
16. Mannino DM, Etzel RA, Parrish RG. Pulmonary fibrosis deaths in the United States, 1979–1991. An analysis of multiple-cause mortality data. Am J Respir Crit Care Med. 1996;153(5):1548–52.
17. Olson AL, et al. Mortality from pulmonary fibrosis increased in the United States from 1992 to 2003. Am J Respir Crit Care Med. 2007;176(3):277–84.
18. Hubbard R, et al. Mortality rates from cryptogenic fibrosing alveolitis in seven countries. Thorax. 1996;51(7):711–6.
19. Hutchinson JP, et al. Increasing global mortality from idiopathic pulmonary fibrosis in the twenty-first century. Ann Am Thorac Soc. 2014;11(8):1176–85.
20. Raphael K. Recall bias: a proposal for assessment and control. Int J Epidemiol. 1987;16(2):167–70.
21. 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.
22. Baumgartner KB, et al. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1997;155(1):242–8.
23. Miyake Y, et al. Occupational and environmental factors and idiopathic pulmonary fibrosis in Japan. Ann Occup Hyg. 2005;49(3):259–65.
24. Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease? Proc Am Thorac Soc. 2006;3(4):293–8.
25. Scott J, Johnston I, Britton J. What causes cryptogenic fibrosing alveolitis? A case-control study of environmental exposure to dust. BMJ. 1990;301(6759):1015–7.
26. Hubbard R, et al. Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. Lancet. 1996;347(8997):284–9.
27. Iwai K, et al. Idiopathic pulmonary fibrosis. Epidemiologic approaches to occupational exposure. Am J Respir Crit Care Med. 1994;150(3):670–5.
28. Garcia-Sancho Figueroa MC, et al. Risk factors for idiopathic pulmonary fibrosis in a Mexican population. A case-control study. Respir Med. 2010;104(2):305–9.

29. Garcia-Sancho C, et al. Familial pulmonary fibrosis is the strongest risk factor for idiopathic pulmonary fibrosis. Respir Med. 2011;105(12):1902–7.
30. Steele MP, et al. Clinical and pathologic features of familial interstitial pneumonia. Am J Respir Crit Care Med. 2005;172(9):1146–52.
31. Baumgartner KB, et al. Occupational and environmental risk factors for idiopathic pulmonary fibrosis: a multicenter case-control study. Collaborating centers. Am J Epidemiol. 2000;152(4):307–15.
32. Hubbard R, et al. Risk of cryptogenic fibrosing alveolitis in metal workers. Lancet. 2000;355(9202):466–7.
33. Pinheiro GA, et al. Occupational risks for idiopathic pulmonary fibrosis mortality in the United States. Int J Occup Environ Health. 2008;14(2):117–23.
34. Gustafson T, et al. Occupational exposure and severe pulmonary fibrosis. Respir Med. 2007;101(10):2207–12.
35. Mullen J, et al. Case-control study of idiopathic pulmonary fibrosis and environmental exposures. J Occup Environ Med. 1998;40(4):363–7.
36. Nett RJ, et al. Dental personnel treated for idiopathic pulmonary fibrosis at a tertiary care center – Virginia, 2000–2015. MMWR Morb Mortal Wkly Rep. 2018;67(9):270–3.
37. Nicholson AG, et al. The prognostic significance of the histologic pattern of interstitial pneumonia in patients presenting with the clinical entity of cryptogenic fibrosing alveolitis. Am J Respir Crit Care Med. 2000;162(6):2213–7.
38. King TE Jr, et al. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med. 2001;164(6):1025–32.
39. King TE Jr, et al. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med. 2001;164(7):1171–81.
40. Rudd RM, et al. British Thoracic Society Study on cryptogenic fibrosing alveolitis: response to treatment and survival. Thorax. 2007;62(1):62–6.
41. Collard HR, et al. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2003;168(5):538–42.
42. Nathan SD, et al. Long-term course and prognosis of idiopathic pulmonary fibrosis in the new millennium. Chest. 2011;140(1):221–9.
43. 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.
44. Wells AU, 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.
45. Ley B, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. Ann Intern Med. 2012;156(10):684–91.
46. Hanson D, et al. Changes in pulmonary function test results after 1 year of therapy as predictors of survival in patients with idiopathic pulmonary fibrosis. Chest. 1995; 108(2):305–10.
47. du Bois RM, et al. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184(4):459–66.
48. Ley B, et al. Predictors of mortality poorly predict common measures of disease progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2016;194(6):711–8.
49. Richeldi L, et al. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med. 2011;365(12):1079–87.
50. King TE Jr, et al. BUILD-3: a randomized, controlled trial of bosentan in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184(1):92–9.
51. Daniels CE, et al. Imatinib treatment for idiopathic pulmonary fibrosis: randomized placebo-controlled trial results. Am J Respir Crit Care Med. 2010;181(6):604–10.
52. Taniguchi H, et al. Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J. 2010;35(4):821–9.
53. Raghu G, et al. Treatment of idiopathic pulmonary fibrosis with etanercept: an exploratory, placebo-controlled trial. Am J Respir Crit Care Med. 2008;178(9):948–55.

54. Azuma A, et al. Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2005;171(9):1040–7.
55. Demedts M, et al. High-dose acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med. 2005;353(21):2229–42.
56. Raghu G, et al. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2004;350(2):125–33.
57. Panos RJ, et al. Clinical deterioration in patients with idiopathic pulmonary fibrosis: causes and assessment. Am J Med. 1990;88(4):396–404.
58. Martinez FJ, et al. The clinical course of patients with idiopathic pulmonary fibrosis. Ann Intern Med. 2005;142(12 Pt 1):963–7.
59. Nathan SD, et al. Prevalence and impact of coronary artery disease in idiopathic pulmonary fibrosis. Respir Med. 2010;104(7):1035–41.
60. Izbicki G, et al. The prevalence of coronary artery disease in end-stage pulmonary disease: is pulmonary fibrosis a risk factor? Respir Med. 2009;103(9):1346–9.
61. Kizer JR, et al. Association between pulmonary fibrosis and coronary artery disease. Arch Intern Med. 2004;164(5):551–6.
62. Ponnuswamy A, et al. Association between ischaemic heart disease and interstitial lung disease: a case-control study. Respir Med. 2009;103(4):503–7.
63. Hubbard RB, et al. The association between idiopathic pulmonary fibrosis and vascular disease: a population-based study. Am J Respir Crit Care Med. 2008;178(12):1257–61.
64. American Heart Association. Heart disease and stroke statistics – 2004 update. In: Coronary heart disease, acute coronary syndrome and angina pectoris. 2004. p. 9–12.
65. Sprunger DB, et al. Pulmonary fibrosis is associated with an elevated risk of thromboembolic disease. Eur Respir J. 2012;39(1):125–32.
66. Sode BF, et al. Venous thromboembolism and risk of idiopathic interstitial pneumonia: a nationwide study. Am J Respir Crit Care Med. 2010;181(10):1085–92.
67. Hubbard R, et al. Lung cancer and cryptogenic fibrosing alveolitis. A population-based cohort study. Am J Respir Crit Care Med. 2000;161(1):5–8.
68. Le Jeune I, et al. The incidence of cancer in patients with idiopathic pulmonary fibrosis and sarcoidosis in the UK. Respir Med. 2007;101(12):2534–40.
69. Aubry MC, et al. Primary pulmonary carcinoma in patients with idiopathic pulmonary fibrosis. Mayo Clin Proc. 2002;77(8):763–70.
70. Turner-Warwick M, Burrows B, Johnson A. Cryptogenic fibrosing alveolitis: clinical features and their influence on survival. Thorax. 1980;35(3):171–80.
71. Carrington CB, et al. Natural history and treated course of usual and desquamative interstitial pneumonia. N Engl J Med. 1978;298(15):801–9.
72. Selman M, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. PLoS One. 2007;2(5):e482.
73. Boon K, et al. Molecular phenotypes distinguish patients with relatively stable from progressive idiopathic pulmonary fibrosis (IPF). PLoS One. 2009;4(4):e5134.
74. Kondo A. Acute exacerbation in idiopathic interstitial pneumonia. In: Interstitial pneumonia of unknown etiology. 1989. p. 33–42.
75. Kondoh Y, et al. Acute exacerbation in idiopathic pulmonary fibrosis. Analysis of clinical and pathologic findings in three cases. Chest. 1993;103(6):1808–12.
76. Colby T. Interstitial lung disease. In: Lombard C, Colby TV, Yousem SA, et al., editors. Atlas of pulmonary surgical pathology. Philadelphia: WB Saunders; 1991. p. 227–306.
77. Collard HR, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2007;176(7):636–43.
78. Kondoh Y, et al. Risk factors of acute exacerbation of idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis. 2010;27(2):103–10.
79. Song JW, et al. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J. 2011;37(2):356–63.
80. King TE Jr, et al. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. Lancet. 2009;374(9685):222–8.
81. Noth I, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186(1):88–95.

82. Zisman DA, et al. A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. N Engl J Med. 2010;363(7):620–8.
83. King TE Jr, et al. BUILD-1: a randomized placebo-controlled trial of bosentan in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2008;177(1):75–81.
84. Bitterman PB, et al. Familial idiopathic pulmonary fibrosis. Evidence of lung inflammation in unaffected family members. N Engl J Med. 1986;314(21):1343–7.
85. Rosas IO, et al. Early interstitial lung disease in familial pulmonary fibrosis. Am J Respir Crit Care Med. 2007;176(7):698–705.
86. El-Chemaly S, Ziegler S, Wilson K, Gahl WA, Moss J, Gochuico BR. Familial pulmonary fibrosis: natural history of preclinical disease. Am J Respir Crit Care Med. 2010;181:A2980.
87. Sakamoto K, et al. Acute exacerbation of idiopathic pulmonary fibrosis as the initial presentation of the disease. Eur Respir Rev. 2009;18(112):129–32.
88. Parambil JG, Myers JL, Ryu JH. Histopathologic features and outcome of patients with acute exacerbation of idiopathic pulmonary fibrosis undergoing surgical lung biopsy. Chest. 2005;128(5):3310–5.
89. Kim DS, et al. Acute exacerbation of idiopathic pulmonary fibrosis: frequency and clinical features. Eur Respir J. 2006;27(1):143–50.
90. Chida M, et al. Subclinical idiopathic pulmonary fibrosis is also a risk factor of postoperative acute respiratory distress syndrome following thoracic surgery. Eur J Cardiothorac Surg. 2008;34(4):878–81.
91. Fukushima K, et al. Prognosis of possible development into diffuse interstitial pneumonia for 127 patients with localized usual interstitial pneumonia. J Jpn Respir Soc. 1999;37:177–82.
92. Araya J, et al. Clinically occult subpleural fibrosis and acute interstitial pneumonia a precursor to idiopathic pulmonary fibrosis? Respirology. 2008;13(3):408–12.
93. Hunninghake GM, et al. MUC5B promoter polymorphism and interstitial lung abnormalities. N Engl J Med. 2013;368(23):2192–200.
94. Putman RK, et al. Association between interstitial lung abnormalities and all-cause mortality. JAMA. 2016;315(7):672–81.
95. Doyle TJ, Hunninghake GM, Rosas IO. Subclinical interstitial lung disease: why you should care. Am J Respir Crit Care Med. 2012;185(11):1147–53.
96. Fell CD. Idiopathic pulmonary fibrosis: phenotypes and comorbidities. Clin Chest Med. 2012;33(1):51–7.
97. Nathan SD, Noble PW, Tuder RM. Idiopathic pulmonary fibrosis and pulmonary hypertension: connecting the dots. Am J Respir Crit Care Med. 2007;175(9):875–80.
98. Kawut SM, et al. Exercise testing determines survival in patients with diffuse parenchymal lung disease evaluated for lung transplantation. Respir Med. 2005;99(11):1431–9.
99. Nathan SD, et al. Pulmonary hypertension and pulmonary function testing in idiopathic pulmonary fibrosis. Chest. 2007;131(3):657–63.
100. Lettieri CJ, et al. Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest. 2006;129(3):746–52.
101. Nadrous HF, et al. Pulmonary hypertension in patients with idiopathic pulmonary fibrosis. Chest. 2005;128(4):2393–9.
102. Cottin V, et al. Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. Eur Respir J. 2005;26(4):586–93.
103. Cottin V, et al. Pulmonary hypertension in patients with combined pulmonary fibrosis and emphysema syndrome. Eur Respir J. 2010;35(1):105–11.
104. Jankowich MD, Rounds SIS. Combined pulmonary fibrosis and emphysema syndrome: a review. Chest. 2012;141(1):222–31.
105. Mejia M, et al. Idiopathic pulmonary fibrosis and emphysema: decreased survival associated with severe pulmonary arterial hypertension. Chest. 2009;136(1):10–5.
106. King TE, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014;370:2083–92.
107. Richeldi L, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370:2071–82.

Chapter 3
Histopathology of IPF and Related Disorders

Amir Lagstein and Jeffrey L. Myers

Introduction

Idiopathic interstitial pneumonias are an important subset of the broader category of diffuse, nonneoplastic interstitial lung diseases [1–3]. Common to all idiopathic interstitial pneumonias is expansion, and potentially distortion, of distal lung interstitium by some combination of inflammation and/or fibrosis. Fibrosis, when present, takes the form of increased numbers of fibroblasts and myofibroblasts and/or collagen deposition. These changes are usually seen in patients with breathlessness or cough, diffuse radiological abnormalities, and evidence of physiologic dysfunction.

Averill Liebow pioneered the notion that morphologic classification of idiopathic interstitial pneumonias is useful in separating them into distinct clinical categories [4]. Since then a number of classification schemes have been proposed. In 2002 an international committee, sponsored by the American Thoracic Society (ATS) and the European Respiratory Society (ERS), proposed a classification scheme reflecting consensus of a large multidisciplinary group of experts [5]. This statement has had a profound impact, influencing management of patients with suspected idiopathic interstitial pneumonias, driving study design for clinical trials, and creating opportunities for research to challenge areas in which evidence was weak. An updated statement published in 2013 highlighted substantial changes that have occurred in the intervening decade that impact the role of biopsy in patients with idiopathic interstitial pneumonia including more refined criteria for identifying patients with nonspecific interstitial pneumonia (NSIP) and the importance of acute exacerbation in our revised understanding of the natural history of untreated idiopathic pulmonary fibrosis (IPF) [6]. The purpose of this

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review is to briefly summarize the relationship between clinical, radiological, and histopathologic features of the idiopathic interstitial pneumonias, focusing primarily on usual interstitial pneumonia (UIP) and IPF. Other forms of diffuse lung disease typically included with the idiopathic interstitial pneumonias are briefly discussed to highlight those features that set them apart from UIP in surgical lung biopsies.

Histopathologic Classification of Idiopathic Interstitial Pneumonias

The previously referenced 2002 consensus classification proposed seven categories of idiopathic interstitial pneumonia, ordering them by relative frequency and separating *histologic patterns* from *clinical-radiologic-pathologic diagnoses* [5]. The 2013 updated statement reorganizes them into "major" (more common) and "minor" (rare) types (see Table 3.1) and subclassifies them into chronic fibrosing, smoking-related, and acute/subacute types. Acute interstitial pneumonia (AIP), a form of rapidly progressive diffuse lung disease first described by Hamman and Rich in the 1930s and 1940s, is not included in this review, which is focused instead on the chronic forms of idiopathic interstitial pneumonia [7, 8].

In the 2013 revision, pleuroparenchymal fibroelastosis (PPFE) is added to lymphoid interstitial pneumonia as rare forms of idiopathic interstitial pneumonia. The authors also recognize that some patients may have unclassifiable disease even after full evaluation. Acute fibrinous and organizing pneumonia (AFOP) and interstitial pneumonias with a bronchiolocentric distribution are added as "rare histologic patterns" of interstitial pneumonia though they are not recognized as specific clinicopathologic entities given the uncertainty as to whether they reflect variants of existing IIP categories rather than distinct diagnostic groups.

Table 3.1 Classification of idiopathic interstitial pneumonias

	International consensus classification
Katzenstein [2]	Clinical-radiologic-pathologic diagnoses [5]
Usual interstitial pneumonia (UIP)	Idiopathic pulmonary fibrosis (IPF)
Desquamative interstitial pneumonia (DIP)/respiratory bronchiolitis interstitial lung disease (RBILD)	Desquamative interstitial pneumonia (DIP)
	Respiratory bronchiolitis interstitial lung disease (RBILD)
Acute interstitial pneumonia (AIP)	Acute interstitial pneumonia (AIP)
Nonspecific interstitial pneumonia (NSIP)	Nonspecific interstitial pneumonia (NSIP)
	Cryptogenic organizing pneumonia (COP)
	Lymphoid interstitial pneumonia (LIP)

Katzenstein has popularized a simplified approach that uses a single unifying terminology and omits cryptogenic organizing pneumonia (COP), also termed idiopathic bronchiolitis obliterans organizing pneumonia (BOOP), and lymphoid interstitial pneumonia (LIP) [2]. The rationale for omitting idiopathic COP is that pathologically it is predominantly an air space, rather than an interstitial, process and clinically mimics infectious pneumonias rather than diffuse interstitial pneumonia. LIP is omitted because it represents a form of lymphoproliferative disorder more closely allied to follicular bronchiolitis on one hand and low-grade lymphoma on the other. Katzenstein's classification scheme serves as a framework for this overview.

Usual Interstitial Pneumonia

Usual interstitial pneumonia (UIP) is the most common of the idiopathic interstitial pneumonias, accounting for about 60% of biopsied patients [9–12]. An ATS consensus statement published in 2000 cemented the link between UIP and IPF by defining the latter as "a specific form of chronic fibrosing interstitial pneumonia limited to the lung and associated with histologic appearance of usual interstitial pneumonia (UIP) on surgical (thoracoscopic or open) lung biopsy" [13]. A revision published in 2011 as a multidisciplinary guideline for diagnosis and management of IPF affirmed UIP as the defining feature of IPF [14]. As these statements imply, UIP and IPF are nearly synonymous terms, exceptions being those patients with underlying systemic connective tissue diseases or occupational/environmental exposures that suggest an etiology for their lung disease (e.g., asbestosis). UIP is also the most common finding in patients with familial interstitial pneumonia [15, 16].

Clinical Features

The clinical features of UIP/IPF are detailed elsewhere in this text (see Chap. 11). Briefly, patients with surgical lung biopsy diagnoses of UIP usually present in the sixth or seventh decade of life with slowly progressive dyspnea and nonproductive cough. Men are affected more commonly than women by a ratio of nearly 2:1. Physical findings include bibasilar inspiratory crackles, a nonspecific but characteristic finding in nearly all patients. Pulmonary function studies show restrictive abnormalities in most patients accompanied by a reduction in the diffusion capacity for carbon monoxide (DL_{CO}) with hypoxemia at rest and/or with exercise (see Chap. 5). No single pharmacologic agent or combination of drugs has shown consistent efficacy in all patients with UIP, although a number of novel antifibrotic therapies are now available and show promise in patients with mild disease (see Chap. 13). Lung transplantation is used in some patients, but its application is limited due to older age and frequent comorbidities. In most patients UIP pursues a progressive

course with median survivals from the time of diagnosis of about 3 years in retrospective observational case-based studies [9, 17].

Occasional patients present with a more acute onset of respiratory symptoms that may mimic the clinical presentation of acute interstitial pneumonia (AIP) [18, 19]. This syndrome has been termed *acute exacerbation of IPF* (or *accelerated UIP*) and occurs in as many as 14% of untreated patients and about half of those who die from respiratory failure (see Chap. 17) [20, 21]. Histopathologic findings consistent with acute exacerbation are common at autopsy in UIP patients [22]. Acute exacerbation is defined as the sudden onset of marked respiratory deterioration in patients with UIP characterized by the development of widespread new alveolar abnormalities on chest imaging [23]. Diagnosis depends on exclusion of other known and potentially treatable causes of clinical worsening, such as cardiac disease, pulmonary embolism, and infection. This revised definition dispenses with the requirement that an episode of acute exacerbation be idiopathic. The definition also links acute exacerbation to more specific radiological (i.e., widespread ground glass opacities/consolidation) and histologic (i.e., diffuse alveolar damage or rarely organizing pneumonia) findings – in a UIP/IPF patient with precipitous respiratory decompensation. This revision is consonant with well-recognized criteria for the acute respiratory distress syndrome (ARDS), and in effect acknowledges that "acute exacerbation" represents the development of ARDS in a patient with UIP/IPF. Most patients are known to have UIP at the time of acute worsening, but some patients with clinically occult IPF present with acute exacerbation without a previously established diagnosis of fibrotic lung disease [19]. Therefore, if and when such patients undergo lung biopsy, the diagnosis of UIP often comes as a diagnostic "surprise" [19, 24]. The prognosis is grim, with short-term mortality rates in excess of 50% in the majority of reported series.

The relative role of imaging studies and surgical lung biopsies in patients with UIP has changed over the last decade and a half, as reflected in the most recently published guideline for diagnosis [14]. High-resolution computed tomography (HRCT) scans have greatly improved diagnostic accuracy over conventional chest radiography, revolutionizing the role of radiology in managing patients with diffuse interstitial lung diseases (see Chap. 4). HRCT scans in about half of patients show a characteristic combination of peripheral (subpleural), irregular, linear ("reticular") opacities involving predominantly the lower lung zones with associated architectural distortion in the form of traction bronchiectasis and bibasilar honeycomb change [25–28]. Experienced radiologists can make a specific diagnosis of UIP with a high degree of accuracy in patients with this combination of findings thus obviating the need for lung biopsy. Lung biopsy is increasingly limited to those patients with atypical radiological findings, meaning that there is a growing selection bias toward reserving surgical lung biopsy for patients with potentially "discordant" or atypical radiological findings. It is this change that has created confusion around the relative roles of clinicians, radiologists, and pathologists in biopsied patients. In this context most of the evidence indicates that a biopsy diagnosis of UIP remains the single most important predictor of outcome at the time of diagnosis and thus remains a diagnostic "gold standard" of sorts [25, 29].

Pathologic Features

Usual interstitial pneumonia is a specific morphologic entity defined by a combination of (1) fibrosis, (2) a heterogeneous ("patchwork") distribution of qualitatively variable abnormalities, (3) architectural distortion in the form of honeycomb change and/or scars, and (4) fibroblast foci [1, 2, 30–32]. The histologic hallmark of UIP in surgical lung biopsies is a heterogeneous or variegated appearance resulting from irregularly distributed fibrotic scarring, honeycomb change, interstitial inflammation, and relatively unaffected lung (Fig. 3.1). This distinctive "patchwork" appearance is fundamental to recognizing UIP at low magnification.

Fibrosis predominates over inflammation in classical UIP and comprises dense eosinophilic collagen deposition, often accompanied by smooth muscle hyperplasia. Fibroblast foci are a characteristic but nonspecific finding, representing small interstitial foci of acute lung injury in which fibroblasts and myofibroblasts are arranged in a linear fashion within a pale-staining matrix (Fig. 3.2) [33]. Overlying

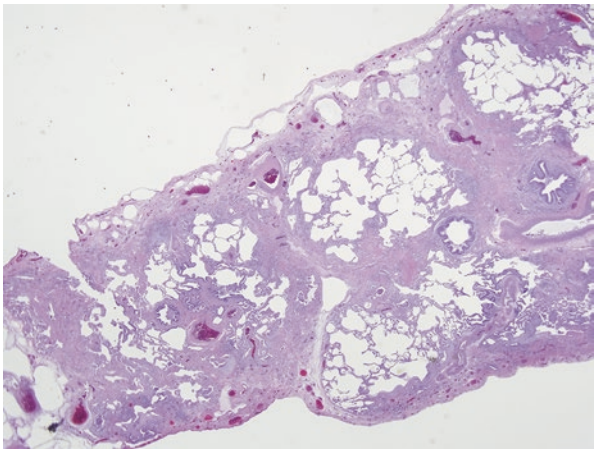

Fig. 3.1 Low magnification photomicrograph of surgical lung biopsy showing UIP (hematoxylin and eosin stain; original magnification 20×). There is patchy fibrosis affecting subpleural and paraseptal parenchyma as well as bronchovascular bundles, leaving intervening lung tissue relatively unaffected. The fibrosis is paucicellular with minimal associated inflammation

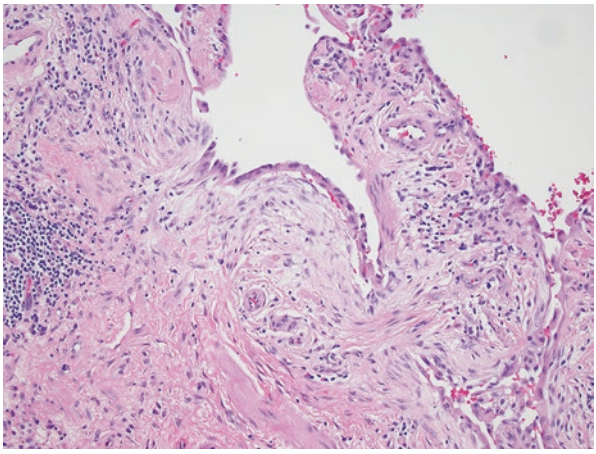

Fig. 3.2 High magnification photomicrograph showing fibroblast focus in UIP (hematoxylin and eosin stain; original magnification 200×). A small area of subepithelial stromal pallor demonstrates plump fibroblasts and myofibroblasts arranged in a vaguely linear fashion. The fibroblast focus is sandwiched between overlying type 2 pneumocytes and adjacent fibrotic scar

epithelium consists of hyperplastic pneumocytes or columnar non-ciliated bronchiolar cells. Fibroblast foci, although seen in other conditions, are characteristic of UIP and an important diagnostic feature when seen in the context of patchy fibrosis and honeycomb change. The presence of these microscopic zones of acute lung injury set against a backdrop of chronic scarring accounts for the *temporal heterogeneity* typical of UIP.

Honeycomb change is present in most surgical lung biopsies and is another important diagnostic feature. Honeycomb change comprises cystic-like, dilated air spaces that are frequently lined by columnar respiratory epithelium in scarred, fibrotic lung tissue (Fig. 3.3). The honeycomb spaces affect primarily peripheral subpleural lung, resulting in a characteristic *cobblestone* appearance of the visceral pleural surface that resembles cirrhotic liver (Fig. 3.4). Fibrotic scars that obscure the underlying lung architecture without associated honeycomb change are another form of architectural distortion characteristic of UIP (Fig. 3.5). Smooth muscle hyperplasia is commonly seen in areas of fibrosis and honeycomb change and can be striking in some patients. The histopathologic findings described for patients with sporadic IPF are indistinguishable from the findings seen in patients with familial disease [15, 16].

Usual interstitial pneumonia is a relatively common finding in patients with underlying systemic connective tissue disease (CTD), especially rheumatoid arthritis, systemic sclerosis, and inflammatory myositis [34–36]. By consensus, UIP in patients with systemic CTD (UIP-CTD) is not considered IPF. While in most patients with UIP-CTD, the diagnosis of CTD precedes diagnosis of UIP, occasionally the interstitial pneumonia is diagnosed before the patient is known to have CTD [37].

In some patients an underlying CTD may be suspected based on a combination of clinical, laboratory, and/or morphological features that fall short of meeting diagnostic criteria for a specific CTD. An ERS/ATS task force published a research statement in 2015 proposing criteria for what was termed *interstitial pneumonia*

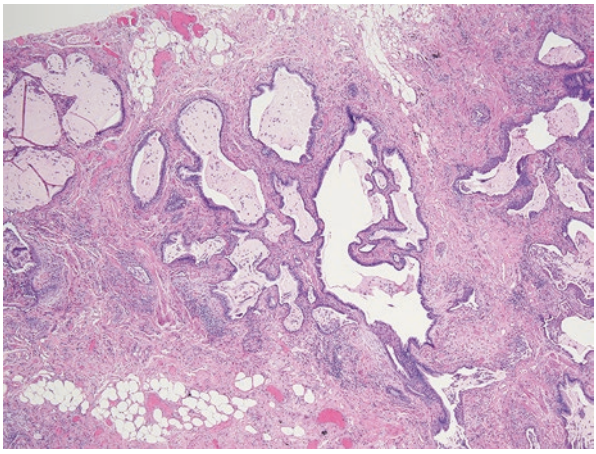

Fig. 3.3 Low magnification photomicrograph showing honeycomb change in a surgical lung biopsy from a patient with UIP/IPF (hematoxylin and eosin stain; original magnification 40×). Cystic spaces situated in densely scarred subpleural lung (visceral pleural surface at upper left) are lined by bronchiolar epithelium

Fig. 3.4 Photograph showing visceral pleural surface (left) and cut surface (right) of autopsy lung from patient with UIP/IPF. Peripheral, subpleural honeycomb change results in a *cobblestone* appearance of the lung surface

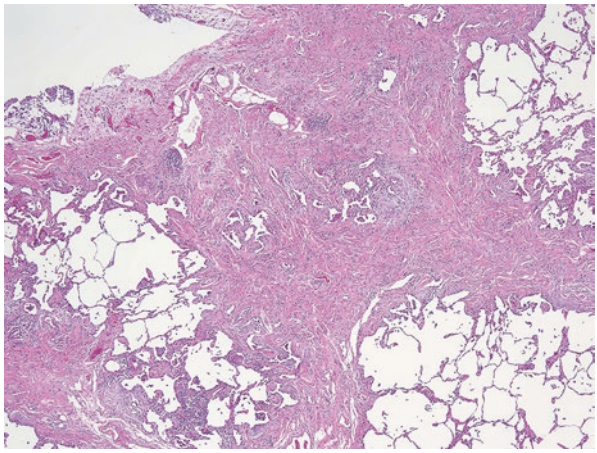

Fig. 3.5 Low magnification photomicrograph showing area of subpleural scarring without well-developed honeycomb change in a patient with UIP (hematoxylin and eosin stain; original magnification 40×). The area of scarring effaces the lung architecture and is characterized by a combination of dense collagen deposition and smooth muscle hyperplasia with minimal inflammation

with autoimmune features (IPAF) [38]. This statement is not meant for clinical decision-making or diagnosis but instead to standardize criteria for defining meaningful patient cohorts for future study. The practical utility of identifying IPAF as a specific entity separate from IPF remains to be proven. UIP was not included as one of the proposed morphologic criteria which instead comprise NSIP, organizing pneumonia (OP), "NSIP with OP overlap," and LIP. Two additional findings were proposed that may apply to otherwise classical UIP: interstitial lymphoid aggregates with germinal centers and diffuse lymphoplasmacytic infiltration with or

without lymphoid follicles. These six criteria were chosen as they were deemed "highly associated with, but not diagnostic for" CTD. Although UIP was not included in the morphologic "domain," a patient with UIP may still be included in the IPAF category based on the presence of one or both of the described morphologic criteria in combination with a criterion from another domain. Patients with histologically classic UIP without either of these two superimposed findings may also be included in the IPAF category by satisfying criteria in the other domains. While the practical utility of IPAF as a diagnostic category remains unknown, from a histologic standpoint, it remains the case that UIP in IPF patients cannot be reliably separated from UIP in patients with underlying systemic CTD on the basis of histology alone. And while lymphoid hyperplasia in the form of peribronchiolar lymphoid aggregates ("follicular bronchiolitis") is more common in patients with underlying rheumatoid arthritis, it also occurs, albeit less commonly, in patients with IPF (Fig. 3.6) [39]. For that reason the presence or absence of associated lymphoid hyperplasia in an individual surgical lung biopsy demonstrating otherwise typical UIP cannot by itself be used to separate IPF from CTD-associated pulmonary fibrosis.

Biopsies from patients with acute exacerbation usually show a combination of UIP and superimposed diffuse alveolar damage (DAD) (Fig. 3.7) [18, 19]. The features of DAD may be patchy and typically include some combination of confluent alveolar septal thickening and distortion by fibroblasts and myofibroblasts with minimal associated inflammatory cells, marked hyperplasia of cytologically atypical type 2 pneumocytes, hyaline membranes, fibrin thrombi in small vessels, and squamous metaplasia of bronchiolar epithelium. In other patients the superimposed pattern of acute lung injury has been reported to more closely resemble organizing pneumonia, though the organizing phase of DAD shows substantial histologic overlap with organizing pneumonia. In some patients with acute exacerbation, the vagaries of sampling may account for the failure to identify diagnostic evidence of DAD on the one hand or of underlying UIP on the other.

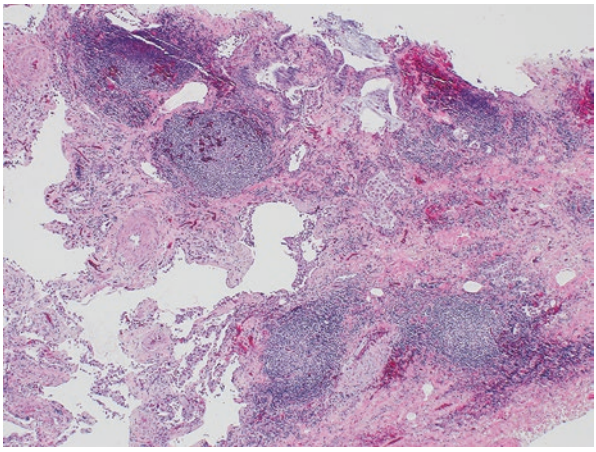

Fig. 3.6 Low magnification photomicrograph of UIP with lymphoid hyperplasia comprising multiple lymphoid follicles with secondary germinal centers localized to the peribronchiolar interstitium and pleura. This finding may be seen in idiopathic UIP/IPF or in connective tissue disease-associated UIP, though somewhat more commonly in the latter (hematoxylin and eosin stain; original magnification 40×)

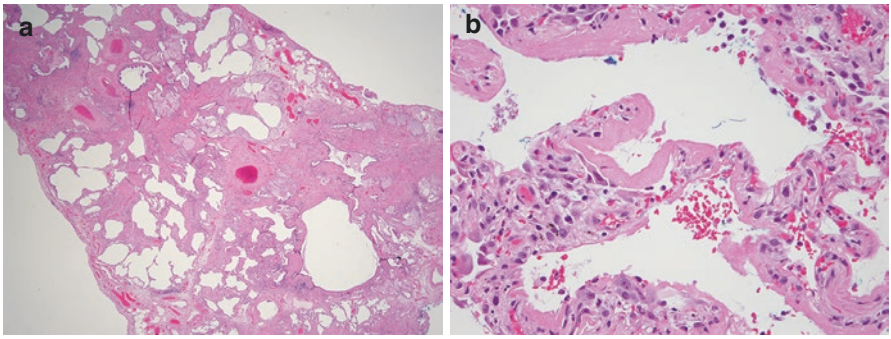

Fig. 3.7 (**a**) Low magnification photomicrograph showing combination of "patchwork fibrosis" and honeycomb change typical of UIP in a patient with IPF (hematoxylin and eosin stain; original magnification 20×). (**b**) High magnification photomicrograph from different area of same biopsy showing an area of diffuse alveolar damage (hematoxylin and eosin stain; original magnification 400×). Alveolar septa show a scant inflammatory infiltrate, myofibroblasts and a few residual pneumocytes associated with distinct eosinophilic hyaline membranes. Hyaline membranes are the histologic hallmark of diffuse alveolar damage, establishing the diagnosis of acute exacerbation in a patient with IPF for whom there is no other identifiable cause for acute respiratory distress

No single histologic finding consistently predicts prognosis in individual patients with UIP. Patients with more extensive fibroblast foci have experienced shorter mean survivals in some studies [40–43], while other investigators have failed to demonstrate the same relationship to survival in patients with neither clinical nor histologic evidence of acute exacerbation [17, 44].

Desquamative Interstitial Pneumonia/Respiratory Bronchiolitis Interstitial Lung Disease

Desquamative interstitial pneumonia (DIP) and respiratory bronchiolitis interstitial lung disease (RBILD) are two highly related and overlapping forms of diffuse interstitial lung disease typically grouped with the idiopathic interstitial pneumonias. Katzenstein has proposed collapsing the two into a single category for reasons described later. DIP/RBILD is uncommon, accounting for only a small minority of surgical lung biopsies from patients with idiopathic interstitial pneumonias [9–11]. They are separated from UIP/IPF because of marked differences in natural history and prognosis [45, 46].

Clinical Features

DIP/RBILD affects younger patients, with a mean age at diagnosis in the fourth or fifth decade of life [1, 2]. Nearly all patients have strong histories of cigarette

smoking prompting many to consider DIP/RBILD a form of smoking-related lung disease rather than an idiopathic condition [26, 47]. Pulmonary function tests in most patients show evidence of mild restrictive disease accompanied by a moderate decrease in diffusing capacity. HRCT scans typically show patchy ground glass opacities, often with a lower lung zone distribution, without the traction bronchiectasis and honeycomb change typical of UIP.

DIP/RBILD is associated with a significantly better prognosis than UIP. Overall survival is nearly 90%, ranging from around 70–80% in older studies to 100% in more recently published series [1, 46]. Higher survival rates in more recent studies may reflect a trend toward assigning cases with associated fibrosis to the category of NSIP. RBILD is associated with an equally good or better prognosis [46, 48, 49]. Retrospective case series suggest smoking cessation as an important therapeutic strategy, but the impact on outcome is controversial [48].

Pathologic Features

DIP/RBILD is characterized by the presence of pigmented ("smoker's") macrophages within the lumens of distal airways (i.e., respiratory bronchioles) and air spaces. The macrophages are distinctive in that they have abundant cytoplasm containing finely granular dusty brown pigment. In RBILD the changes are patchy at low magnification and limited to the airways with only minimal or mild interstitial inflammation or fibrosis (Fig. 3.8). The appearance is indistinguishable from isolated respiratory bronchiolitis (RB), a common, incidental finding in otherwise asymptomatic cigarette smokers without clinical evidence of restrictive lung disease. RBILD may include mild fibrotic thickening of alveolar septa without

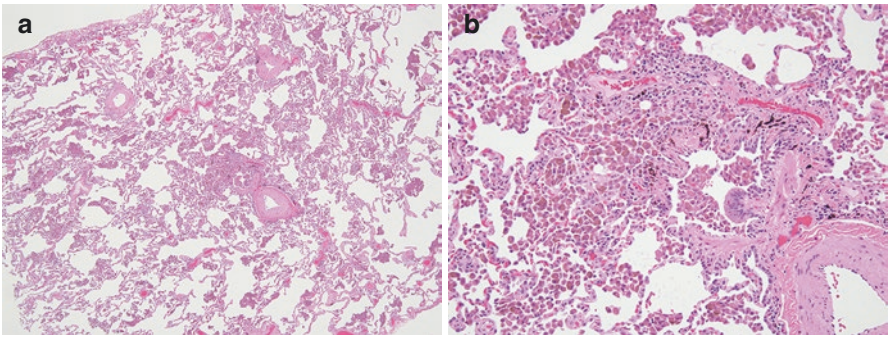

Fig. 3.8 (**a**) Low magnification photomicrograph showing respiratory bronchiolitis (hematoxylin and eosin; original magnification 40×). Pigmented alveolar macrophages are clustered within the lumens of distal bronchioles and peribronchiolar air spaces without the fibrosis or architectural distortion typical of UIP. (**b**) High magnification photomicrograph from same biopsy illustrated in A showing respiratory bronchiolitis (hematoxylin and eosin; original magnification 400×). Pigmented ("smoker's") macrophages are loosely clustered within the lumen of a respiratory bronchiole and peribronchiolar alveolar spaces

architectural distortion immediately adjacent to the visceral pleura and bronchovascular bundles in some patients (Fig. 3.9) [50]. This pattern of concomitant fibrosis has been referred to using a variety of terms, most recently smoking-related interstitial fibrosis (SRIF), and like respiratory bronchiolitis, SRIF does not by itself predict for clinically or physiologically significant lung disease [51].

Historically DIP was defined by not only the airway-centered changes described in RBILD but also uniform alveolar septal thickening due to a combination of mild fibrosis and inflammation (i.e., interstitial pneumonia). The advent of SRIF as a form of fibrosis in patients who otherwise fit comfortably in the category of RBILD and recognition of NSIP as a form of interstitial pneumonia distinctly different from UIP have combined to effectively eliminate DIP as a modern category of idiopathic interstitial pneumonia. Patients historically labeled as having DIP are increasingly assigned to the categories of either RBILD (with SRIF) or NSIP. As originally defined, the key feature that separated DIP from UIP was that the interstitial changes were more uniform at low magnification with a focally bronchiolocentric distribution and without honeycomb change or fibrotic scarring (Fig. 3.10) [4, 52].

Significance of Pathological Diagnoses of DIP or RBILD

Neither RBILD nor DIP should be viewed as free-standing histopathologic entities, since areas resembling both commonly occur as incidental findings in cigarette smokers with other lung diseases, including UIP [31, 53]. In addition, there are no histologic changes that reliably separate patients with DIP/RBILD from those with other lung diseases in whom RB and "DIP-like reactions" represent incidental findings [53]. For that reason, DIP/RBILD should be diagnosed only when other forms

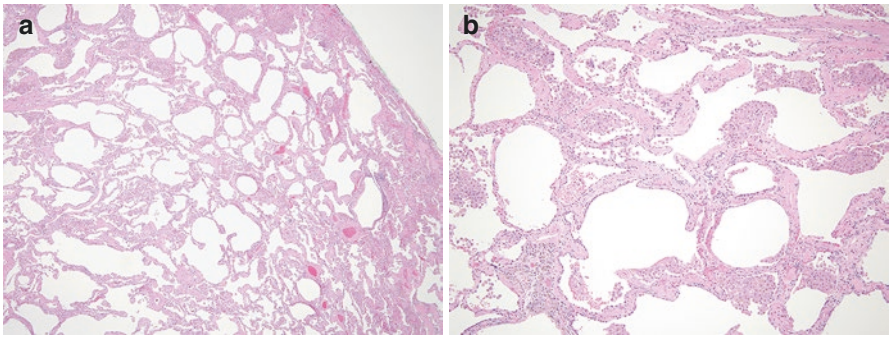

Fig. 3.9 (**a**) Low magnification photomicrograph showing subpleural smoking-related interstitial fibrosis (SRIF) in a patient with RBILD (hematoxylin and eosin stain; original magnification 40×). Subpleural alveolar septa are mildly and diffusely thickened by paucicellular, eosinophilic collagen deposition without architectural distortion in the form of tissue-destructive scarring or honeycomb change. (**b**) Intermediate magnification photomicrograph illustrating uniform alveolar septal thickening by dense eosinophilic collagen deposition with minimal associated interstitial inflammation in SRIF complicating RBILD (hematoxylin and eosin stain; original magnification 100×)

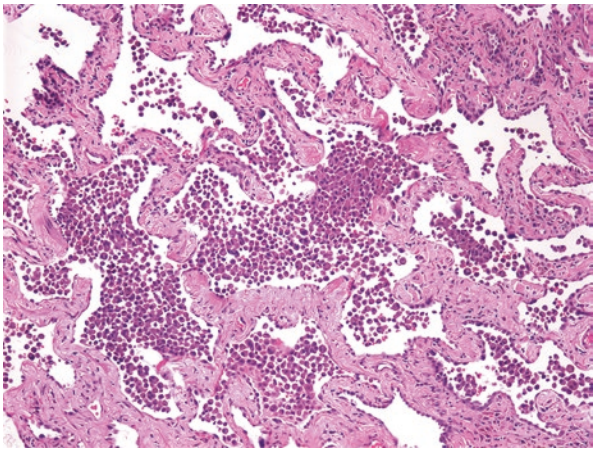

Fig. 3.10 Intermediate magnification photomicrograph showing the features that historically defined DIP: an interstitial pneumonia characterized by mild fibrosis and inflammation resulting in uniform thickening of alveolar septa lined by reactive type 2 pneumocytes and prominent pigmented macrophages within alveolar spaces (hematoxylin and eosin stain; original magnification 100×). Increasingly patients historically assigned to the category of DIP are more likely to be classified as either RBILD (with *smoking-related interstitial fibrosis* – SRIF) or NSIP depending on the characteristics and extent of the interstitial changes

of interstitial lung disease have been vigorously excluded by carefully examining all aspects of the microscopic slides and by correlating the surgical lung biopsy diagnosis with clinical and radiological features to establish the presence of physiologically meaningful restrictive lung disease [54]. While incidental RB can be recognized on transbronchial biopsy (TBB), this technique cannot be used to diagnose DIP/RBILD.

Nonspecific Interstitial Pneumonia

Nonspecific interstitial pneumonia/fibrosis (NSIP) was proposed in 1994 as a form of chronic interstitial pneumonia characterized by relatively uniform expansion of alveolar septa by inflammation and/or fibrosis without the geographic and temporal heterogeneity of UIP [55]. As the term implies, the histologic findings in NSIP are not specific. Findings indistinguishable from NSIP can occur focally in other conditions, most importantly UIP. The findings are also nonspecific from a clinical perspective given that identical changes can occur in surgical lung biopsies from patients with a variety of underlying causes or associations, including hypersensitivity pneumonia and various systemic connective tissue diseases [47, 55, 56]. Recognizing idiopathic NSIP as a distinct entity is therefore a process of exclusion that, like DIP/RBILD, requires careful correlation with clinical and radiological information. While the previously referenced 2002 consensus classification suggested that NSIP be considered "a *provisional diagnosis* until there is further clarity

on the nature of the corresponding clinical condition," the 2013 updated statement recognizes NSIP as a distinct clinicopathological entity that should be separated from UIP due to important differences in natural history, treatment, and outcome [6, 30, 32, 47, 56].

Clinical Features

NSIP is the second most common idiopathic interstitial pneumonia, accounting for as many as a third of patients undergoing surgical lung biopsy in retrospective series [9–12, 47]. NSIP fails to show the gender predilection for men seen in UIP, and in some series is more common in women [56]. NSIP also differs from UIP in that it tends to affect younger patients, with an average age at diagnosis of around 50 years [47, 56]. Shortness of breath and dry cough are the most common complaints, often developing in an insidious fashion indistinguishable from that described for patients with UIP. Pulmonary function studies show restricted lung volumes and abnormalities of oxygenation, although the degree of abnormality tends to be less severe compared to patients with UIP. CT scans show a nonspecific but characteristic combination of ground glass opacities, irregular lines, and traction bronchiectasis, occasionally with subpleural sparing.

Multiple studies have now confirmed the survival advantage associated with a diagnosis of NSIP compared to UIP [47, 56]. Median survival for all NSIP cases is over 9 years, with the best prognosis occurring in patients with minimal fibrosis (i.e., "cellular NSIP"). Most patients with cellular NSIP survive, but about half have persistent stable disease. Patients in whom fibrosis predominates in surgical lung biopsies do worse than those with more cellular lesions, although still better than UIP [11, 55, 57–60]. Mortality rates for patients with fibrotic NSIP vary widely, ranging from 11% to 68% in various studies (mean ± STD, 30.4 ± 18.9%) [10, 11, 55–58, 61]. Reported 5-year survivals of such patients are about 76% compared to about 45% for UIP [37, 60]. Survivors typically have persistent lung disease. To some extent variation in mortality rates reported for patients with fibrotic NSIP reflects differences in histologic definitions and the difficulty in separating fibrotic NSIP from UIP. Corticosteroids have not been prospectively evaluated in a randomized fashion but may be effective in a subset of patients, especially those with minimal associated fibrosis [57].

Pathologic Features

A diagnosis of NSIP in surgical lung biopsies requires the presence of a chronic interstitial pneumonia without findings to prompt diagnosis of a more specific pathologic process. Unlike UIP, NSIP is in many respects a diagnosis of exclusion. Defined in this way, NSIP spans a range of histologic abnormalities ranging from a predominantly cellular process (i.e., cellular NSIP) to paucicellular lung fibrosis (i.e., fibrotic NSIP). The most cellular forms are characterized by an alveolar septal

infiltrate of mononuclear cells that may be patchy or diffuse (Fig. 3.11). Whether patchy or diffuse, the qualitative features of the interstitial abnormalities remain constant without the geographic and temporal heterogeneity associated with UIP. The inflammatory infiltrate consists of lymphocytes and variable numbers of admixed plasma cells. Neutrophils, eosinophils, and histiocytes are relatively inconspicuous. Granulomas are rare in NSIP and, if present, should raise other considerations such as infection or hypersensitivity pneumonia.

The relative frequency of fibrosis in NSIP is variable. Patients with fibrotic NSIP outnumber patients with cellular NSIP by a ratio of nearly 4:1 in published studies, but this may reflect selection bias in that most reports are from tertiary referral centers where patients with fibrotic interstitial lung disease may be overrepresented. In addition, there are no clearly articulated criteria for separating cellular from fibrotic NSIP. The term fibrotic NSIP should be limited to those cases in which paucicellular fibrosis with minimal or mild inflammation is the predominant feature. Defined in this way, the extent of interstitial fibrosis is variable. Fibrosis takes the form of uniform collagen accumulation resulting in expansion of alveolar septa and peribronchiolar interstitium (Fig. 3.12) without the patchwork distribution characteristic of UIP. Pathology reports should comment on the presence and extent of interstitial fibrosis, since it is associated with significantly increased risk for disease-specific mortality [1, 2, 47, 55, 56]. Associated smooth muscle hyperplasia tends to be less extensive than that seen in UIP. Fibroblast foci may be present but are typically less numerous and are inconspicuous compared to UIP. Honeycomb change and broad zones of scarring should be absent. The absence of honeycomb change is perhaps the single most important feature in distinguishing fibrotic NSIP from UIP. Patchy intraluminal fibrosis resembling organizing pneumonia is common but should be a focal rather than a dominant finding.

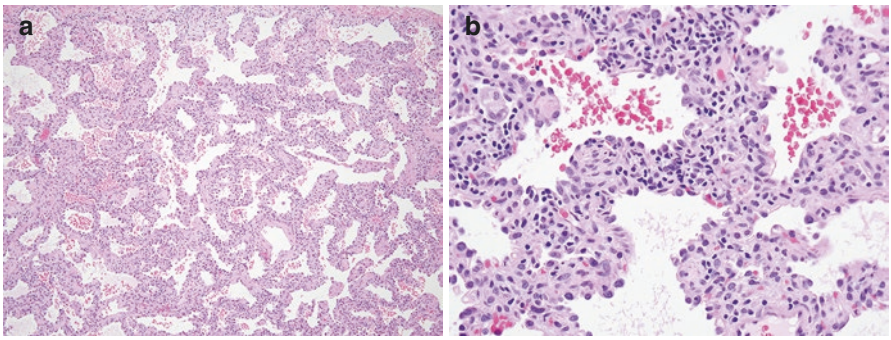

Fig. 3.11 (**a**) Intermediate magnification photomicrograph of cellular NSIP (hematoxylin and eosin stain; original magnification 100×). Alveolar septa are uniformly thickened by an infiltrate of mononuclear inflammatory cells with minimal fibrosis and preservation of lung architecture. (**b**) High magnification photomicrograph showing expansion of alveolar septa by an interstitial infiltrate of predominantly lymphocytes and occasional plasma cells in the same patient with cellular NSIP (hematoxylin and eosin stain; original magnification 400×)

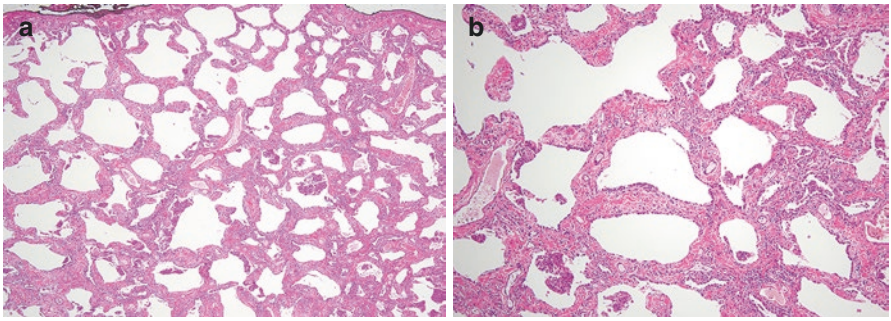

Fig. 3.12 (**a**) Low magnification photomicrograph illustrating fibrotic NSIP (hematoxylin and eosin stain; original magnification 40×). Alveolar septa are uniformly expanded by collagen deposition with mild inflammation. There is no associated scarring or honeycomb change. (**b**) Intermediate magnification photomicrograph from the same patient with fibrotic NSIP illustrating expansion of alveolar septa by eosinophilic collagen with a mild and patchy associated infiltrate of mononuclear inflammatory cells (hematoxylin and eosin stain; original magnification 100×). Thickened alveolar septa are lined by reactive pneumocytes, a nonspecific but common manifestation of interstitial injury

Pleuroparenchymal Fibroelastosis

The newest addition to the group of idiopathic interstitial pneumonias is idiopathic pleuroparenchymal fibroelastosis (PPFE), regarded (along with LIP) as one of the rare subtypes. Indeed, in the authors' cumulative experiences, we have encountered only a handful of cases of PPFE on surgical lung biopsy or explant pneumonectomy in transplant patients. The condition is thought to have been first described in the Japanese literature in 1992 as a form of idiopathic upper lobe fibrosis but acquired its current name in 2004 in a case series of five patients [62]. PPFE is also reported as a rare manifestation of chronic graft-versus-host disease in bone marrow transplant patients [63], as well as the histologic correlate to an uncommon form of chronic lung transplant rejection termed restrictive allograft syndrome [64].

Clinical Features

Patients present with persistent shortness of breath, dyspnea on exertion, dry cough, and restrictive physiology on pulmonary function testing [65, 66]. Interestingly, up to a third of patients experienced spontaneous pneumothorax. Patients have ranged in age quite widely from 13 to 87 years (53 years, median), without significant gender predilection. There is no significant association with smoking, and serum titers of various autoantibodies are elevated in a subset of cases. Chest CTs typically show an upper and middle lung zone-predominant distribution of pleural and subpleural reticular parenchymal fibrosis with clear demarcation between affected and unaffected zones. The natural history of PPFE is not known for certain, ranging from

long-term stability, to slow mild progression, to death from progressive disease. This inconsistency is likely due, in part, to the inclusion of non-PPFE cases in some reports of PPFE given the lack of standardized diagnostic criteria.

Pathologic Features

PPFE is characterized by the presence of pleural fibrosis and subpleural parenchymal fibroelastotic scarring which lends the process its name (Fig. 3.13a, b). The most distinctive feature of the scarring is the combination of admixed elastin and collagen fibers, with an overabundance of the former over the latter, endowing the lesion with a pale basophilic hue at all magnifications. While the fibrosis is most prominent in the lung immediately beneath the pleura, there should be evidence of deeper involvement of the lung parenchyma consistent with a diffuse fibrosing process rather than a superficial (localized) phenomenon. Fibrotic zones are typically sharply demarcated from the non-fibrotic areas, and the fibrotic process often seems to take the appearance of a sweeping or pushing "front." Fibroblast foci may be found. It is most prominent in the upper lobes, but there should be histologic evidence of involvement of other lobes in well-sampled cases with multiple-lobe biopsies.

The most important differential diagnosis for PPFE is UIP, especially as there are occasional cases of UIP that are rich in elastic fibers (Fig. 3.14). This is straightforward in most cases since PPFE lacks the "patchwork," haphazard pattern of fibrosis characteristic of UIP. In addition, though PPFE results in confluent fibrous scarring, it does not cause either macroscopic or microscopic honeycombing.

The histologic findings in PPFE are indistinguishable from the much more common pulmonary apical cap [67]. Distinction hinges on the clinical context and the radiologic extent and distribution of disease.

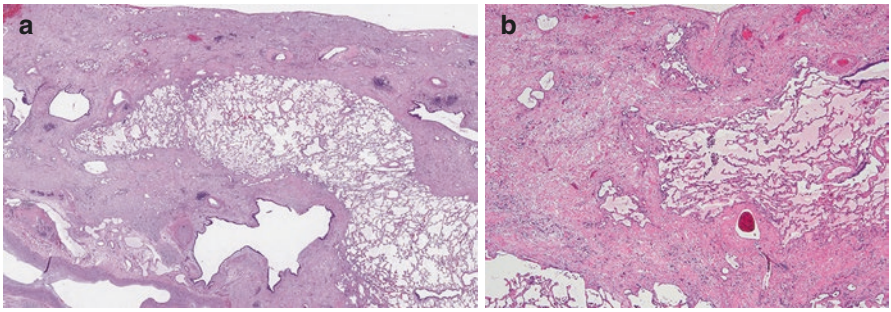

Fig. 3.13 (**a**) Low magnification photomicrograph demonstrating pleuroparenchymal fibroelastosis (PPFE) (hematoxylin and eosin stain; original magnification 10×). There is diffuse fibroelastotic scarring with subpleural accentuation. Notice the sharp demarcation between fibrotic and non-fibrotic zones. (**b**) Intermediate magnification view highlighting the distinctive mix of elastosis and collagen fibrosis in PPFE (hematoxylin and eosin stain; original magnification 40×)

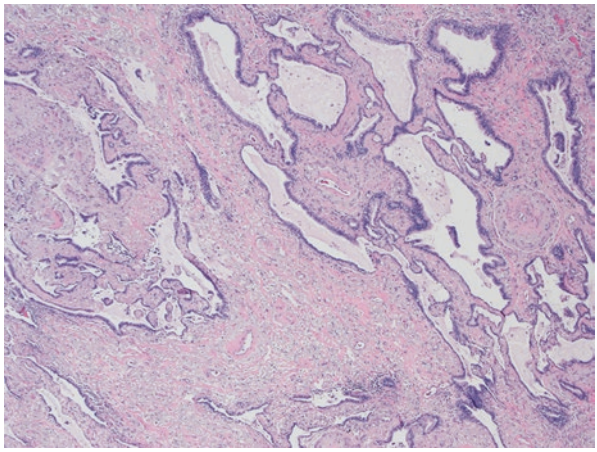

Fig. 3.14 Low magnification photomicrograph of a case of UIP rich in elastotic fibrosis. The appearance is deceptively similar to PPFE; however, microscopic honeycomb change is evident. Furthermore, other foci (not shown) demonstrated a haphazard distribution to the fibroelastosis which is inconsistent with PPFE (hematoxylin and eosin stain; original magnification 40×)

The Role of Surgical Lung Biopsy in Classification and Diagnosis of Idiopathic Interstitial Pneumonias

"Pattern" Versus "Diagnosis" for Reporting the Results of Surgical Lung Biopsy

The authors of the 2002 consensus classification advocated the use of the term *pattern* when reporting lung biopsy findings in order to distinguish the pathological diagnosis from a final "clinico-radiologic-pathologic diagnosis," a recommendation that remains unaltered by the 2013 updated classification. This recommendation emphasizes the value of an iterative dynamic multidisciplinary process that correlates histologic findings with other relevant data, as reviewed in greater detail in Chap. 12, but such a multidisciplinary process may be unnecessary and in some cases potentially dangerous [32]. It is unnecessary in that many pathological diagnoses are not isolated events but rather essential components of an iterative process in which final interpretation is dynamic and framed by ongoing data collection. For example, a lung biopsy diagnosis of adenocarcinoma may be reinterpreted as metastatic adenocarcinoma after discovery of a previously occult primary malignancy outside the lung. This possibility should not drive an argument for substituting the term "adenocarcinoma pattern," terminology that may interfere with the end-user's recognition that the diagnosis of malignancy is certain. The use of the term "pattern" may result in confusion regarding the circumstances in which the specificity of the histopathologic findings is, in fact, the primary driver of a final diagnosis.

UIP stands alone among the idiopathic interstitial pneumonias in being a specific histopathological entity. Several studies have demonstrated the primary role of a lung biopsy diagnosis of UIP in establishing a clinical diagnosis of IPF [25, 26, 29, 68, 69]. This is especially important given that increasingly patients are selected for lung biopsy because there is some level of doubt regarding the likelihood of IPF,

usually based on an atypical radiological pattern of disease. It is precisely in this context that a biopsy diagnosis of UIP establishes the clinical diagnosis with certainty, and in this context, the biopsy result remains the single most powerful predictor of disease-specific mortality at the time of diagnosis [10, 25]. The histopathologic findings are less specific in all other forms of idiopathic interstitial pneumonia, and perhaps for these a stronger argument can be made for using the term "pattern." In the biased view of these authors, however, this diminishes the role of the pathologist to a technician who merely provides histologic descriptions rather than a physician who engages in proactively integrating histological observations with clinical information to arrive at a diagnosis. This proactive approach is common in other areas of medicine in which the pathology report serves as a platform for integrating relevant clinical, laboratory and radiological information that facilitates accurate interpretation of microscopic findings.

The second argument for using the term "pattern" in reporting diagnoses of UIP is that it occurs in patients for whom the term IPF is deemed inappropriate. The implication is that sorting patients with UIP into different clinical groups impacts therapeutic options and outcome. The preponderance of evidence suggests that patients with a biopsy diagnosis of UIP have a form of fibrotic lung disease that is relatively insensitive to conventional immunosuppressive therapy and likely to be associated with a progressive course regardless of the underlying or associated condition. Although a number of studies have indicated a better prognosis for UIP associated with connective tissue diseases, others have failed to demonstrate the same survival advantage [17, 70–72]. The differences observed in some studies may be related to confounding factors such as younger age, greater prevalence of women, and lower smoking rates in patients with connective tissue diseases, factors that themselves are associated with a better prognosis in patients with UIP/IPF. In addition the survival advantage does not apply to patients with rheumatoid arthritis, the largest subset of patients with connective tissue disease-associated UIP [72]. Similarly, asbestos can be viewed as a potential cause of UIP that carries significant legal ramifications but with few if any meaningful differences between asbestosis and IPF in terms of signs and symptoms, morphology, treatment response, or natural history [73, 74]. Even in patients with an exposure history suggesting chronic hypersensitivity pneumonias as an alternative, a biopsy diagnosis of UIP predicts a natural history indistinguishable from IPF [75–79].

Distinguishing Fibrotic NSIP from UIP

Separating fibrotic NSIP from UIP is perhaps the greatest challenge when it comes to making meaningful distinctions among the idiopathic interstitial pneumonias [30]. Separating fibrotic NSIP from UIP hinges on recognition of the patchwork distribution, fibroblast foci, and honeycomb change typical of UIP. Recognition of any one of these features in a biopsy for which a diagnosis of fibrotic NSIP is being

contemplated is reason for caution. In this circumstance correlation with other clinical data, especially HRCT findings, may be helpful.

The primary problem is that areas typical of NSIP can occur as a focal phenomenon in other conditions making sampling bias a potential barrier to accurate diagnosis. In a review of 20 explanted lungs with UIP, for example, all but 3 showed isolated areas that were indistinguishable from NSIP ("NSIP-like areas") [31]. Other studies have shown that the presence of UIP in even a single piece of tissue defined a survival curve typical of IPF in patients from whom surgical lung biopsies taken from more than one site demonstrated both UIP and NSIP ("discordant UIP") [80, 81]. For these reasons establishing a diagnosis of idiopathic NSIP requires the absence of clinical, radiological, or pathological findings to suggest an alternative. For example, a biopsy diagnosis of fibrotic NSIP in a patient with bibasilar honeycomb change on HRCT is almost certainly a sampling error in a patient with UIP. While the 2002 consensus classification would suggest that this issue be resolved by producing a pathology report with a diagnosis of *fibrotic NSIP pattern*, it may be more prudent to instead offer a descriptive diagnosis in the pathology report that synthesizes histopathologic, clinical, and radiological data (e.g., *chronic interstitial pneumonia with fibrosis most consistent with UIP*) with a comment acknowledging that the biopsy is not by itself diagnostic but that correlation with imaging studies indicates UIP as the correct diagnosis. This approach avoids the risk of others who are engaged in a patient's care having to reconcile seemingly discordant information when comparing pathology reports with other clinical or radiological data.

Role of Transbronchial Biopsies

Transbronchial biopsies may be useful in managing selected patients suspected of having idiopathic interstitial pneumonias, but its role in establishing a diagnosis of UIP remains controversial [82, 83]. The 2011 ATS/ERS/JRS/ALAT statement on IPF recommends that "Transbronchial biopsy should not be used in the evaluation of IPF in the majority of patients, but may be appropriate in a minority (weak recommendation, low-quality evidence)" [14]. In a retrospective case study limited to patients with UIP, about a third of transbronchial biopsies showed some combination of fibrosis distributed in a patchwork pattern, fibroblast foci, and honeycomb change considered diagnostic or at least suggestive of UIP [82]. Two recent trends have sparked renewed interest in the potential value of transbronchial biopsy in the diagnosis of patients for whom the cost/risk of surgical lung biopsy outweighs the benefits. The first is a novel biopsy technique termed *cryobiopsy* in which a freezing probe is introduced bronchoscopically and the tissue is then frozen to the probe and retrieved. When compared to traditional forceps biopsy, cryobiopsy is reported to increase the tissue yield and improve readability by decreasing "crush" artifact [84]. This in turn has improved diagnostic confidence according to at least one study [85]. The second major trend stems from the continued emphasis on an integrated

multidisciplinary approach utilizing specialists in pulmonary medicine, thoracic radiology, and pathology (multidisciplinary discussion [MDD]) in the diagnosis of patients with unexplained diffuse lung disease. Specifically, a recent study demonstrated that MDD was able to achieve a confident diagnosis in 20–30% of patients utilizing review of TBB alone [86]. Additional studies are necessary to more fully understand the diagnostic sensitivity and specificity of transbronchial lung biopsy in this setting.

Summary

Surgical lung biopsy diagnosis is an essential component of the diagnostic algorithm for the majority of patients with idiopathic interstitial pneumonia. Differentiating these entities is important because of significant differences in therapeutic options and outcome. As HRCT gains widespread acceptance as a primary diagnostic modality for a subset of patients with UIP, lung biopsies will be increasingly limited to patients with atypical and nondiagnostic radiological findings. It is in this subset of patients that surgical lung biopsy plays a key role in diagnosis and management.

References

1. Myers J. Idiopathic interstitial pneumonias. In: Churg A, Myers J, Tazelaar HD, Wright JL, editors. Thurlbeck's pathology of the lung. New York: Thieme; 2004. p. 563–600.
2. Katzenstein AL. Katzenstein and Askin's surgical pathology of non-neoplastic lung disease. 4th ed. Philadelphia: Saunders Elsevier; 2006.
3. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. Am J Respir Crit Care Med. 1998;157(4 Pt 1):1301–15.
4. Liebow AA, Steer A, Billingsley JG. Desquamative interstitial pneumonia. Am J Med. 1965;39:369–404.
5. American Thoracic Society/European Respiratory Society international multidisciplinary consensus classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2002;165(2):277–304.
6. Travis WD, Costabel U, Hansell DM, King TE Jr, Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013;188(6):733–48.
7. Hamman BL, Rich AR. Acute diffuse interstitial fibrosis of the lung. Bull Johns Hopkins Hosp. 1944;74:177–212.
8. Katzenstein AL, Myers JL, Mazur MT. Acute interstitial pneumonia. A clinicopathologic, ultrastructural, and cell kinetic study. Am J Surg Pathol. 1986;10(4):256–67.
9. Bjoraker JA, Ryu JH, Edwin MK, Myers JL, Tazelaar HD, Schroeder DR, et al. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998;157(1):199–203.
10. Flaherty KR, Toews GB, Travis WD, Colby TV, Kazerooni EA, Gross BH, et al. Clinical significance of histological classification of idiopathic interstitial pneumonia. Eur Respir J. 2002;19(2):275–83.

11. Nicholson AG, Colby TV, du Bois RM, Hansell DM, Wells AU. The prognostic significance of the histologic pattern of interstitial pneumonia in patients presenting with the clinical entity of cryptogenic fibrosing alveolitis. Am J Respir Crit Care Med. 2000;162(6):2213–7.
12. Riha RL, Duhig EE, Clarke BE, Steele RH, Slaughter RE, Zimmerman PV. Survival of patients with biopsy-proven usual interstitial pneumonia and nonspecific interstitial pneumonia. Eur Respir J. 2002;19(6):1114–8.
13. Idiopathic pulmonary fibrosis: diagnosis and treatment. Am J Respir Crit Care Med. 2000;161(2):646–64.
14. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. 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.
15. Lee HL, Ryu JH, Wittmer MH, Hartman TE, Lymp JF, Tazelaar HD, et al. Familial idiopathic pulmonary fibrosis: clinical features and outcome. Chest. 2005;127(6):2034–41.
16. Steele MP, Speer MC, Loyd JE, Brown KK, Herron A, Slifer SH, et al. Clinical and pathologic features of familial interstitial pneumonia. Am J Respir Crit Care Med. 2005;172(9):1146–52.
17. Flaherty KR, Colby TV, Travis WD, Toews GB, Mumford J, Murray S, et al. Fibroblastic foci in usual interstitial pneumonia: idiopathic versus collagen vascular disease. Am J Respir Crit Care Med. 2003;167(10):1410–5.
18. Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, King TE Jr, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2007;176(7):636–43.
19. Parambil JG, Myers JL, Ryu JH. Histopathologic features and outcome of patients with acute exacerbation of idiopathic pulmonary fibrosis undergoing surgical lung biopsy. Chest. 2005;128(5):3310–5.
20. Azuma A, Nukiwa T, Tsuboi E, Suga M, Abe S, Nakata K, et al. Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2005;171(9):1040–7.
21. Martinez FJ, Safrin S, Weycker D, Starko KM, Bradford WZ, King TE Jr, et al. The clinical course of patients with idiopathic pulmonary fibrosis. Ann Intern Med. 2005;142(12 Pt 1):963–7.
22. Daniels C, Yi E, Ryu JH. Autopsy findings in 42 consecutive patients with idiopathic pulmonary fibrosis. Eur Respir J. 2008;32:170–4.
23. Collard HR, Ryerson CJ, Corte TJ, Jenkins G, Kondoh Y, Lederer DJ, et al. Acute exacerbation of idiopathic pulmonary fibrosis. An international working group report. Am J Respir Crit Care Med. 2016;194(3):265–75.
24. Lagstein A, Myers JL. Role of lung biopsy in identifying patients with acute exacerbation of usual interstitial pneumonia: a case review. AJSP Rev Rep. 2017;22:8–14.
25. Flaherty KR, Thwaite EL, Kazerooni EA, Gross BH, Toews GB, Colby TV, et al. Radiological versus histological diagnosis in UIP and NSIP: survival implications. Thorax. 2003;58(2):143–8.
26. Hunninghake GW, Zimmerman MB, Schwartz DA, King TE Jr, Lynch J, Hegele R, et al. Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2001;164(2):193–6.
27. Johkoh T, Muller NL, Cartier Y, Kavanagh PV, Hartman TE, Akira M, et al. Idiopathic interstitial pneumonias: diagnostic accuracy of thin-section CT in 129 patients. Radiology. 1999;211(2):555–60.
28. Sumikawa H, Johkoh T, Ichikado K, Taniguchi H, Kondoh Y, Fujimoto K, et al. Usual interstitial pneumonia and chronic idiopathic interstitial pneumonia: analysis of CT appearance in 92 patients. Radiology. 2006;241(1):258–66.
29. Sumikawa H, Johkoh T, Colby TV, Ichikado K, Suga M, Taniguchi H, et al. Computed tomography findings in pathological usual interstitial pneumonia: relationship to survival. Am J Respir Crit Care Med. 2008;177(4):433–9.
30. Katzenstein AL, Mukhopadhyay S, Myers JL. Diagnosis of usual interstitial pneumonia and distinction from other fibrosing interstitial lung diseases. Hum Pathol. 2008;39(9):1275–94.
31. Katzenstein AL, Zisman DA, Litzky LA, Nguyen BT, Kotloff RM. Usual interstitial pneumonia: histologic study of biopsy and explant specimens. Am J Surg Pathol. 2002;26(12):1567–77.

32. Myers JL, Katzenstein AL. Beyond a consensus classification for idiopathic interstitial pneumonias: progress and controversies. Histopathology. 2009;54(1):90–103.
33. Myers JL, Katzenstein AL. Fibroblasts in focus. Am J Respir Crit Care Med. 2006;174(6):623–4.
34. Tazelaar HD, Viggiano RW, Pickersgill J, Colby TV. Interstitial lung disease in polymyositis and dermatomyositis. Clinical features and prognosis as correlated with histologic findings. Am Rev Respir Dis. 1990;141(3):727–33.
35. Wells AU, Cullinan P, Hansell DM, Rubens MB, Black CM, Newman-Taylor AJ, et al. Fibrosing alveolitis associated with systemic sclerosis has a better prognosis than lone cryptogenic fibrosing alveolitis. Am J Respir Crit Care Med. 1994;149(6):1583–90.
36. Yousem SA, Colby TV, Carrington CB. Lung biopsy in rheumatoid arthritis. Am Rev Respir Dis. 1985;131(5):770–7.
37. Tzelepis GE, Toya SP, Moutsopoulos HM. Occult connective tissue diseases mimicking idiopathic interstitial pneumonias. Eur Respir J. 2008;31(1):11–20.
38. Fischer A, Antoniou KM, Brown KK, Cadranel J, Corte TJ, du Bois RM, et al. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. Eur Respir J. 2015;46(4):976–87.
39. Atkins SR, Turesson C, Myers JL, Tazelaar HD, Ryu JH, Matteson EL, et al. Morphologic and quantitative assessment of CD20+ B cell infiltrates in rheumatoid arthritis-associated nonspecific interstitial pneumonia and usual interstitial pneumonia. Arthritis Rheum. 2006;54(2):635–41.
40. Enomoto N, Suda T, Kato M, Kaida Y, Nakamura Y, Imokawa S, et al. Quantitative analysis of fibroblastic foci in usual interstitial pneumonia. Chest. 2006;130(1):22–9.
41. King TE Jr, Schwarz MI, Brown K, Tooze JA, Colby TV, Waldron JA Jr, et al. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med. 2001;164(6):1025–32.
42. Nicholson AG, Fulford LG, Colby TV, du Bois RM, Hansell DM, Wells AU. The relationship between individual histologic features and disease progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2002;166(2):173–7.
43. Tiitto L, Bloigu R, Heiskanen U, Paakko P, Kinnula VL, Kaarteenaho-Wiik R. Relationship between histopathological features and the course of idiopathic pulmonary fibrosis/usual interstitial pneumonia. Thorax. 2006;61(12):1091–5.
44. Hanak V, Ryu JH, de Carvalho E, Limper A, Hartman TE, Decker PA, et al. Profusion of fibroblast foci in patients with idiopathic pulmonary fibrosis does not predict outcome. Respir Med. 2008;102:852–6.
45. Myers JL, Veal CF Jr, Shin MS, Katzenstein AL. Respiratory bronchiolitis causing interstitial lung disease. A clinicopathologic study of six cases. Am Rev Respir Dis. 1987;135(4):880–4.
46. Ryu JH, Myers JL, Capizzi SA, Douglas WW, Vassallo R, Decker PA. Desquamative interstitial pneumonia and respiratory bronchiolitis-associated interstitial lung disease. Chest. 2005;127(1):178–84.
47. Myers JL. Nonspecific interstitial pneumonia: pathologic features and clinical implications. Semin Diagn Pathol. 2007;24(3):183–7.
48. Portnoy J, Veraldi KL, Schwarz MI, Cool CD, Curran-Everett D, Cherniack RM, et al. Respiratory bronchiolitis-interstitial lung disease: long-term outcome. Chest. 2007;131(3):664–71.
49. Craig PJ, Wells AU, Doffman S, Rassl D, Colby TV, Hansell DM, et al. Desquamative interstitial pneumonia, respiratory bronchiolitis and their relationship to smoking. Histopathology. 2004;45(3):275–82.
50. Yousem SA. Respiratory bronchiolitis-associated interstitial lung disease with fibrosis is a lesion distinct from fibrotic nonspecific interstitial pneumonia: a proposal. Mod Pathol. 2006;19(11):1474–9.
51. Katzenstein AL. Smoking-related interstitial fibrosis (SRIF), pathogenesis and treatment of usual interstitial pneumonia (UIP), and transbronchial biopsy in UIP. Mod Pathol. 2012;25(Suppl 1):S68–78.

52. Gaensler EA, Goff AM, Prowse CM. Desquamative interstitial pneumonia. N Engl J Med. 1966;274(3):113–28.
53. Fraig M, Shreesha U, Savici D, Katzenstein AL. Respiratory bronchiolitis: a clinicopathologic study in current smokers, ex-smokers, and never-smokers. Am J Surg Pathol. 2002;26(5):647–53.
54. Wells AU, Nicholson AG, Hansell DM. Challenges in pulmonary fibrosis 4: smoking-induced diffuse interstitial lung diseases. Thorax. 2007;62(10):904–10.
55. Katzenstein AL, Fiorelli RF. Nonspecific interstitial pneumonia/fibrosis. Histologic features and clinical significance. Am J Surg Pathol. 1994;18(2):136–47.
56. Travis WD, Hunninghake G, King TE Jr, Lynch DA, Colby TV, Galvin JR, et al. Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. Am J Respir Crit Care Med. 2008;177(12):1338–47.
57. Nagai S, Kitaichi M, Itoh H, Nishimura K, Izumi T, Colby TV. Idiopathic nonspecific interstitial pneumonia/fibrosis: comparison with idiopathic pulmonary fibrosis and BOOP.[erratum appears in Eur Respir J 1999 Mar;13(3):711]. Eur Respir J. 1998;12(5):1010–9.
58. Travis WD, Matsui K, Moss J, Ferrans VJ. Idiopathic nonspecific interstitial pneumonia: prognostic significance of cellular and fibrosing patterns: survival comparison with usual interstitial pneumonia and desquamative interstitial pneumonia. Am J Surg Pathol. 2000;24(1):19–33.
59. Latsi PI, du Bois RM, Nicholson AG, Colby TV, Bisirtzoglou D, Nikolakopoulou A, et al. Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. Am J Respir Crit Care Med. 2003;168(5):531–7.
60. Shin KM, Lee KS, Chung MP, Han J, Bae YA, Kim TS, et al. Prognostic determinants among clinical, thin-section CT, and histopathologic findings for fibrotic idiopathic interstitial pneumonia: tertiary hospital study. Radiology. 2008;249(1):328–37.
61. Jegal Y, Kim DS, Shim TS, Lim CM, Do Lee S, Koh Y, et al. Physiology is a stronger predictor of survival than pathology in fibrotic interstitial pneumonia. Am J Respir Crit Care Med. 2005;171(6):639–44.
62. Frankel SK, Cool CD, Lynch DA, Brown KK. Idiopathic pleuroparenchymal fibroelastosis: description of a novel clinicopathologic entity. Chest. 2004;126(6):2007–13.
63. von der Thusen JH, Hansell DM, Tominaga M, Veys PA, Ashworth MT, Owens CM, et al. Pleuroparenchymal fibroelastosis in patients with pulmonary disease secondary to bone marrow transplantation. Mod Pathol. 2011;24(12):1633–9.
64. Ofek E, Sato M, Saito T, Wagnetz U, Roberts HC, Chaparro C, et al. Restrictive allograft syndrome post lung transplantation is characterized by pleuroparenchymal fibroelastosis. Mod Pathol. 2013;26(3):350–6.
65. Cheng SK, Chuah KL. Pleuroparenchymal fibroelastosis of the lung: a review. Arch Pathol Lab Med. 2016;140(8):849–53.
66. Bonifazi M, Montero MA, Renzoni EA. Idiopathic pleuroparenchymal fibroelastosis. Curr Pulmonol Rep. 2017;6(1):9–15.
67. Lagstein A. Pulmonary apical cap-what's old is new again. Arch Pathol Lab Med. 2015;139(10):1258–62.
68. Flaherty KR, Andrei AC, King TE Jr, Raghu G, Colby TV, Wells A, et al. Idiopathic interstitial pneumonia: do community and academic physicians agree on diagnosis? Am J Respir Crit Care Med. 2007;175(10):1054–60.
69. Flaherty KR, King TE Jr, Raghu G, Lynch JP 3rd, Colby TV, Travis WD, et al. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? Am J Respir Crit Care Med. 2004;170(8):904–10.
70. Hubbard R, Venn A. The impact of coexisting connective tissue disease on survival in patients with fibrosing alveolitis. Rheumatology. 2002;41(6):676–9.
71. Kocheril SV, Appleton BE, Somers EC, Kazerooni EA, Flaherty KR, Martinez FJ, et al. Comparison of disease progression and mortality of connective tissue disease-related interstitial lung disease and idiopathic interstitial pneumonia. Arthritis Rheum. 2005;53(4):549–57.

72. Park JH, Kim DS, Park IN, Jang SJ, Kitaichi M, Nicholson AG, et al. Prognosis of fibrotic interstitial pneumonia: idiopathic versus collagen vascular disease-related subtypes. Am J Respir Crit Care Med. 2007;175(7):705–11.
73. Copley SJ, Wells AU, Sivakumaran P, Rubens MB, Lee YC, Desai SR, et al. Asbestosis and idiopathic pulmonary fibrosis: comparison of thin-section CT features. Radiology. 2003;229(3):731–6.
74. Gaensler EA, Jederlinic PJ, Churg A. Idiopathic pulmonary fibrosis in asbestos-exposed workers. Am Rev Respir Dis. 1991;144(3 Pt 1):689–96.
75. Perez-Padilla R, Salas J, Chapela R, Sanchez M, Carrillo G, Perez R, et al. Mortality in Mexican patients with chronic pigeon breeder's lung compared with those with usual interstitial pneumonia. Am Rev Respir Dis. 1993;148(1):49–53.
76. Churg A, Sin DD, Everett D, Brown K, Cool C. Pathologic patterns and survival in chronic hypersensitivity pneumonitis. Am J Surg Pathol. 2009;33(12):1765–70.
77. Hayakawa H, Shirai M, Sato A, Yoshizawa Y, Todate A, Imokawa S, et al. Clinicopathological features of chronic hypersensitivity pneumonitis. Respirology. 2002;7(4):359–64.
78. Ohtani Y, Saiki S, Kitaichi M, Usui Y, Inase N, Costabel U, et al. Chronic bird fancier's lung: histopathological and clinical correlation. An application of the 2002 ATS/ERS consensus classification of the idiopathic interstitial pneumonias. Thorax. 2005;60(8):665–71.
79. Trahan S, Hanak V, Ryu JH, Myers JL. Role of surgical lung biopsy in separating chronic hypersensitivity pneumonia from usual interstitial pneumonia/idiopathic pulmonary fibrosis: analysis of 31 biopsies from 15 patients. Chest. 2008;134(1):126–32.
80. Flaherty KR, Travis WD, Colby TV, Toews GB, Kazerooni EA, Gross BH, et al. Histopathologic variability in usual and nonspecific interstitial pneumonias. Am J Respir Crit Care Med. 2001;164(9):1722–7.
81. Monaghan H, Wells AU, Colby TV, du Bois RM, Hansell DM, Nicholson AG. Prognostic implications of histologic patterns in multiple surgical lung biopsies from patients with idiopathic interstitial pneumonias. Chest. 2004;125(2):522–6.
82. Berbescu EA, Katzenstein AL, Snow JL, Zisman DA. Transbronchial biopsy in usual interstitial pneumonia. Chest. 2006;129(5):1126–31.
83. Ensminger SA, Prakash UB. Is bronchoscopic lung biopsy helpful in the management of patients with diffuse lung disease? Eur Respir J. 2006;28(6):1081–4.
84. Hetzel J, Eberhardt R, Herth FJ, Petermann C, Reichle G, Freitag L, et al. Cryobiopsy increases the diagnostic yield of endobronchial biopsy: a multicentre trial. Eur Respir J. 2012;39(3):685–90.
85. Tomassetti S, Wells AU, Costabel U, Cavazza A, Colby TV, Rossi G, et al. Bronchoscopic lung cryobiopsy increases diagnostic confidence in the multidisciplinary diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2016;193(7):745–52.
86. Sheth JS, Belperio JA, Fishbein MC, Kazerooni EA, Lagstein A, Murray S, et al. Utility of transbronchial versus surgical lung biopsy in the diagnosis of suspected fibrotic interstitial lung disease. Chest. 2017;151(2):389–99.

Chapter 4
Imaging of Idiopathic Pulmonary Fibrosis

Jonathan H. Chung and Jeffrey P. Kanne

Introduction

Idiopathic pulmonary fibrosis (IPF) is the most common cause of fibrotic lung disease. Approximately, 1–2/10,000 people are diagnosed with IPF, with an increased prevalence in elderly patients. Men are affected nearly twice as often as women [1]. There is a strong association between IPF and cigarette smoking, especially in patients with a >20 pack-year smoking history [2]. Additionally, gastroesophageal reflux disease (GERD) is also very common in patients with IPF; 90% of patients with IPF have GERD, and treatment for GERD has been associated with increased survival [3]. The prognosis of patients with IPF is poor (approaching levels similar to non-small cell lung cancer), with a median survival of approximately 3 years [4]. The clinical presentation is nonspecific and includes progressive dyspnea (especially upon exertion), dry cough, early inspiratory crackles on chest auscultation, and digital clubbing. Usual interstitial pneumonia (UIP) is the most common imaging correlate in patients with IPF [5, 6]. Based on imaging, a confident diagnosis of UIP can often be made, obviating the need for biopsy.

Radiography

Given its poor contrast resolution compared to computerized tomography (CT), routine use of radiography in the work-up of patients with known or suspected IPF has

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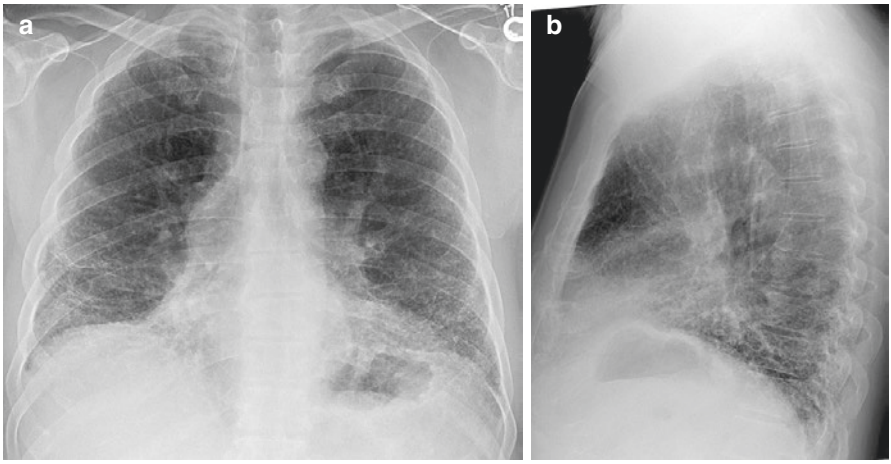

Fig. 4.1 PA (**a**) and lateral (**b**) chest radiographs show small lung volumes and basilar-predominant reticulation highly suggestive of pulmonary fibrosis. Further evaluation with HRCT would be necessary to more accurately characterize underlying lung disease

markedly decreased with the widespread availability of multidetector CT. However, because imaging findings of UIP may be detected before patients become symptomatic, recognition of the radiographic pattern of UIP remains important, because the radiologist may be the first to suggest underlying pulmonary fibrosis. The main pattern on chest radiography is that of bilateral symmetric reticulation and irregular linear opacities [7]. Superimposition of reticulation on radiography may lead to apparent reticulonodular opacities, though no nodules are actually present. Traction bronchiectasis may also be evident. UIP favors the subpleural and basal lung regions (Fig. 4.1). In typical cases, pulmonary fibrosis will lead to basilar-predominant volume loss. In cases of concomitant upper lobe-predominant emphysema from smoking, total lung volume may be normal. In more advanced cases, subpleural honeycombing, which manifests as basilar-predominant cystic spaces, may be apparent [8]. Honeycombing implies local areas of advanced pulmonary fibrosis and is highly specific for the diagnosis of UIP [9]. In a study of 16 patients with UIP, 15 had interstitial opacities on the chest radiograph, 10 patients had reticular opacities, 2 had reticulonodular opacities, 3 had frank honeycombing, and 1 patient had mixed alveolar and interstitial opacities (although the distinction between "interstitial" and "alveolar" opacities has fallen out of favor among many thoracic radiologists). Lung volumes were decreased in the majority of patients (12/16 = 75%). No patients had increased lung volumes in keeping with the restrictive nature of pulmonary fibrosis [10]. If pulmonary fibrosis is suspected on radiography, the next step is further evaluation with high-resolution CT (HRCT) of the chest.

Technical Aspects of HRCT

The rapid growth of CT in the late 1990s revolutionized lung imaging. Because images are acquired in cross section with CT, contrast resolution is superior to

radiography (where overlapping structures complicate an accurate assessment of the lung parenchyma) [11]. HRCT is the reference standard for imaging the lungs in the setting of diffuse lung disease, and with current multidetector scanners, images can be reconstructed in any plane given the near-isovolumetric acquisition. Modern CT scanners are able to acquire volumetric data of the entire chest in a single breath hold and reconstruct high-resolution images [12].

Unfortunately, there is no standard HRCT protocol. CT scans can be acquired in a helical manner (most common) or in a sequential or "step-and-shoot" fashion. Helical CT acquisition allows for more diverse reconstruction parameters and images the entire chest as opposed to the step-and-shoot strategy. However, the step-and-shoot method allows for gapped imaging such that significant portions of the chest are not scanned, leading to substantial reduction in radiation dose. This is most advantageous in the setting of diffuse lung diseases where complete imaging of the thorax is not usually necessary [7]. However, given the short life spans of most patients with pulmonary fibrosis and the long lead time for the development of radiation-induced malignancy (the risk of which is controversial at doses used with diagnostic imaging), volumetric HRCT is preferred, as it can detect subtle fibrosis and honeycombing, which may alter management. Different centers use different acquisition parameters (CT scanner make and model, tube peak kilovoltage (kVp), tube current (mA), tube rotation time, table speed) as well as different image reconstruction parameters (slice thickness, slice interval, reconstruction kernel and method, field of view). Typically, the kVp should be 80–120 kV, depending on patient size, and the mA should be less than 250 mA. Tube current modulation, available on most modern scanners, has become the standard of care because it significantly reduces patient radiation exposure [13–15]. Field of view should include both lungs, while the inclusion of an excess amount of overlying air should be avoided. Prone and expiratory imaging can be helpful in distinguishing mild pulmonary fibrosis from peripheral atelectasis (particularly in the dependent aspect of the lungs) and to assess for air trapping, respectively. A dynamic expiratory scan can also be included to assess for tracheobronchomalacia. Although there are many variations, any HRCT scan should include a number of mandatory requirements that include (1) thin-section reconstruction (0.5–1.5 mm), (2) high spatial frequency (edge-enhancing) reconstruction kernel, (3) full inspiration, and (4) absence of motion artifact.

Typical HRCT Pulmonary Findings

The vast majority of patients with UIP have reticulation in a subpleural and basilar-predominant distribution. A small percentage of patients have upper lobe-predominant fibrosis, although this pattern is more suggestive of non-UIP conditions such as sarcoidosis [16]. Associated architectural distortion with traction bronchiectasis and bronchiolectasis are the rule. Honeycombing occurs in the subpleural lung and typically manifests as "clustered cystic air spaces, typically of comparable diameters on the order of 3–10 mm" [17]. Honeycombing, in addition to upper lobe, subpleural linear lines, is the most specific finding of UIP on HRCT and is quite common, occurring in up to 90% of UIP cases [9, 18]. A small amount of ground-glass

opacity is not uncommon [19] (Fig. 4.2). When there are other findings of frank fibrosis (traction bronchiectasis and bronchiolectasis, reticulation, and honeycombing), ground-glass opacity almost assuredly represents microscopic pulmonary fibrosis. In cases in which ground-glass opacity is isolated, it may alternatively represent active inflammation [20, 21].

Mild mediastinal and hilar lymph node enlargement is present on HRCT in up to 70–86% of patients with UIP [22–24] (Fig. 4.3). Lymph node size usually does not exceed 1.5 cm in short axis and is typically isolated to one or two lymph node stations, most commonly levels 4 (lower paratracheal), 5 (subaortic), 7 (subcarinal), and 10R (right hilar) [22]. In one study of 30 patients with pulmonary fibrosis (25 of whom had IPF), patients with more ground-glass opacity tended to have larger individual lymph nodes, while those with more fibrosis had an overall greater number of enlarged lymph nodes [24]. However, a larger study with similar design showed that the presence of lymph node enlargement did not correlate to any specific pattern or to the extent of disease on HRCT [23].

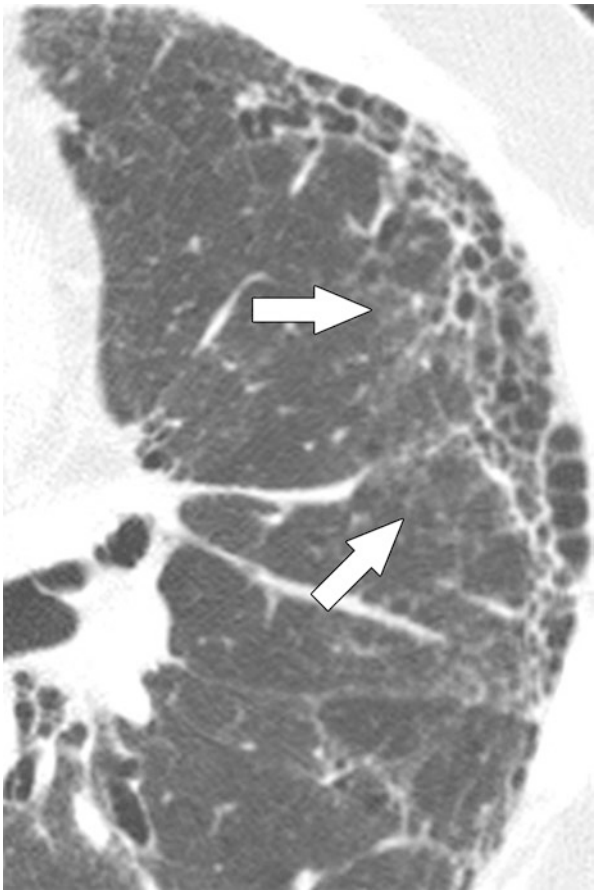

Fig. 4.2 Axial HRCT image shows a small amount of ground-glass opacity (*arrows*) in the left upper lobe. Given the large degree of adjacent pulmonary fibrosis, ground-glass opacity likely represents microscopic pulmonary fibrosis rather than inflammation

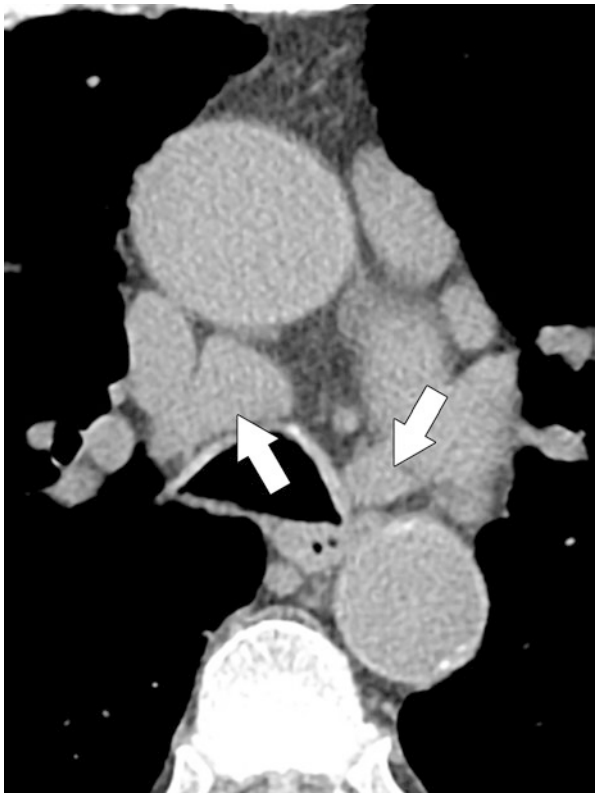

Fig. 4.3 Axial CT image shows mild mediastinal lymph node enlargement (*arrows*) in this patient with UIP

The syndrome of combined pulmonary fibrosis and emphysema (CPFE) has recently gained increased recognition. Approximately one-third of patients with IPF also have emphysema [25]. This association is not surprising, considering that smoking is a common risk factor for both emphysema and IPF (Fig. 4.4). As is typical for smoking-related emphysema, emphysema predominates in the upper lobes and has a centrilobular distribution. Fibrosis is peripheral and basilar predominant and has typical findings of UIP. Pulmonary function testing in patients with combined IPF and emphysema usually shows little or modest decreases in forced vital capacity and forced expiratory volume in 1 s, but a marked decrease in the diffusion capacity is typically present [26]. Interestingly, there is a strong association with combined disease and pulmonary hypertension; in one series, 47% of patients with combined emphysema and IPF had pulmonary hypertension on initial diagnosis, which increased to 55% on follow-up [26]. Patients with CPFE tend to have a poor prognosis. This is especially true if there is concomitant pulmonary hypertension; one study showed that patients with CPFE and pulmonary hypertension have a 1-year survival of only 60% [26].

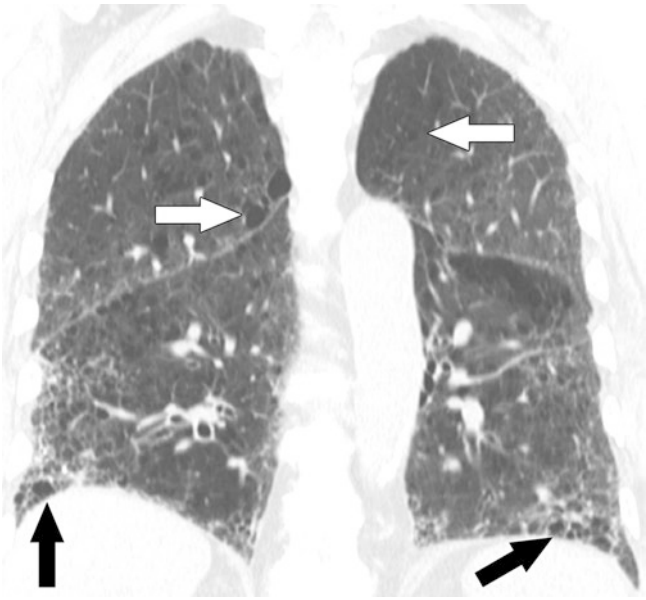

Fig. 4.4 Coronal reformatted HRCT image shows basilar-predominant pulmonary fibrosis (*black arrows*) with upper lung zone emphysema (*white arrows*) consistent with combined pulmonary fibrosis and emphysema

Accuracy of HRCT

The accuracy of HRCT in the setting of UIP is approximately 80–90% [9, 27–30] when UIP is the first-choice diagnosis. However, when a confident diagnosis of UIP can be made on HRCT, the accuracy increases to 90–100%. Unfortunately, HRCT is not a perfect tool for the diagnosis of pulmonary fibrosis because different conditions may manifest with similar imaging findings. A confident diagnosis of UIP cannot be established by HRCT in approximately 50% of patients who are ultimately diagnosed with IPF [31, 32].

The most recent consensus statement from the American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Association (ATS/ERS/JRS/ALAT) on idiopathic pulmonary fibrosis suggested guidelines for radiologists when interpreting and reporting cases in which UIP is being considered [33]. In the setting of fibrotic interstitial lung diseases, the three classes of UIP diagnoses on HRCT are (definite) UIP pattern, possible UIP pattern, and inconsistent with UIP pattern. A confident diagnosis of UIP can be made on HRCT if the following four imaging parameters are met: (1) basilar and subpleural predominance, (2) reticulation, (3) honeycombing (with or without traction bronchiectasis), and (4) absence of features to suggest another diagnosis (inconsistent with UIP pattern) (Fig. 4.5). When there is a definite UIP pattern, the diagnosis will almost always be IPF, although a definite UIP pattern can occasionally be seen with collagen vascular disease, asbestosis, familial fibrosis, chronic hypersensitivity pneumonitis, or drug-related pulmonary fibrosis. The possible UIP

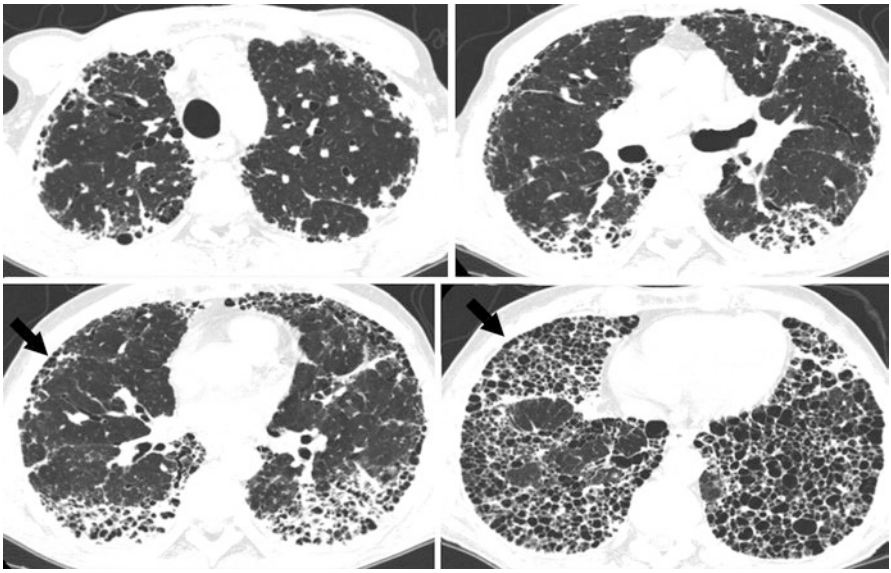

Fig. 4.5 Multiple axial HRCT images show basilar- and peripheral-predominant pulmonary fibrosis characterized by reticulation, traction bronchiolectasis, and subpleural honeycombing (*arrows*), diagnostic of UIP

pattern on HRCT includes all the imaging parameters of the definite UIP pattern with the exception of honeycombing, which is absent (Fig. 4.6). The distinction between a confident UIP diagnosis and possible UIP diagnosis can be challenging, given that the main distinction between these two groups is the presence or absence of honeycombing, which may be difficult to identify when honeycombing is subtle or when HRCT images are noncontiguous. This is highlighted by the finding that only fair-to-moderate agreement exists among expert readers for the identification of honeycombing (mean kappa of 0.45 in one study) [34]. A larger study of 112 international observers (96 of whom were thoracic radiologists) who reviewed 150 HRCT scans found mean kappa of 0.59 for honeycombing and mean kappa values of 0.48 and 0.52 for general and thoracic radiologists, respectively, for ATS/ERS/JRS/ALAT categories [35]. The pattern should be considered as inconsistent with UIP if any one of the following imaging parameters is present: (1) upper or mid-lung predominance (Fig. 4.7), (2) peribronchovascular predominance (Fig. 4.8), (3) extensive ground-glass opacity (more extensive than reticulation) (see Fig. 4.8), (4) profuse micronodules (Fig. 4.9), (5) discrete cysts (multiple, not consistent with honeycombing) (Fig. 4.10), (6) diffuse mosaic attenuation or air trapping (involving three or more lobes and bilateral) (Fig. 4.11), or (7) consolidation (Fig. 4.12). The presence of any of these findings is much more suggestive of an alternative diagnosis to UIP (Table 4.1). Patients with a HRCT pattern of possible UIP or inconsistent with UIP need further work-up and will often require biopsy to establish a confident diagnosis.

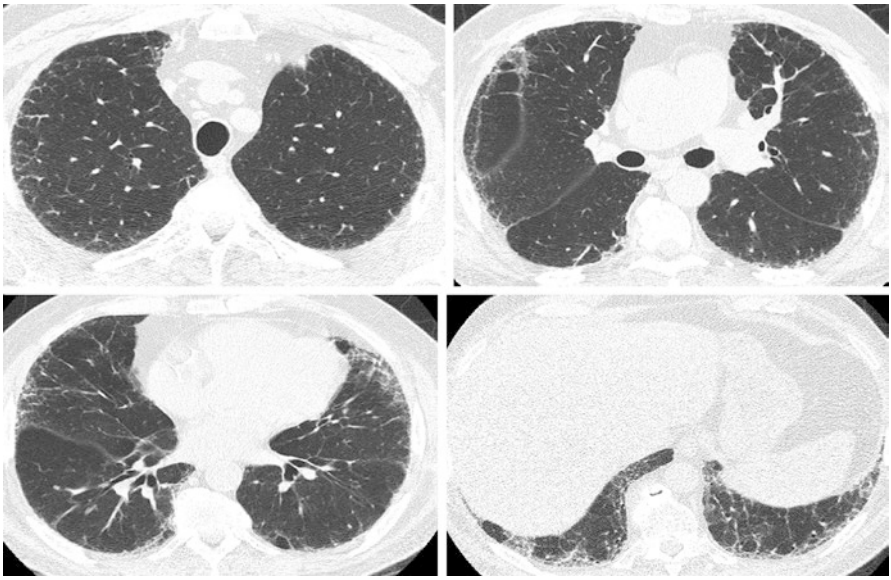

Fig. 4.6 Multiple axial HRCT images show basilar- and peripheral-predominant pulmonary fibrosis characterized by reticulation, mild ground-glass opacity, and traction bronchiolectasis, meeting criteria for possible usual interstitial pneumonia. The main distinction between a HRCT diagnosis of definite UIP and possible UIP is the presence or absence of honeycombing

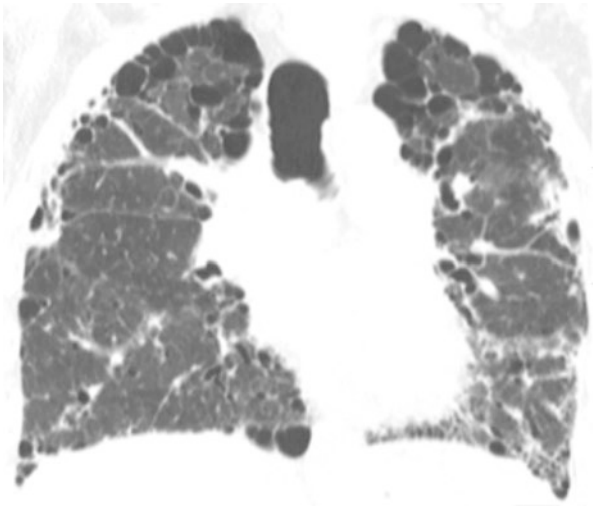

Fig. 4.7 Coronal reformatted HRCT image shows peripheral-predominant pulmonary fibrosis. However, as opposed to typical cases of UIP, fibrosis in this case predominates in the upper lungs. This patient was shown to have sarcoidosis-related pulmonary fibrosis

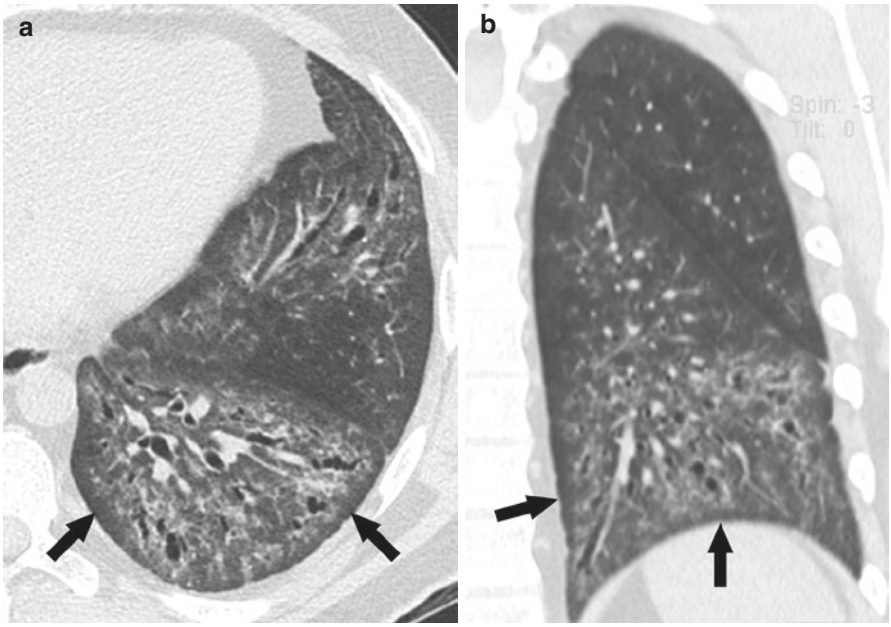

Fig. 4.8 Axial (**a**) and coronal (**b**) HRCT images show basilar- and bronchovascular-predominant pulmonary fibrosis characterized by ground-glass opacity, mild reticulation, and traction bronchiectasis. There is relative sparing of the subpleural lung (*arrows*). These findings strongly favor NSIP over UI

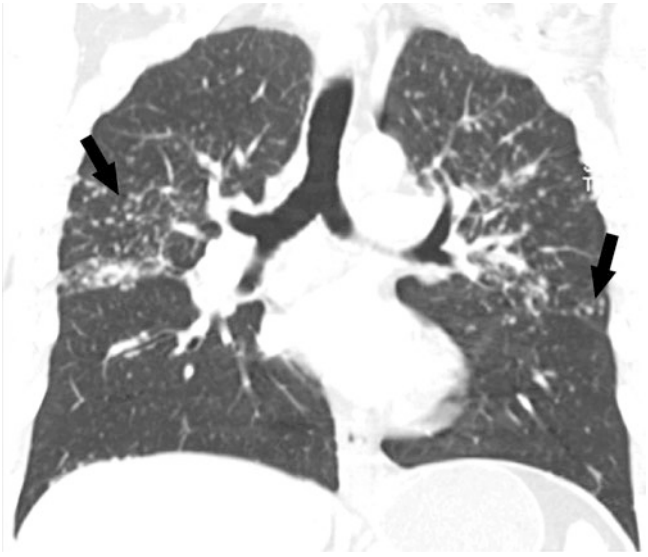

Fig. 4.9 Coronal reformatted HRCT image shows multiple nodules (*arrows*) in the mid- and upper lungs in a perilymphatic distribution along bronchovascular structures, interlobular septa, and subpleural lung in this patient with sarcoidosis

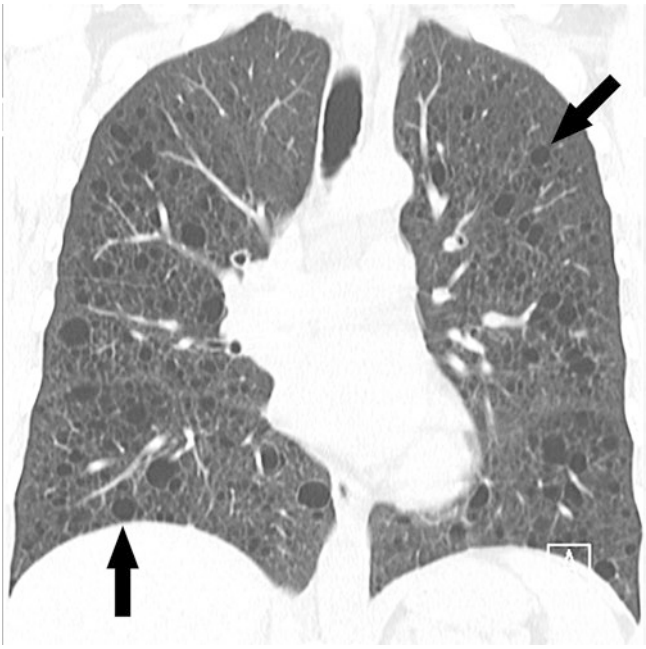

Fig. 4.10 Coronal reformatted HRCT image shows multiple uniform thin-walled lung cysts (*arrows*), which are more profuse in the mid- and lower lungs in this patient with lymphangioleiomyomatosis

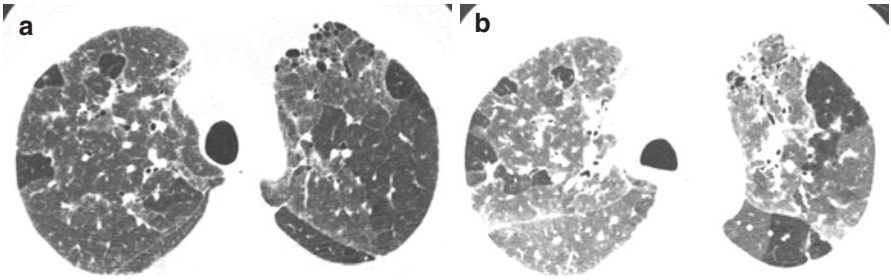

Fig. 4.11 Axial HRCT images taken during inspiration (**a**) and end-expiration (**b**) show lobular areas of air trapping with adjacent pulmonary fibrosis, typical of hypersensitivity pneumonitis. The combination of fibrosis and air trapping represents a combination of the subacute and chronic phases of hypersensitivity pneumonitis

Recent publications have addressed the fact that many patients with a possible UIP pattern on HRCT findings have a histologic diagnosis of UIP on surgical biopsy, which is currently recommended by ATS/ERS/JRS/ALAT guidelines [36, 37]. However, avoiding surgical biopsies in patients with pulmonary fibrosis when possible is important given associated morbidity and mortality. One recent study of 201 subjects with HRCT scans and surgical biopsies performed within 1 year grouped subjects into four UIP categories (definite, probable, indeterminate, and inconsistent) [36]. The 34 subjects (16.9%) in the probable UIP group had all of the typical UIP findings except honeycombing, while the 72 subjects (35.8%) in the

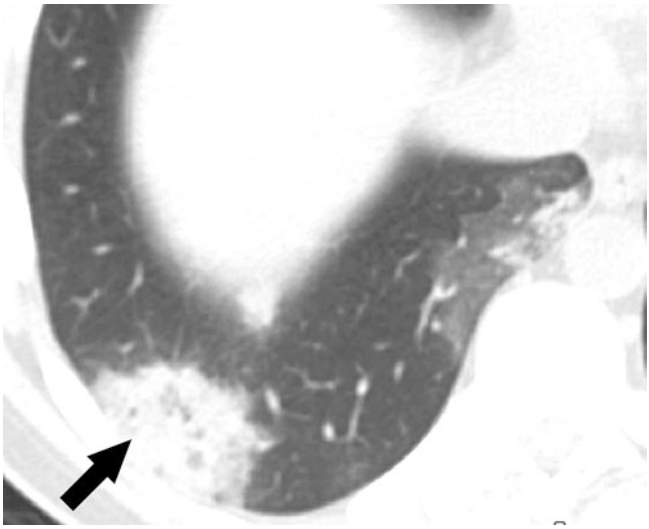

Fig. 4.12 Axial HRCT image shows subpleural consolidation (*arrow*) and ground-glass opacity in the right lower lobe in this patient with organizing pneumonia

Table 4.1 Differential diagnosis of imaging features that are considered to be inconsistent with a diagnosis of UIP

Imaging finding	Differential diagnosis (diffuse lung diseases)
Upper or mid-lung predominance	Sarcoidosis, HP, familial pulmonary fibrosis, pleuroparenchymal fibroelastosis
Peribronchovascular predominance	NSIP
Extensive ground-glass abnormality	NSIP, HP, DIP, PAP
Profuse micronodules (predominantly in upper lobes)	Ground-glass: HP, RB
	Solid: sarcoidosis, silicosis/CWP
Discrete cysts (not consistent with honeycombing)	Cystic lung disease (LAM, LCH, LIP)
Diffuse mosaic attenuation/air trapping	HP, OB
Consolidation	COP, CEP

CEP chronic eosinophilic pneumonia, *COP* cryptogenic organizing pneumonia, *CWP* coal workers' pneumoconiosis, *DIP* desquamative interstitial pneumonitis, *HP* hypersensitivity pneumonitis, *LAM* lymphangioleiomyomatosis, *LCH* Langerhans cell histiocytosis, *LIP* lymphocytic interstitial pneumonitis, *NSIP* nonspecific interstitial pneumonitis, *PAP* pulmonary alveolar proteinosis, *RB* respiratory bronchiolitis, *OB* obliterative bronchiolitis

indeterminate group had fibrosis findings not sufficiently characteristic to reach a definite, probable, or inconsistent with UIP level. There was a statistically significant difference between the proportion of UIP diagnosis on histology in these two subgroups, with 82.4% of subjects with probable UIP on HRCT having a probable or definite UIP diagnosis on histology as opposed to 54.2% of subjects with indeterminate UIP [36]. This study suggests that it may be useful to separate the possible UIP pattern group of patients into two categories, so that some patients in the possible UIP group could, after careful multidisciplinary discussion, avoid unnecessary biopsies.

Prognosis

Unfortunately, a diagnosis of UIP carries a poor prognosis. In a study in patients with various interstitial lung diseases, UIP histopathology was shown to have the worst prognosis [38]. Interestingly, imaging findings, which may be discordant with histopathologic findings, correlate with survival, even in patients with known histopathology; patients with a nonspecific interstitial pneumonia (NSIP) pattern on HRCT but UIP on histopathology have survival rates that are more similar to patients with NSIP. Patients with indeterminate HRCT patterns with UIP on histopathology also have 3.68 years of increased median survival relative to patients with a UIP HRCT pattern and histopathologic UIP [27]. The extent of honeycombing and pulmonary fibrosis has been shown to be associated with prognosis in the setting of pulmonary fibrosis [18, 39–42]. Lead time bias may play a role in the longer survival of patients with milder fibrosis, because honeycombing and more extensive fibrosis suggest that the fibrosis has likely been present for a longer duration.

Nonetheless, the ability to predict survival from the time of CT scanning still has importance. Therefore, either qualitative or quantitative assessment of the degree of pulmonary fibrosis and of honeycombing is mandatory. It is intuitive that the rate of progression of fibrosis and honeycombing would be associated with survival in the setting of fibrosing interstitial pneumonitis; a recent study demonstrated that progression of honeycombing on follow-up CT is an important determinant of survival in patients with fibrosing interstitial pneumonia [43].

Thoracic Complications of IPF

An acute exacerbation of IPF carries a poor prognosis, with most patients eventually dying within weeks to months after the initial onset of acute respiratory worsening [44–46]. The most common histological correlate is diffuse alveolar damage, with organizing pneumonia occurring less commonly [47]. The HRCT manifestations reflect the underlying histology; ground-glass opacity, consolidation, or both are superimposed on underlying pulmonary fibrosis [48, 49] (Fig. 4.13). Given the somewhat nonspecific pattern of HRCT abnormalities, pneumonia and pulmonary edema must first be excluded. The distribution of lung disease may be peripheral, patchy, or diffuse. Based on limited data, it appears that a peripheral pattern of disease is less often fatal than multifocal or diffuse patterns [48, 49]. Patients with an organizing pneumonia have a better prognosis than those with diffuse alveolar damage. Therefore, one would expect patients with more consolidation, which is a pattern that is more typical of organizing pneumonia (peripheral and bronchovascular), to have a better prognosis than those patients with ground-glass opacities that are more typical for diffuse alveolar damage (Fig. 4.14). However, this has not been shown conclusively.

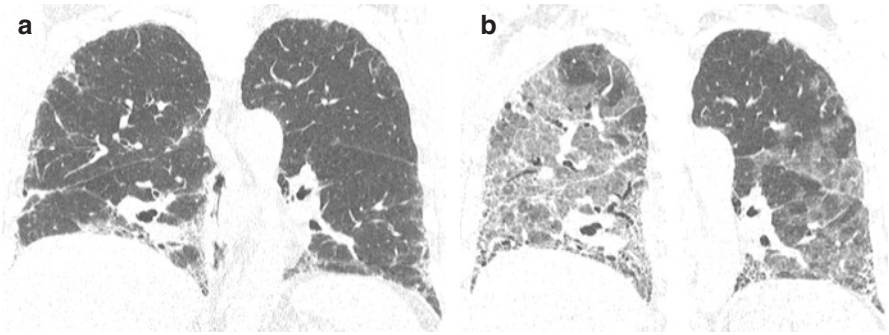

Fig. 4.13 Coronal reformatted HRCT image (**a**) shows typical findings of basilar- and peripheral-predominant pulmonary fibrosis in UIP. Coronal reformatted HRCT image obtained approximately 18 months later (**b**) shows diffuse ground-glass opacity in this patient with acute exacerbation of IPF. Ground-glass opacity in this case is consistent with diffuse alveolar damage histopathology

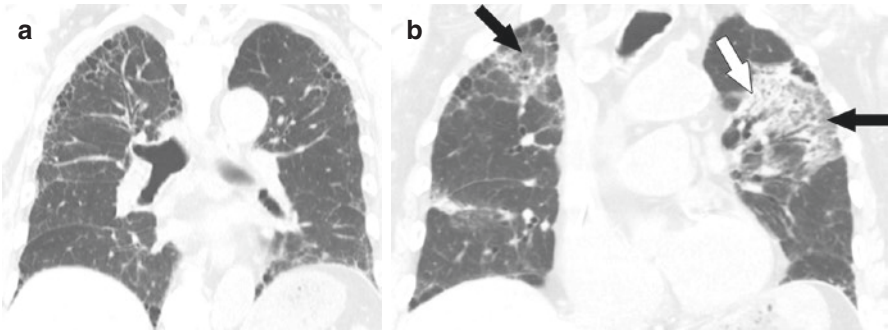

Fig. 4.14 Coronal reformatted HRCT image (**a**) shows pulmonary fibrosis in this patient with UIP. Coronal reformatted HRCT image obtained approximately 2 years later (**b**) shows bronchovascular-predominant ground-glass opacity (*black arrows*) and consolidation (*white arrow*) in this patient with acute exacerbation of IPF. The pattern of bronchovascular ground-glass opacity and consolidation is consistent with organizing pneumonia histopathology

Patients with IPF are at fivefold increased risk of developing lung cancer than the general population [50], and older men with a history of smoking are most often affected. Synchronous cancers are not uncommon and occur in up to 15% of patients [51]. Lung cancers in these patients arise most frequently in the peripheral lung in areas of more severe fibrosis or at the junction of fibrosis and normal lung [52–55] (Fig. 4.15). With regard to lobar distribution, lung cancers in patients with IPF have been reported to occur more often in the lower lobes [56, 57], but other studies report a more balanced distribution of cancer between the upper and lower lobes [52, 58]. The most common types of primary lung cancer in IPF are adenocarcinoma and squamous cell carcinoma [52]. On HRCT, the most common manifestation of

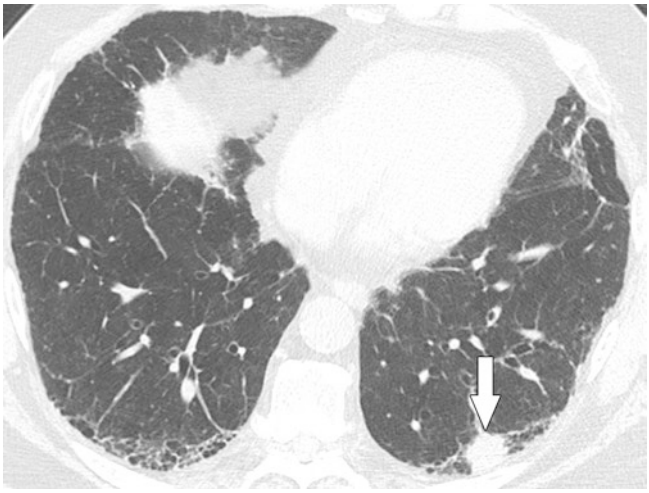

Fig. 4.15 Axial HRCT image shows typical findings of UIP (peripheral-predominant reticulation and honeycombing). The nodule (*arrow*) in the peripheral left lower lobe was new. Transcutaneous needle biopsy showed primary lung adenocarcinoma

lung cancer in association with IPF is an ill- or well-defined nodule or mass. At times, lung cancer can present as air-space consolidation, which usually represents a mucinous adenocarcinoma. Given that lung cancer tends to arise in or adjacent to areas of fibrosis [59], the early detection of lung cancer in IPF can be challenging. Therefore, comparison of current images to previous studies to assess for any new focal nodular or consolidative opacity is, therefore, paramount. In one retrospective study, the authors found that there was a 409-day median delay in lung cancer diagnosis in patients with pulmonary fibrosis, indicating the subtle nature of early lung cancer in this setting [55].

Patients with pulmonary fibrosis are also predisposed to pneumonia, especially from mycobacterial and *Aspergillus* species as well as *Pneumocystis jirovecii* pneumonia (PJP). These tend to develop during periods of immunosuppression in patients with worsening fibrosis and clinical disease progression [50]. *Aspergillus* infection in patients with IPF usually manifests as an aspergilloma in areas of preexisting fibrocavitary disease or as chronic necrotizing aspergillosis [60, 61]. Aspergillomas represent a saprophytic infection in which the fungus ball can shift freely within a lung cavity or dilated bronchus (Fig. 4.16). Because the associated inflammatory response leads to friable and hypervascular cavity walls, patients can develop hemoptysis, which may be life-threatening. Chronic necrotizing aspergillosis presents as focal consolidation, usually within the upper lobes, that eventually cavitates [60]. Patients with secondary pulmonary tuberculosis in the setting of IPF may present with an atypical imaging pattern. Rather than classic upper lobe-predominant cavitary disease with tree-in-bud opacities and centrilobular nodules, subpleural nodules, masses, coalescent consolidation, or a combination of these findings may be seen [62]. Although patients with IPF are unlikely to be at significantly increased risk for PJP if not immunosuppressed, individuals on even mild corticosteroid therapy are more susceptible to PJP. Unfortunately, the HRCT manifestations of PJP in IPF may mimic the findings of an acute exacerbation, with bilateral,

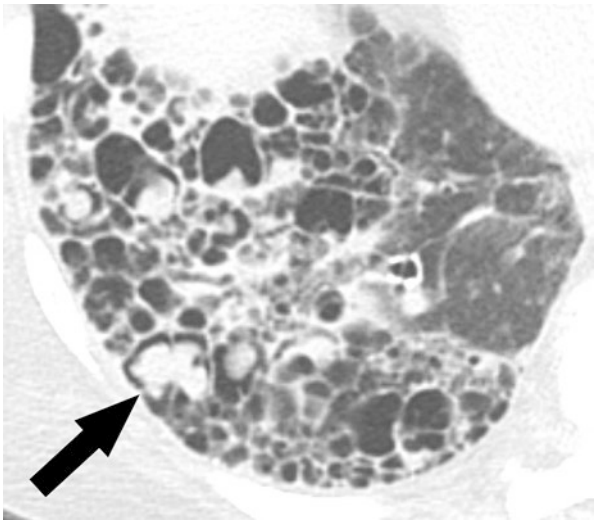

Fig. 4.16 Axial HRCT image shows nodular filling defects (*arrow*) in cystic areas of bronchiectasis and honeycombing shown to represent aspergillomas

diffuse ground-glass opacities, reticulation, and mild consolidation all possible on HRCT imaging. Patients with IPF (especially those on immunosuppression) who present with acute to subacute dyspnea in the context of one of the latter HRCT patterns should be evaluated for infection (including PJP) before the initiation or augmentation of immunosuppression is considered.

Spontaneous pneumomediastinum and pneumothorax develop in up to 11.5% of patients with IPF [63–65] (Fig. 4.17). Pneumothoraces are likely caused by rupture of honeycomb cysts into the pleural space. Pneumomediastinum may be caused by the Macklin effect, in which increased intrathoracic pressure results in alveolar rupture with subsequent dissection of gas along the peribronchial sheaths centrally into the mediastinum. Accurate estimates of the incidence of events where gas gains access to extra-alveolar spaces are difficult to make, because in many cases, patients may be only mildly symptomatic or even asymptomatic. The clinical significance of pneumomediastinum and pneumothorax in asymptomatic patients is unclear. However, when patients present with cough, dyspnea, or chest pain, extra-alveolar gas may portend a poor prognosis, although the evidence for this is weak [64].

Pulmonary hypertension (PH) is present in up to 46% of patients with IPF referred for lung transplantation [66, 67]. In addition, patients with concomitant IPF and PH have a worse prognosis compared to patients with IPF without PH [68]. In one study, the 1-year mortality rate was 28.0% for patients with IPF and PH compared to 5.5% for patients with IPF but without PH [69]. In another study, IPF patients with mean systolic pulmonary artery pressure above 50 mmHg had a mean survival of only 0.7 years compared to 4.8 years for IPF patients with mean systolic pulmonary artery pressure below 35 mmHg [70]. The pathophysiological relationship between IPF and PH is complex and likely includes fibrotic destruction of the vasculature and chronic hypoxic vasoconstriction of small pulmonary vessels. However, these factors in isolation may not explain the relationship between IPF

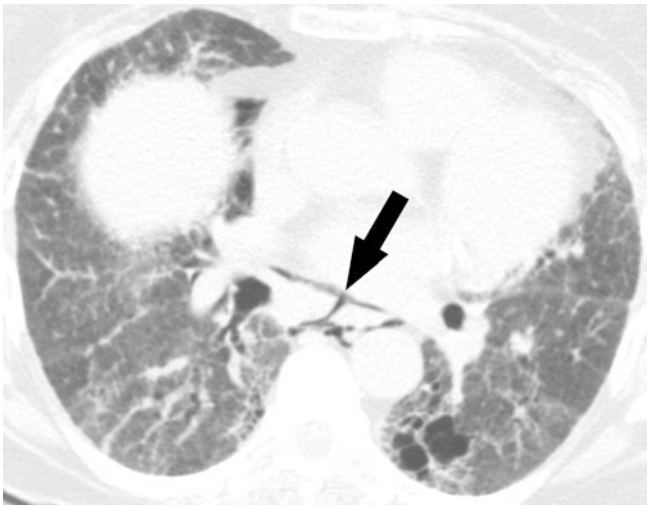

Fig. 4.17 Axial HRCT image shows pneumomediastinum (*arrow*) in this patient with UIP

and PH. There are a significant number of cases in which there is discordance between the degree of IPF or oxygen saturation and PH, which implies that other underlying factors may be present that have not yet been fully identified [71]. Although a correlation between pulmonary arterial diameter and mean pulmonary artery pressure has been shown in the general population, it appears that this relationship may not be extrapolated to patients with IPF. One study showed that the diameter of the main pulmonary artery and the pulmonary artery to aorta diameter ratio did not differ between those with and without PH, and no significant correlation was found between the mean pulmonary artery pressure and pulmonary arterial diameter [72]. Another study also showed that pulmonary artery diameters or ratios were unreliable in predicting mean pulmonary artery pressure. In fact, pulmonary artery dilation may occur in the absence of significant pulmonary hypertension [73].

Atypical UIP on HRCT and How to Distinguish It from Other Common Fibrotic Lung Diseases

In addition to indeterminate HRCT patterns in patients with UIP, the pattern of lung disease on HRCT in UIP may mimic other interstitial lung diseases, most commonly NSIP or chronic (fibrotic) hypersensitivity pneumonitis (HP) and less commonly sarcoidosis (Figs. 4.18 and 4.19). In one study of 55 patients with biopsy-proven UIP, UIP was considered low probability (<30%) by at least two out of three observers on HRCT for 34 of the 55 patients, and NSIP (18/34 = 53%), chronic HP (4/34 = 12%), and sarcoidosis (3/34 = 9%) were scored as the most likely (high degree of probability) first-choice diagnoses. Additionally, NSIP, chronic HP, and sarcoidosis were also most often included in the differential

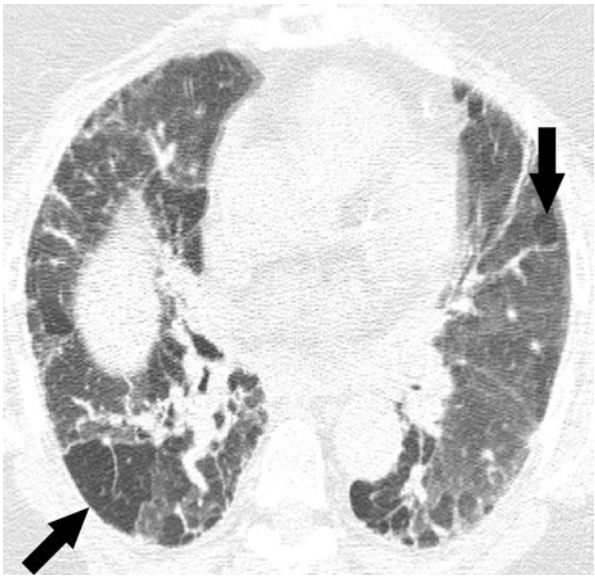

Fig. 4.18 Axial HRCT image during expiration shows lobular areas of air trapping (*arrows*) as well as mild pulmonary fibrosis, consistent with chronic hypersensitivity pneumonitis; however, open lung biopsy showed UIP. Based on clinical work-up, the patient was diagnosed with IPF

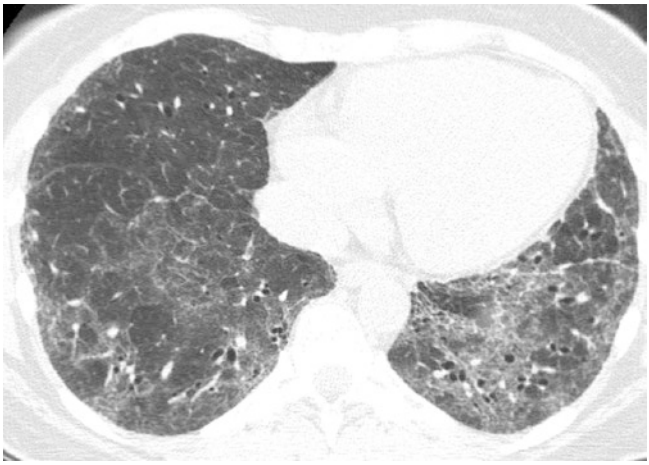

Fig. 4.19 Axial HRCT image shows basilar ground-glass opacity, reticulation, and traction bronchiectasis, most consistent with NSIP. However, open lung biopsy showed UIP. Based on clinical work-up, the patient was diagnosed with IPF

diagnosis, even when these were not scored as the first-choice diagnosis [74]. Silva et al. also compared HRCT appearances of patients with IPF, NSIP, and chronic HP and found that in 23 cases of histopathologically proven UIP, observers chose NSIP or chronic HP as a first-choice diagnosis 25.7% of the time (exclusive of cases in which the first-choice diagnosis was "indeterminate") [30].

Findings suggestive of NSIP include ground-glass opacity (the salient feature, which is present in nearly all cases), fine reticulation, traction bronchiectasis, and lower lobe volume loss [75–79]. Basilar and peribronchovascular predominance is the rule, and upper lobe-predominant disease favors an alternative diagnosis such as

sarcoidosis, HP, or familial pulmonary fibrosis. Because UIP is also nearly always basilar preponderant, the cranio-caudad distribution of disease is not helpful in distinguishing NSIP from UIP. However, the axial distribution of disease can be quite helpful in distinguishing NSIP from IPF. Specifically, although the axial distribution of fibrosis in NSIP can be peripheral, diffuse, or peribronchovascular, the latter pattern combined with relative sparing of the subpleural lung is much more suggestive of NSIP rather than UIP [30] (see Fig. 4.8).

Chronic HP may have findings on HRCT that are identical to those of UIP [80]. However, a confident diagnosis of chronic HP can be made if certain imaging parameters are present. The most specific findings for chronic HP include centrilobular ground-glass nodules, mosaic attenuation (reflecting air trapping), and mid- to upper lobe-predominant pulmonary fibrosis [30, 81, 82]. This combination of findings actually represents overlap of the subacute and chronic phases of HP [83] (see Fig. 4.11). In more advanced cases of chronic HP, honeycombing is quite common, and the HRCT pattern may mimic that of UIP [30, 84].

In the absence of a high-confidence diagnosis of UIP on HRCT, no single test or set of tests has proven to be adequately sensitive and specific in the diagnosis of UIP. In fact, because of the difficulty in establishing a firm diagnosis of UIP (as well as other diffuse lung diseases), a multidisciplinary review of cases by pulmonologists, radiologists, and pathologists is essential in establishing the most accurate diagnosis. One study of 58 patients with suspected interstitial lung disease showed that after consensus review of the clinical, radiological, and pathological data, radiologists changed their initial diagnosis in 50% of cases, pulmonologists in 30% of cases, and pathologists in 20% of cases [85]. Radiologists most commonly changed their initial diagnoses of NSIP to UIP as well as respiratory bronchiolitis or desquamative interstitial pneumonia, and HP was often changed to NSIP. In a study of patients with IPF diagnosed locally by international consensus criteria, the diagnosis of IPF was rejected by an expert panel in 12.8% of cases based on their review of the HRCT and histopathologic findings [86]. Interestingly, the mean kappa value for three expert thoracic radiologists' HRCT evaluations was 0.40, and the kappa value was even lower at 0.30 for two expert pulmonary pathologists' histopathologic evaluations. This further supports the importance of a multidisciplinary diagnostic approach, as disagreements clearly occur even among experts. By increasing opportunities for the pulmonologist, radiologist, or pathologist to make a confident diagnosis of a specific diagnosis (often UIP), a more accurate diagnosis can be established in a greater percentage of patients with diffuse lung disease.

Summary

UIP is the imaging and histopathologic correlate of IPF. If the typical pattern of UIP is present on HRCT, a confident and accurate diagnosis of UIP can be made, obviating the need for lung biopsy. However, in up to half of patients, who ultimately are

proven to have UIP on biopsy, a confident diagnosis of UIP cannot be made by HRCT; these patients often require further work-up with a surgical lung biopsy. The most common diseases that mimic UIP are NSIP and chronic HP. Although there is often overlap in radiographic appearance among these conditions, HRCT can often distinguish UIP from NSIP or chronic HP if certain imaging patterns are present.

References

1. Coultas DB, Zumwalt RE, Black WC, Sobonya RE. The epidemiology of interstitial lung diseases. Am J Respir Crit Care Med. 1994;150:967–72.
2. Baumgartner KB, Samet JM, Stidley CA, Colby TV, Waldron JA. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1997;155:242–8.
3. Raghu G, Freudenberger TD, Yang S, Curtis JR, Spada C, Hayes J, et al. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. Eur Respir J. 2006;27:136–42.
4. Collard HR, King TE Jr, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2003;168:538–42.
5. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. Am J Respir Crit Care Med. 1998;157:1301–15.
6. Bjoraker JA, Ryu JH, Edwin MK, Myers JL, Tazelaar HD, Schroeder DR, et al. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998;157:199–203.
7. Mathieson JR, Mayo JR, Staples CA, Muller NL. Chronic diffuse infiltrative lung disease: comparison of diagnostic accuracy of CT and chest radiography. Radiology. 1989;171:111–6.
8. Johnson TH Jr. Radiology and honeycomb lung disease. Am J Roentgenol Radium Therapy Nucl Med. 1968;104:810–21.
9. Hunninghake GW, Lynch DA, Galvin JR, Gross BH, Müller N, Schwartz DA, et al. Radiologic findings are strongly associated with a pathologic diagnosis of usual interstitial pneumonia. Chest. 2003;124:1215–23.
10. Chandler PW, Shin MS, Friedman SE, Myers JL, Katzenstein AL. Radiographic manifestations of bronchiolitis obliterans with organizing pneumonia vs usual interstitial pneumonia. AJR Am J Roentgenol. 1986;147:899–906.
11. Staples CA, Muller NL, Vedal S, Abboud R, Ostrow D, Miller RR. Usual interstitial pneumonia: correlation of CT with clinical, functional, and radiologic findings. Radiology. 1987;162:377–81.
12. Mayo JR. CT evaluation of diffuse infiltrative lung disease: dose considerations and optimal technique. J Thorac Imaging. 2009;24:252–9.
13. Papadakis AE, Perisinakis K, Damilakis J. Automatic exposure control in pediatric and adult multidetector CT examinations: a phantom study on dose reduction and image quality. Med Phys. 2008;35:4567–76.
14. Tack D, De Maertelaer V, Gevenois PA. Dose reduction in multidetector CT using attenuation based online tube current modulation. AJR Am J Roentgenol. 2003;181:331–4.
15. Kalra MK, Rizzo S, Maher MM, Halpern EF, Toth TL, Shepard JA, et al. Chest CT performed with z-axis modulation: scanning protocol and radiation dose. Radiology. 2005;237:303–8.
16. Sumikawa H, Johkoh T, Ichikado K, Taniguchi H, Kondoh Y, Fujimoto K, et al. Usual interstitial pneumonia and chronic idiopathic interstitial pneumonia: analysis of CT appearance in 92 patients. Radiology. 2006;241:258–66.
17. Hansell DM, Bankier AA, MacMahon H, McLoud TC, Muller NL, Remy J. Fleischner Society: glossary of terms for thoracic imaging. Radiology. 2008;246:697–722.

18. Lynch DA, Godwin JD, Safrin S, Starko KM, Hormel P, Brown KK, et al. High-resolution computed tomography in idiopathic pulmonary fibrosis: diagnosis and prognosis. Am J Respir Crit Care Med. 2005;172:488–93.
19. Remy-Jardin M, Giraud F, Remy J, Copin MC, Gosselin B, Duhamel A. Importance of ground-glass attenuation in chronic diffuse infiltrative lung disease: pathologic-CT correlation. Radiology. 1993;189:693–8.
20. Nishimura K, Kitaichi M, Izumi T, Nagai S, Kanaoka M, Itoh H. Usual interstitial pneumonia: histologic correlation with high-resolution CT. Radiology. 1992;182:337–42.
21. Leung AN, Miller RR, Muller NL. Parenchymal opacification in chronic infiltrative lung diseases: CT-pathologic correlation. Radiology. 1993;188:209–14.
22. Niimi H, Kang EY, Kwong JS, Carignan S, Muller NL. CT of chronic infiltrative lung disease: prevalence of mediastinal lymphadenopathy. J Comput Assist Tomogr. 1996;20:305–8.
23. Souza CA, Muller NL, Lee KS, Johkoh T, Mitsuhiro H, Chong S. Idiopathic interstitial pneumonias: prevalence of mediastinal lymph node enlargement in 206 patients. AJR Am J Roentgenol. 2006;186:995–9.
24. Jung JI, Kim HH, Jung YJ, Park SH, Lee JM, Hahn ST. Mediastinal lymphadenopathy in pulmonary fibrosis: correlation with disease severity. J Comput Assist Tomogr. 2000;24:706–10.
25. 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;167:962–9.
26. Cottin V, Nunes H, Brillet PY, Delaval P, Devouassoux G, Tillie-Leblond I, et al. Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. Eur Respir J. 2005;26:586–93.
27. Flaherty KR, Thwaite EL, Kazerooni EA, Gross BH, Toews GB, Colby TV, et al. Radiological versus histological diagnosis in UIP and NSIP: survival implications. Thorax. 2003;58:143–8.
28. Tsubamoto M, Muller NL, Johkoh T, Ichikado K, Taniguchi H, Kondoh Y, et al. Pathologic subgroups of nonspecific interstitial pneumonia: differential diagnosis from other idiopathic interstitial pneumonias on high-resolution computed tomography. J Comput Assist Tomogr. 2005;29:793–800.
29. Johkoh T, Muller NL, Cartier Y, Kavanagh PV, Hartman TE, Akira M, et al. Idiopathic interstitial pneumonias: diagnostic accuracy of thin-section CT in 129 patients. Radiology. 1999;211:555–60.
30. Silva CI, Muller NL, Lynch DA, Curran-Everett D, Brown KK, Lee KS, et al. Chronic hypersensitivity pneumonitis: differentiation from idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia by using thin-section CT. Radiology. 2008;246:288–97.
31. Hunninghake GW, Zimmerman MB, Schwartz DA, King TE Jr, Lynch J, Hegele R, et al. Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2001;164:193–6.
32. Swensen SJ, Aughenbaugh GL, Myers JL. Diffuse lung disease: diagnostic accuracy of CT in patients undergoing surgical biopsy of the lung. Radiology. 1997;205:229–34.
33. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183:788–824.
34. Sundaram B, Gross BH, Martinez FJ, Oh E, Müller NL, Schipper M, et al. Accuracy of high-resolution CT in the diagnosis of diffuse lung disease: effect of predominance and distribution of findings. AJR Am J Roentgenol. 2008;191:1032–9.
35. Walsh SL, Calandriello L, Sverzellati N, Wells AU, Hansell DM, UIP Observer Consort. Interobserver agreement for the ATS/ERS/JRS/ALAT criteria for a UIP pattern on CT. Thorax. 2016 Jan;71(1):45–51.
36. Chung JH, Chawla A, Peljto AL, et al. CT scan findings of probable usual interstitial pneumonitis have a high predictive value for histologic usual interstitial pneumonitis. Chest. 2015;147:450–9.
37. Raghu G, Lynch D, Godwin JD, et al. Diagnosis of idiopathic pulmonary fibrosis with high-resolution CT in patients with little or no radiological evidence of honeycombing: secondary analysis of a randomised, controlled trial. Lancet Respir Med. 2014;2:277–84.

38. Flaherty KR, Toews GB, Travis WD, Colby TV, Kazerooni EA, Gross BH, et al. Clinical significance of histological classification of idiopathic interstitial pneumonia. Eur Respir J. 2002;19:275–83.
39. Gay SE, Kazerooni EA, Toews GB, Lynch JP 3rd, Gross BH, Cascade PN, et al. Idiopathic pulmonary fibrosis: predicting response to therapy and survival. Am J Respir Crit Care Med. 1998;157:1063–72.
40. Nagao T, Nagai S, Hiramoto Y, Hamada K, Shigematsu M, Hayashi M, et al. Serial evaluation of high-resolution computed tomography findings in patients with idiopathic pulmonary fibrosis in usual interstitial pneumonia. Respiration. 2002;69:413–9.
41. Sumikawa H, Johkoh T, Colby TV, Ichikado K, Suga M, Taniguchi H, et al. Computed tomography findings in pathological usual interstitial pneumonia: relationship to survival. Am J Respir Crit Care Med. 2008;177:433–9.
42. Shin KM, Lee KS, Chung MP, Han J, Bae YA, Kim TS, et al. Prognostic determinants among clinical, thin-section CT, and histopathologic findings for fibrotic idiopathic interstitial pneumonias: tertiary hospital study. Radiology. 2008;249:328–37.
43. Hwang JH, Misumi S, Curran-Everett D, Brown KK, Sahin H, Lynch DA. Longitudinal follow-up of fibrosing interstitial pneumonia: relationship between physiologic testing, computed tomography changes, and survival rate. J Thorac Imaging. 2011;26:209–17.
44. Parambil JG, Myers JL, Ryu JH. Histopathologic features and outcome of patients with acute exacerbation of idiopathic pulmonary fibrosis undergoing surgical lung biopsy. Chest. 2005;128:3310–5.
45. Kim DS, Park JH, Park BK, Lee JS, Nicholson AG, Colby T. Acute exacerbation of idiopathic pulmonary fibrosis: frequency and clinical features. Eur Respir J. 2006;27:143–50.
46. Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, King TE Jr, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2007;176:636–43.
47. Churg A, Muller NL, Silva CI, Wright JL. Acute exacerbation (acute lung injury of unknown cause) in UIP and other forms of fibrotic interstitial pneumonias. Am J Surg Pathol. 2007;31:277–84.
48. Akira M, Hamada H, Sakatani M, Kobayashi C, Nishioka M, Yamamoto S. CT findings during phase of accelerated deterioration in patients with idiopathic pulmonary fibrosis. AJR Am J Roentgenol. 1997;168:79–83.
49. Silva CI, Muller NL, Fujimoto K, Kato S, Ichikado K, Taniguchi H, et al. Acute exacerbation of chronic interstitial pneumonia: high-resolution computed tomography and pathologic findings. J Thorac Imaging. 2007;22:221–9.
50. Hubbard R, Venn A, Lewis S, Britton J. Lung cancer and cryptogenic fibrosing alveolitis. A population-based cohort study. Am J Respir Crit Care Med. 2000;161:5–8.
51. Mizushima Y, Kobayashi M. Clinical characteristics of synchronous multiple lung cancer associated with idiopathic pulmonary fibrosis. A review of Japanese cases. Chest. 1995;108:1272–7.
52. Kishi K, Homma S, Kurosaki A, Motoi N, Yoshimura K. High-resolution computed tomography findings of lung cancer associated with idiopathic pulmonary fibrosis. J Comput Assist Tomogr. 2006;30:95–9.
53. Matsushita H, Tanaka S, Saiki Y, Hara M, Nakata K, Tanimura S, et al. Lung cancer associated with usual interstitial pneumonia. Pathol Int. 1995;45:925–32.
54. Aubry MC, Myers JL, Douglas WW, Tazelaar HD, Washington Stephens TL, Hartman TE, et al. Primary pulmonary carcinoma in patients with idiopathic pulmonary fibrosis. Mayo Clin Proc. 2002;77:763–70.
55. Yoshida R, Arakawa H, Kaji Y. Lung cancer in chronic interstitial pneumonia: early manifestation from serial CT observations. AJR Am J Roentgenol. 2012;199:85–90.
56. Lee HJ, Im JG, Ahn JM, Yeon KM. Lung cancer in patients with idiopathic pulmonary fibrosis: CT findings. J Comput Assist Tomogr. 1996;20:979–82.
57. Sakai S, Ono M, Nishio T, Kawarada Y, Nagashima A, Toyoshima S. Lung cancer associated with diffuse pulmonary fibrosis: CT-pathologic correlation. J Thorac Imaging. 2003;18:67–71.
58. Park J, Kim DS, Shim TS, Lim CM, Koh Y, Lee SD, et al. Lung cancer in patients with idiopathic pulmonary fibrosis. Eur Respir J. 2001;17:1216–9.

59. Lloyd CR, Walsh SL, Hansell DM. High-resolution CT of complications of idiopathic fibrotic lung disease. Br J Radiol. 2011;84:581–92.
60. Saraceno JL, Phelps DT, Ferro TJ, Futerfas R, Schwartz DB. Chronic necrotizing pulmonary aspergillosis: approach to management. Chest. 1997;112:541–8.
61. Roberts CM, Citron KM, Strickland B. Intrathoracic aspergilloma: role of CT in diagnosis and treatment. Radiology. 1987;165:123–8.
62. Chung MJ, Goo JM, Im JG. Pulmonary tuberculosis in patients with idiopathic pulmonary fibrosis. Eur J Radiol. 2004;52:175–9.
63. Picado C, Gomez de Almeida R, Xaubet A, Montserrat J, Letang E, Sanchez-Lloret J. Spontaneous pneumothorax in cryptogenic fibrosing alveolitis. Respiration. 1985;48:77–80.
64. Fujiwara T. Pneumomediastinum in pulmonary fibrosis. Detection by computed tomography. Chest. 1993;104:44–6.
65. Franquet T, Gimenez A, Torrubia S, Sabate JM, Rodriguez-Arias JM. Spontaneous pneumothorax and pneumomediastinum in IPF. Eur Radiol. 2000;10:108–13.
66. Shorr AF, Wainright JL, Cors CS, Lettieri CJ, Nathan SD. Pulmonary hypertension in patients with pulmonary fibrosis awaiting lung transplant. Eur Respir J. 2007;30:715–21.
67. King TE Jr, Tooze JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med. 2001;164:1171–81.
68. Hamada K, Nagai S, Tanaka S, Handa T, Shigematsu M, Nagao T, et al. Significance of pulmonary arterial pressure and diffusion capacity of the lung as prognosticator in patients with idiopathic pulmonary fibrosis. Chest. 2007;131:650–6.
69. Lettieri CJ, Nathan SD, Barnett SD, Ahmad S, Shorr AF. Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest. 2006;129:746–52.
70. Nadrous HF, Pellikka PA, Krowka MJ, Swanson KL, Chaowalit N, Decker PA, et al. Pulmonary hypertension in patients with idiopathic pulmonary fibrosis. Chest. 2005;128:2393–9.
71. Corte TJ, Wort SJ, Wells AU. Pulmonary hypertension in idiopathic pulmonary fibrosis: a review. Sarcoidosis Vasc Diffuse Lung Dis. 2009;26:7–19.
72. Zisman DA, Karlamangla AS, Ross DJ, Keane MP, Belperio JA, Saggar R, et al. High-resolution chest CT findings do not predict the presence of pulmonary hypertension in advanced idiopathic pulmonary fibrosis. Chest. 2007;132:773–9.
73. Devaraj A, Wells AU, Meister MG, Corte TJ, Hansell DM. The effect of diffuse pulmonary fibrosis on the reliability of CT signs of pulmonary hypertension. Radiology. 2008;249:1042–9.
74. Sverzellati N, Wells AU, Tomassetti S, Desai SR, Copley SJ, Aziz ZA, et al. Biopsy-proved idiopathic pulmonary fibrosis: spectrum of nondiagnostic thin-section CT diagnoses. Radiology. 2010;254:957–64.
75. Elliot TL, Lynch DA, Newell JD Jr, Cool C, Tuder R, Markopoulou K, et al. High-resolution computed tomography features of nonspecific interstitial pneumonia and usual interstitial pneumonia. J Comput Assist Tomogr. 2005;29:339–45.
76. Johkoh T, Muller NL, Colby TV, Ichikado K, Taniguchi H, Kondoh Y, et al. Nonspecific interstitial pneumonia: correlation between thin-section CT findings and pathologic subgroups in 55 patients. Radiology. 2002;225:199–204.
77. Hartman TE, Swensen SJ, Hansell DM, Colby TV, Myers JL, Tazelaar HD, et al. Nonspecific interstitial pneumonia: variable appearance at high-resolution chest CT. Radiology. 2000;217:701–5.
78. MacDonald SL, Rubens MB, Hansell DM, Copley SJ, Desai SR, du Bois RM, et al. Nonspecific interstitial pneumonia and usual interstitial pneumonia: comparative appearances at and diagnostic accuracy of thin-section CT. Radiology. 2001;221:600–5.
79. Kligerman SJ, Groshong S, Brown KK, Lynch DA. Nonspecific interstitial pneumonia: radiologic, clinical, and pathologic considerations. Radiographics. 2009;29:73–87.
80. Lynch DA, Newell JD, Logan PM, King TE Jr, Muller NL. Can CT distinguish hypersensitivity pneumonitis from idiopathic pulmonary fibrosis? AJR Am J Roentgenol. 1995;165:807–11.
81. Hansell DM, Wells AU, Padley SP, Muller NL. Hypersensitivity pneumonitis: correlation of individual CT patterns with functional abnormalities. Radiology. 1996;199:123–8.

82. Small JH, Flower CD, Traill ZC, Gleeson FV. Air-trapping in extrinsic allergic alveolitis on computed tomography. Clin Radiol. 1996;51:684–8.
83. Silva CI, Churg A, Muller NL. Hypersensitivity pneumonitis: spectrum of high-resolution CT and pathologic findings. AJR Am J Roentgenol. 2007;188:334–44.
84. Remy-Jardin M, Remy J, Wallaert B, Muller NL. Subacute and chronic bird breeder hypersensitivity pneumonitis: sequential evaluation with CT and correlation with lung function tests and bronchoalveolar lavage. Radiology. 1993;189:111–8.
85. Flaherty KR, King TE Jr, Raghu G, Lynch JP 3rd, Colby TV, Travis WD, et al. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? Am J Respir Crit Care Med. 2004;170:904–10.
86. Thomeer M, Demedts M, Behr J, Buhl R, Costabel U, Flower CD, et al. Multidisciplinary interobserver agreement in the diagnosis of idiopathic pulmonary fibrosis. Eur Respir J. 2008;31:585–91.

Chapter 5
Pulmonary Function Tests in Idiopathic Pulmonary Fibrosis

Francesco Bonella, Fabiano di Marco, and Paolo Spagnolo

Pulmonary Function Tests in IPF

Pulmonary fibrosis affects both the mechanical properties of the lung as well as gas exchange. Impairment of the mechanical properties is due to decreased lung compliance (i.e., the lungs become "stiff" and have a high level of elastic recoil), which leads to restrictive abnormalities. A pure restrictive ventilatory defect is characterized by a reduction in total lung capacity (TLC) and a normal forced expiratory volume in 1 s/vital capacity (FEV1/VC) ratio [1]. An example of a restrictive ventilatory pattern is shown in Fig. 5.1. As expected, flow rates are often increased due to the increased elastic recoil, with the presence of a concomitant chronic airflow obstruction component only in smokers with significant small airway disease [2–4]. Another condition that can impact the extent of the expected restrictive ventilatory defect in "pure" pulmonary fibrosis is the contemporaneous presence of emphysema, as seen in combined pulmonary fibrosis and emphysema (CPFE) [5]. Indeed, emphysema leads to a significant increase of lung compliance (i.e., reduction of elastic recoil), which can counterbalance the "mechanical" effects of pulmonary fibrosis, leading to "pseudonormalized" lung volumes and flows [5]. In the case of idiopathic pulmonary fibrosis (IPF), impairment of gas exchange properties, as demonstrated by a reduction of lung diffusion for carbon monoxide (DL_{CO}), is

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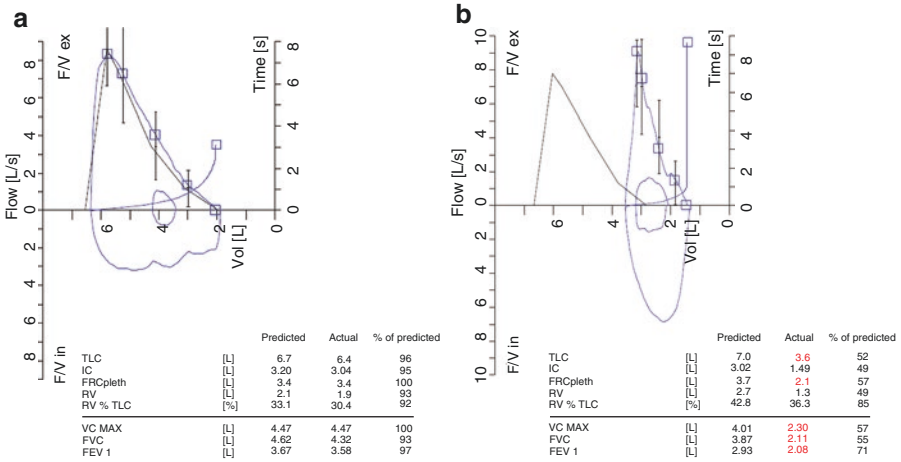

Fig. 5.1 A normal flow-volume loop in a healthy subject (**a**) compared with a patient with IPF (**b**). The ventilatory pattern in this IPF patient is characterized by a moderate reduction in the total and forced vital capacity (about 50% of predicted). A slight reduction of forced expiratory flow is also present. The predicted flow-volume loop is represented by the black line, and the actual loop of the examined subjects is in blue

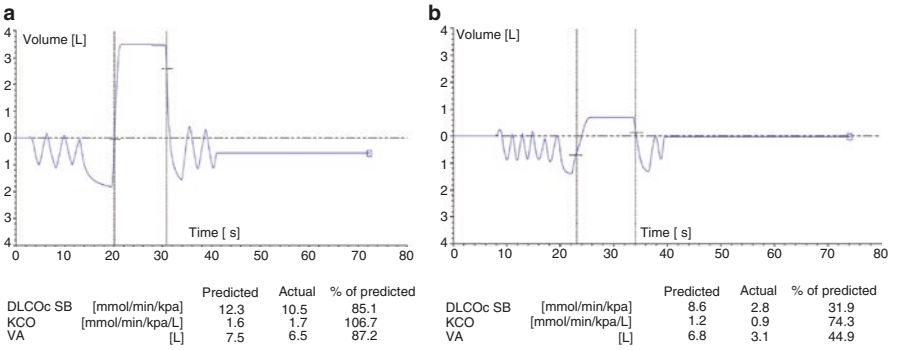

Fig. 5.2 Diffusing capacity of the lung for carbon monoxide (DL_{CO}) in a healthy subject (**a**) and in a patient with IPF (**b**). The diffusion is clearly also reduced in the patient after adjustment per alveolar volume (Kco)

caused by loss of pulmonary capillary volume and by ventilation and perfusion abnormalities (Fig. 5.2). Increased ventilation/perfusion mismatching results in an increased ratio of dead space ventilation to tidal volume (V_D/V_T) due to hypoperfusion of ventilated alveoli, while a reduced pulmonary capillary bed results in shortened red blood cell transit times. In addition, right-to-left shunt through a patent foramen oval may also cause further gas exchange impairment. Also, a frequent complication/comorbidity of IPF is pulmonary hypertension (PH), which can further worsen gas exchange. In the case of CPFE, unlike well-maintained lung volumes, the DL_{CO} is substantially reduced, since the effects of the two conditions (i.e.,

fibrosis and emphysema) have additive effects on gas exchange. Moreover, CPFE has a greater propensity for complicating PH. At rest, the ventilatory pattern of patients with IPF is characterized by an increased respiratory rate, with a rapid shallow breathing pattern [2, 4]. Since no defined "chemical" reasons for this modification of breathing pattern have been clearly demonstrated, the main reason can be the altered mechanical reflexes due to the increased elastic recoil of the lung.

Additional sources of ventilatory stimulation include early metabolic acidosis with activity due to deconditioning, increased peripheral muscle ergo-receptor activation, altered reflex afferent activation of vagal receptors in the lung parenchyma and airways [6], or the presence of comorbidities/complications, such as PH, emphysema, cardiovascular diseases, or obesity. Arterial blood gas analysis at rest can be normal in some IPF patients with mild disease, but in most cases it shows hypoxemia with a reduced $PaCO_2$ (i.e., respiratory alkalosis), which reflects the increase of minute ventilation. However, exercise desaturation is very common, including cases of mild disease with normal arterial blood gas analysis at rest. During exercise the capillary transit time is shortened due to the rise in cardiac output. In normal subjects mixed venous oxygen levels fall due to increased tissue oxygen extraction, but hypoxemia does not develop due to the recruitment and distension of capillaries and a rise in alveolar oxygen tension. In contrast to normal subjects, patients with IPF fail to recruit additional capillaries, leading to exercise-induced hypoxemia/desaturation.

Exercise intolerance is a cardinal feature of IPF and is often associated with significant exertional dyspnea and fatigue that typically progress over time and cause impairment of patients' quality of life. Healthy subjects increase ventilation mainly by increasing tidal volume during mild-to-moderate exercise. In contrast, the typical rapid, shallow breathing pattern that is present in patients with IPF worsens during exercise. The low tidal volume accompanying IPF precludes a normal decrement in the V_D/V_T ratio, which worsens ventilatory inefficiency and increases inspiratory neural drive, leaving an increased respiratory rate as the only option to meet the higher ventilatory needs [6]. Therefore, in IPF the efficiency of ventilation during exercise is impaired, as demonstrated by the increased minute ventilation/CO_2 production (V_E/V_{CO2}) ratio. The higher level of ventilation needed during exercise for the elimination of the same amount of CO_2 can be the result of two mechanisms, which are both potentially present in patients with IPF as previously discussed: (1) increased wasted ventilation (i.e., increased V_D/V_T) and (2) reduction of CO_2 set point (i.e., change in neural drive with relative alveolar hyperventilation). Inspiratory muscle function is often preserved in patients with IPF as a result of the combination of the training effect due to mechanical loading and the mechanical advantage due to the lower than normal operational lung volume. However, some conditions can lead to impairment of muscle function, such as the effect of systemic inflammation, malnutrition, cachexia or electrolyte disturbances, side effects of drugs, or deconditioning. Despite this abnormal ventilatory pattern, respiratory mechanics are not thought to be the major contributor to exercise limitation in all patients, as demonstrated by the presence of a large ventilatory reserve (i.e., the difference between ventilation at peak of exercise and the maximal ventilation possible) at the

end of exercise. Thus, other factors, including impairment of gas exchange and circulatory limitation, may play important roles in exercise limitation for patients with IPF. During exercise, patients with IPF show a larger increase in the alveolar-arterial oxygen pressure gradient than those with other interstitial lung disease (such as sarcoidosis or asbestosis) due to the generally greater extent of interstitial fibrosis seen in IPF. In IPF an increased pulmonary vascular resistance (PVR) is common, leading in some cases to right ventricular hypertrophy and PH (*cor pulmonale*). Even if, in absence of a significant comorbidity, left ventricular ejection fraction can be preserved with normal values of systolic pressure and pulmonary artery occlusion pressure, the rate of rise of cardiac output can diminish at higher work rate in some patients with IPF, which is partially due to the increase of pulmonary vascular resistances. Pulmonary hypertension at rest or during exercise can lead to a further worsening of ventilatory efficiency, as demonstrated by a very high V_E/V_{CO2}.

FVC and DLco in Routine Clinical Practice and Clinical Trials: Strengths and Pitfalls

FVC is widely used both in clinical practice and clinical trials to evaluate disease status/severity, progression, and response to treatment in patients with IPF. Measuring FVC is simple, widely available, and easily obtainable. In addition, reproducibility of FVC is excellent, with 90% of patients being able to repeat the test with <5% variation [7]. Accordingly, current guidelines recommend measuring the FVC every 3–4 months for monitoring disease status and behavior. While the guideline document does not specify the absolute minimum magnitude of FVC change required for determining disease progression [8], a 10% decline in an individual's FVC has been correlated with increased mortality in multiple studies [9–12]. Du Bois and colleagues measured FVC and other measures of functional status at baseline and 24-week intervals in a large cohort of patients enrolled in two clinical trials of IFN-γ 1b ($N = 1156$) [13]. They assessed FVC reliability (based on two proximal measures of FVC), validity (based on correlations between FVC and other measures of functional status), and responsiveness (based on the relationship between 24-week changes in FVC and other measures of functional status). Correlation of percent-predicted FVC between measurements was high ($r = 0.93$; $p < 0.001$), while correlations of FVC with other parameters were generally weak, with the strongest correlation being observed between FVC and the DL_{CO} ($r = 0.38$; $p < 0.001$). Correlations between change in FVC and changes in other parameters were slightly stronger ($r = 0.16$–0.37; $p < 0.001$). Importantly, patients experiencing a 24-week decline in FVC between 5% and 10% had a more than twofold higher risk of death at 1 year. In addition, du Bois and colleagues showed that a decline in FVC of 2–6% (minimal clinically important difference) is associated with clinically relevant changes of disease status. The observation that marginal changes in FVC over a 24-week period predict mortality during the subsequent 1-year period corroborates previously published data by Zappala and co-workers [14]. The categorical 6-month

changes in FVC and DL_{CO} regarded as "significant" (FVC >10%, DL_{CO} >15%) or "marginal" (FVC 5–10%, DL_{CO} 7.5–15%) in a cohort of patients with IPF ($N = 84$) and nonspecific interstitial pneumonia (NSIP) ($N = 72$) demonstrated that IPF patients with a *significant* decline in FVC and those with a *marginal* decline in FVC had a higher mortality compared with patients with stable disease (hazard ratio (HR) 2.80; $p < 0.001$ and HR 2.31; $p = 0.01$, respectively). More recently, Reichmann and co-workers performed a retrospective chart review to examine change in FVC across IPF patients ($N = 490$) in the 6 months after diagnosis and its association with clinical and healthcare resource utilization (HRU) outcomes in a real-world setting in the USA [15]. Patients were categorized as stable (decline <5%), marginal decline (decline ≥5% and < 10%), or significant decline (decline ≥10%) based on the relative change in percent-predicted FVC. At 6 months, 250 (51%) patients were stable, 98 (20%) experienced a marginal decline, and 142 (29%) a significant decline. In both unadjusted and multivariable analysis, greater FVC decline was associated with significantly increased risk of worse clinical outcomes, including further disease progression, suspected acute exacerbations, mortality, and higher rate of HRU. On the other hand, a marginal (i.e., 5%) decline in FVC was not significantly associated with increased risk of death in a large cohort of patients randomized to placebo from six pirfenidone and nintedanib trials ($N = 1132$), although this was probably due to the shorter duration of observation [16]. In the same study, Paterniti and colleagues evaluated the association between FVC decline and mortality and, consistent with previous studies, confirmed that an absolute decline in FVC of >10% (at any time point during follow-up) increased the risk of death significantly [17].

In clinical trials of IPF, change in FVC has been the most widely used primary endpoint, the rationale being that, due to the archetypal pathophysiology of IPF (i.e., a fibrotic process that reduces the size of the lung), decline in FVC over time is likely to represent disease progression. Change in FVC is analyzed either as a continuous variable or by predefined thresholds for change over time. Analyses of continuous change are more sensitive, but evaluation of FVC as a continuous variable may not capture disease progression occurring in a stepwise fashion. An absolute decline in percent-predicted FVC ≥10% (i.e., from 60% predicted to 50% predicted) at 24 weeks is associated with a nearly fivefold increase in the risk of mortality over the subsequent year [9, 18]. Yet, the optimal threshold for FVC change in patients with IPF is unknown. Similarly unclear is whether the 10% threshold for an FVC decline to be significant refers to "relative change" (i.e., a reduction in percent-predicted values from 60% to 54%) or "absolute change" (i.e., a reduction in percent-predicted values from 60% to 50%) [19]. An obvious disadvantage of absolute thresholds for change is they may have different implications in mild and severe disease. For instance, a 10% absolute change would arguably be regarded as a relatively minor decline in patients with mild disease, but a considerable fall in those with advanced disease (i.e., a fall in FVC from 40% to 30% and, thus, a 25% fall from baseline) [20]. Relative change in FVC does not suffer from this problem and deals more closely with the confounding effect of measurement variation, which is expressed as the standard deviation of change from measured baseline values [20]. Richeldi and colleagues compared the prognostic value of

absolute and relative FVC change thresholds of 10% and 5% in 142 patients with IPF with baseline and 12-month follow-up FVC data from two prospective cohorts [21]. The relative and absolute methods were compared in their ability to predict 2-year transplant-free survival. The frequency of any given FVC decline was significantly greater using the relative change in FVC method. However, for ≥10% decline, both methods predicted 2-year transplant-free survival with similar accuracy and remained significant predictors after adjusting for baseline characteristics. Therefore, using the relative change in FVC maximizes the chance of identifying a ≥10% decline in FVC without sacrificing prognostic accuracy.

The physiological effect of coexisting emphysema on the predictive values of serial changes in FVC is unclear, but emphysema is likely to be a confounding factor by artificially preserving lung volumes [22]. Yet, a reduction in FVC can also be caused by progressive hyperinflation and must therefore be interpreted in the light of other lung function parameters, primarily DL_{CO} [22]. In a post hoc analysis of data derived from a subset of patients ($N = 455$) from two phase III trials of IFN-γ-1b in IPF (GIPF-001 [NCT00047645] and GIPF-007 [NCT00075998]), Cottin and colleagues investigated the relationship between baseline emphysema and fibrosis extent, as well as pulmonary function changes, over 48 weeks [23]. Patients with the greatest emphysema extent (28–65%) showed the smallest FVC decline, with a difference of 3.32% at week 48 versus patients with no emphysema ($p = 0.047$). More importantly, emphysema extent ≥15% was associated with significantly reduced FVC decline over 48 weeks versus no emphysema or emphysema <15%, suggesting that FVC measurements may not be appropriate for monitoring disease progression in IPF patients with extent of emphysema ≥15% [23].

Since a large body of evidence supports that decline in FVC within 6–12 months increases the subsequent risk of mortality [9–12], FVC has been incorporated into several cross-sectional and longitudinal indexes for staging IPF [24–27]. The most recent one is the gender-age-physiology (GAP) index, which had favorable performance characteristics in terms of correlation with mortality in IPF and other ILDs [25, 28, 29]. What makes the clinical assessment of disease progression and therapeutic response challenging is the marginal decline in FVC, given that an annualized decline of ≥5% in FVC is also associated with mortality [14] and that the intra-subject variability in patients with IPF can be high [30].

A recent retrospective study examined the variability in the rate of disease progression and evaluated the effect of treatment continuation in patients enrolled in the ASCEND and CAPACITY trials who experienced meaningful progression during treatment [31]. Analysis of longitudinal FVC data showed only a weak inverse correlation between changes in FVC during two consecutive 6-month intervals, highlighting the variability in both the magnitude and direction of change in this prospective, clinical trial population (Fig. 5.3). A similar conclusion has been drawn from a pooled analysis of data from the phase III trials with nintedanib. FVC declines of ≥5% or ≥10% predicted in the first 24 weeks did not predict FVC decline from week 24 to 52, but these declines were associated with higher mortality [32]. These results are similar to observations from a retrospective analysis of a real-world IPF cohort, which suggested that FVC decline in the 1st year of follow-up after diagnosis was not predictive of future declines in physiology [33].

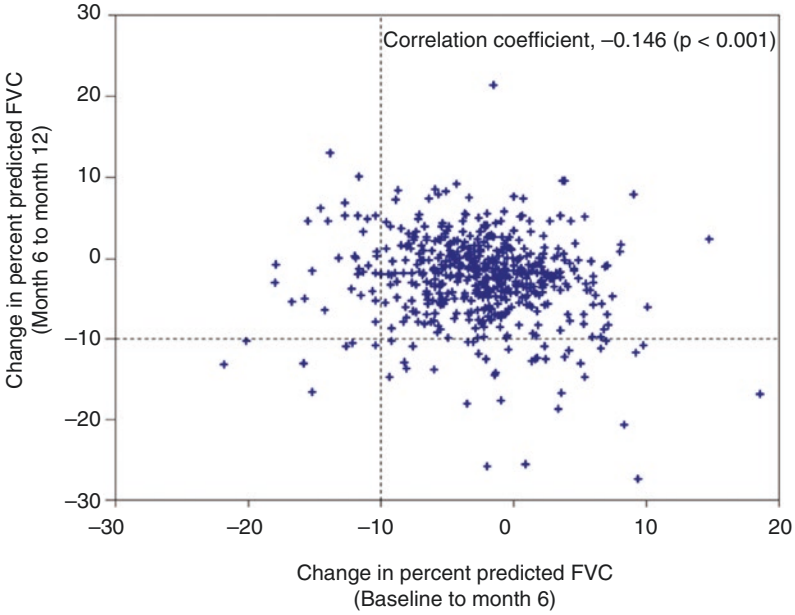

Fig. 5.3 Relationship between changes in percent-predicted FVC during two consecutive 6-month intervals*. *Pooled placebo population, CAPACITY and ASCEND studies ($N = 540$) [31]

Summarizing, the reliability to predict the expected rate of change in FVC during subsequent periods based on prior trends is precluded by the intrinsic variability in the rate of this biomarker [31]. Last but not least, FVC is not a "patient-centered" outcome, and treatment-induced reduction in the rate of functional decline is not perceived as a tangible benefit from the patient's perspective [34].

The measurement of the single-breath DLco is more problematic than FVC and requires a breath hold that can be difficult for more symptomatic patients and has greater intrinsic variability, which is reported as high as 15% [35]. The threshold of 15% change has therefore been utilized to define a significant change. DLco has also been integrated into the GAP index [25], given the fact that stratification of patients on the basis of their DLco allows discrimination of groups with distinctly different long-term survivals [36]. The issue of collinearity between FVC and unadjusted DLco is well known and has raised the notion that the Kco value, which represents the DLco value adjusted for the alveolar volume, might be a better biomarker to serve as an endpoint or to be included in staging indexes [35].

Interestingly, a recent analysis of 416 patients with mild IPF from the Australian IPF Registry has pointed out that there was only fair concordance between FVC and DLco in classifying disease severity, with the FVC ≥80% classifying more patients as mild than DLco ≥55%. A better concordance in classification was reached with composite values (GAP and CPI), as opposed to between single measures such as FVC and DLco, probably due to the integration of both these single measures into

the calculation of the composite scores. It was also highlighted that the DLco <55% and the composite scores were better at predicting survival in comparison to FVC <80% suggesting again that, while it is commonly used, the FVC threshold may not be the most clinically useful criterion [17, 37, 38].

Future Directions: Home Daily Spirometry in IPF

Within respiratory medicine home peak flow measurement is already a feature of asthma self-management, and home spirometry in lung transplant recipients is now an established method to detect early changes in graft function [39–46]. It is not known whether the adoption of a similar approach in IPF might be either feasible or clinically useful. Potential clinical advantages of routine home monitoring in IPF include early detection of rapidly declining patients or those with acute exacerbation and monitoring of response to novel therapies.

While transplant patients receive frequent and lifelong medical outpatient follow-up care at the transplant center, most IPF patients, excluding those participating in clinical trials, usually undergo follow-up visits every 3 months. This raises the issue of adherence to daily measurement and what patients' perception of changes in the FVC might be, given that they are prone to develop anxiety and depression [47].

In a recent study by Russell and colleagues, 50 IPF patients were asked to use the spirometry device once daily, and 13 completed the 70-week follow-up [48]. Subjects were adequately trained on how to perform spirometry, in order to ensure regularity and timing of self-monitoring. Daily home monitoring of FVC resulted in a well-accepted, feasible, and reliable assessment tool, and the readings were comparable to those from healthy volunteers and COPD patients. It was possible to identify patterns of disease behavior (Fig. 5.4), and the rate of decline in FVC was highly predictive of outcome and subsequent mortality when measured at 3, 6, and 12 months. This study of home spirometry, in general, underestimated the values

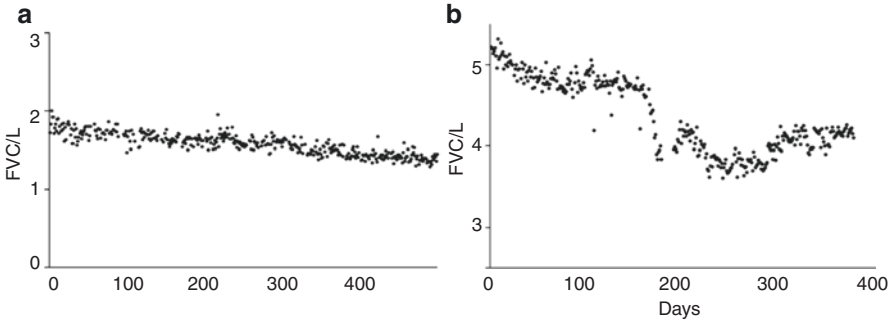

Fig. 5.4 Daily FVC measurements for IPF patients with (**a**) progressive disease and (**b**) an acute exacerbation. Each point represents a single FVC measurement. (Reprinted with permission of the American Thoracic Society. Copyright © 2018 American Thoracic Society. Russell et al. [48])

obtained on hospital-based lung function equipment. The extent of the underestimate was consistent across all levels of baseline FVC, remained stable over time, and, interestingly, did not significantly affect the predictive value of serial measurement of the FVC [48].

With regard to compliance, some subjects dropped out of the study due to cough that was triggered by the maneuver itself. Moreover, in a minority of cases asking patients to record their own FVC so frequently caused psychological distress due to increasing awareness about the rapidity of their own disease progression.

Although this study is promising, additional data in larger cohorts are needed before daily home spirometry can be used routinely in IPF management. Patient compliance, misperformance of spirometry maneuvers, and validation of the quality of individual daily readings are crucial issues to be addressed, especially if treatment assessment is based on these values [49]. A major challenge is, in general, the fact that the maneuver is performed without supervision, and patients should therefore receive adequate training with ongoing verification over time (or refresher training). In addition, spirometers with electronic records of results should be used to reduce errors. In a recent study on posttransplant patients receiving long-term macrolides, difficulties in performing the forced expiratory maneuver were evident in 60% of subjects showing greater variability in home spirometry measurements [50]. Implausible values were also observed, suggesting alternative explanations (including use of the device by another person) [50].

A study comparing unsupervised daily home-based spirometry with hospital-based readings is mandatory in IPF. Until confirmatory data are available, changes in home spirometry should be confirmed by office spirometry, for example, every 4 weeks. It would also be of interest to investigate whether a close correlation exists between changes in FVC measured by home spirometry and quality of life or other patient-centered outcome measures.

In summary, home-based daily monitoring of lung function represents a major step forward in IPF, since it has the potential to improve prediction of disease behavior and response to treatment [49]. From a research perspective, increasing frequency of FVC monitoring may provide an earlier treatment efficacy signal than the classical 3-month follow-up period, making daily spirometry a suitable tool for future clinical trials.

References

1. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005;26:948–68.
2. Kornbluth RS, Turino GM. Respiratory control in diffuse interstitial lung disease and diseases of the pulmonary vasculature. Clin Chest Med. 1980;1:91–102.
3. McCarthy DS, Ostrow DN, Hershfield ES. Chronic obstructive pulmonary disease following idiopathic pulmonary fibrosis. Chest. 1980;77:473–7.
4. Renzi G, Milic-Emili J, Grassino AE. The pattern of breathing in diffuse lung fibrosis. Bull Eur Physiopathol Respir. 1982;18:461–72.
5. Cottin V. The impact of emphysema in pulmonary fibrosis. Eur Respir Rev. 2013;22:153–7.

6. O'Donnell DE, Elbehairy AF, Berton DC, Domnik NJ, Neder JA. Advances in the evaluation of respiratory pathophysiology during exercise in chronic lung diseases. Front Physiol. 2017;8:82.
7. Enright PL, Beck KC, Sherrill DL. Repeatability of spirometry in 18,000 adult patients. Am J Respir Crit Care Med. 2004;169:235–8.
8. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183:788–824.
9. du Bois RM, Weycker D, Albera C, et al. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184:459–66.
10. Latsi PI, du Bois RM, Nicholson AG, et al. Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. Am J Respir Crit Care Med. 2003;168:531–7.
11. Collard HR, King TE Jr, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2003;168:538–42.
12. King TE Jr, Safrin S, Starko KM, et al. Analyses of efficacy end points in a controlled trial of interferon-gamma1b for idiopathic pulmonary fibrosis. Chest. 2005;127:171–7.
13. du Bois RM, Weycker D, Albera C, et al. Forced vital capacity in patients with idiopathic pulmonary fibrosis: test properties and minimal clinically important difference. Am J Respir Crit Care Med. 2011;184:1382–9.
14. Zappala CJ, Latsi PI, Nicholson AG, et al. Marginal decline in forced vital capacity is associated with a poor outcome in idiopathic pulmonary fibrosis. Eur Respir J. 2010;35:830–6.
15. Reichmann WM, Yu YF, Macaulay D, Wu EQ, Nathan SD. Change in forced vital capacity and associated subsequent outcomes in patients with newly diagnosed idiopathic pulmonary fibrosis. BMC Pulm Med. 2015;15:167.
16. Paterniti MO, Bi Y, Rekic D, Wang Y, Karimi-Shah BA, Chowdhury BA. Acute exacerbation and decline in forced vital capacity are associated with increased mortality in idiopathic pulmonary fibrosis. Ann Am Thorac Soc. 2017;14:1395–402.
17. Jo HE, Glaspole I, Moodley Y, et al. Disease progression in idiopathic pulmonary fibrosis with mild physiological impairment: analysis from the Australian IPF registry. BMC Pulm Med. 2018;18:19.
18. King TE Jr, Albera C, Bradford WZ, et al. All-cause mortality rate in patients with idiopathic pulmonary fibrosis. Implications for the design and execution of clinical trials. Am J Respir Crit Care Med. 2014;189:825–31.
19. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med. 2000;161:646–64.
20. Wells AU. Forced vital capacity as a primary end point in idiopathic pulmonary fibrosis treatment trials: making a silk purse from a sow's ear. Thorax. 2013;68:309–10.
21. Richeldi L, Ryerson CJ, Lee JS, et al. Relative versus absolute change in forced vital capacity in idiopathic pulmonary fibrosis. Thorax. 2012;67:407–11.
22. Akagi T, Matsumoto T, Harada T, et al. Coexistent emphysema delays the decrease of vital capacity in idiopathic pulmonary fibrosis. Respir Med. 2009;103:1209–15.
23. Cottin V, Hansell DM, Sverzellati N, et al. Effect of emphysema extent on serial lung function in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2017;196:1162–71.
24. Wells AU, Desai SR, Rubens MB, et al. Idiopathic pulmonary fibrosis: a composite physiologic index derived from disease extent observed by computed tomography. Am J Respir Crit Care Med. 2003;167:962–9.
25. Ley B, Ryerson CJ, Vittinghoff E, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. Ann Intern Med. 2012;156:684–91.
26. Ley B, Elicker BM, Hartman TE, et al. Idiopathic pulmonary fibrosis: CT and risk of death. Radiology. 2014;273:570–9.
27. Ley B, Bradford WZ, Weycker D, Vittinghoff E, du Bois RM, Collard HR. Unified baseline and longitudinal mortality prediction in idiopathic pulmonary fibrosis. Eur Respir J. 2015;45:1374–81.

28. Morisset J, Vittinghoff E, Lee BY, et al. The performance of the GAP model in patients with rheumatoid arthritis associated interstitial lung disease. Respir Med. 2017;127:51–6.
29. Tomassetti S, Ryu JH, Poletti V. Staging systems and disease severity assessment in interstitial lung diseases. Curr Opin Pulm Med. 2015;21:463–9.
30. Martinez FJ, Safrin S, Weycker D, et al. The clinical course of patients with idiopathic pulmonary fibrosis. Ann Intern Med. 2005;142:963–7.
31. Nathan SD, Albera C, Bradford WZ, et al. Effect of continued treatment with pirfenidone following clinically meaningful declines in forced vital capacity: analysis of data from three phase 3 trials in patients with idiopathic pulmonary fibrosis. Thorax. 2016;71:429–35.
32. Richeldi L, Azuma A, Selman M, et al. Twenty-four week decline in forced vital capacity predicts mortality at week 52 in the INPULSIS® trials. Eur Respir J. 2016;48:OA1814.
33. Schmidt SL, Tayob N, Han MK, et al. Predicting pulmonary fibrosis disease course from past trends in pulmonary function. Chest. 2014;145:579–85.
34. Ley B. Clarity on endpoints for clinical trials in idiopathic pulmonary fibrosis. Ann Am Thorac Soc. 2017;14:1383–4.
35. Nathan SD, Meyer KC. IPF clinical trial design and endpoints. Curr Opin Pulm Med. 2014;20:463–71.
36. Nathan SD, Shlobin OA, Weir N, et al. Long-term course and prognosis of idiopathic pulmonary fibrosis in the new millennium. Chest. 2011;140:221–9.
37. Spagnolo P, Luppi F, Maher TM, Wuyts WA, Grutters JC. Primary endpoints in phase 3 clinical trials in idiopathic pulmonary fibrosis: one step at a time. Am J Respir Crit Care Med. 2013;187:1271–2.
38. Raghu G, Collard HR, Anstrom KJ, et al. Idiopathic pulmonary fibrosis: clinically meaningful primary endpoints in phase 3 clinical trials. Am J Respir Crit Care Med. 2012;185:1044–8.
39. Finkelstein SM, Lindgren B, Prasad B, et al. Reliability and validity of spirometry measurements in a paperless home monitoring diary program for lung transplantation. Heart & lung :J Crit Care. 1993;22:523–33.
40. Bjortuft O, Johansen B, Boe J, Foerster A, Holter E, Geiran O. Daily home spirometry facilitates early detection of rejection in single lung transplant recipients with emphysema. Eur Respir J. 1993;6:705–8.
41. Lindgren BR, Finkelstein SM, Prasad B, et al. Determination of reliability and validity in home monitoring data of pulmonary function tests following lung transplantation. Res Nurs Health. 1997;20:539–50.
42. Chlan L, Snyder M, Finkelstein S, et al. Promoting adherence to an electronic home spirometry research program after lung transplantation. Appl Nurs Res. 1998;11:36–40.
43. Finkelstein SM, Snyder M, Stibbe CE, et al. Staging of bronchiolitis obliterans syndrome using home spirometry. Chest. 1999;116:120–6.
44. Finkelstein SM, Scudiero A, Lindgren B, Snyder M, Hertz MI. Decision support for the triage of lung transplant recipients on the basis of home-monitoring spirometry and symptom reporting. Heart & Lung : J Crit Care. 2005;34:201–8.
45. Lavelle MB, Finkelstein SM, Lindgren BR, et al. Newsletters and adherence to a weekly home spirometry program after lung transplant. Prog Transplant. 2010;20:329–34.
46. Finkelstein SM, Lindgren BR, Robiner W, et al. A randomized controlled trial comparing health and quality of life of lung transplant recipients following nurse and computer-based triage utilizing home spirometry monitoring. Telemed J E Health. 2013;19:897–903.
47. Lindell KO, Olshansky E, Song MK, et al. Impact of a disease-management program on symptom burden and health-related quality of life in patients with idiopathic pulmonary fibrosis and their care partners. Heart & Lung : J Crit Care. 2010;39:304–13.
48. Russell AM, Adamali H, Molyneaux PL, et al. Daily home spirometry: an effective tool for detecting progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2016;194:989–97.
49. Wuyts WA, Bonella F, Costabel U, et al. An important step forward, but still a way to go. Am J Respir Crit Care Med. 2016;193:340–1.
50. de Wall C, Sabine D, Gregor W, et al. Home spirometry as early detector of azithromycin refractory bronchiolitis obliterans syndrome in lung transplant recipients. Respir Med. 2014;108:405–12.

Chapter 6
The Role of Immunity and Inflammation in IPF Pathogenesis

Marcus W. Butler and Michael P. Keane

There has been a revolution in the prevailing concensus regarding the pathogenesis of IPF over the course of the past couple of decades, with a retreat from paradigms solely based on IPF as an immune-mediated disorder involving chronic inflammation of the lower airways which progresses to fibrosis, towards a view of IPF as a disease of abnormal pulmonary fibroproliferation/disorganised matrix deposition in the face of repetitive injury to an ageing alveolar epithelium that is genetically predisposed to UIP formation [1–4] (Fig. 6.1). The historical term "cryptogenic fibrosing alveolitis" used interchangeably with IPF encapsulates the thinking decades ago when much of the available evidence pointed to a likely dominant role for chronic alveolar epithelial inflammation progressing to injury and dysregulated repair resulting in fibrosis, not least of all because alveolar inflammation appeared to precede fibrotic lesion development [5]. Initial enthusiasm for a chronic inflammatory basis for IPF also stemmed from observations of an excess of neutrophils within alveolar walls and the alveolar epithelial surfaces in IPF [6]. In addition, immune complexes of mainly IgG were found in the epithelial lining fluid of IPF individuals [7]. In an older study, alveolar inflammation was found to occur in approximately half of clinically unaffected family members who are at risk of inheriting autosomal dominant idiopathic pulmonary fibrosis, termed familial interstitial pneumonia (FIP) [8]; however a more recent larger study (FIP defined as at least two family members with IIP including IPF in at least one affected individual per family) failed to replicate this finding, with no difference seen in inflammatory cell proportions in BAL fluid among at-risk (asymptomatic first-degree relatives of FIP patients) and healthy control subjects [9]. Part of the shift away from the notion of chronic inflammation as a basis for IPF came with the tighter concensus surrounding the pathologic classification of the disease two decades ago, which up to then had included

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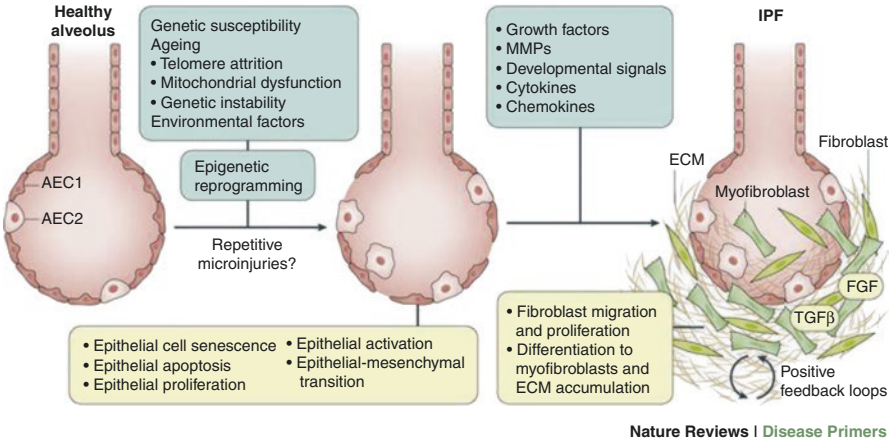


Fig. 6.1 A proposed pathogenetic model of idiopathic pulmonary fibrosis. (From Ref. [4])

what are now widely accepted as being separate forms of idiopathic interstitial pneumonia such as non-specific interstitial pneumonia and acute interstitial pneumonia, for which anti-inflammatory treatments may show more benefit [10–12]. A sentinel event in shaping the current prevailing view that immunomodulatory therapies are to be avoided in IPF came with the discovery in the PANTHER study that a then standard-of-care though unproven therapy for IPF, the combination of oral corticosteroid, azathioprine and N-acetylcysteine was not only inefficacious in IPF but led to higher mortality and hospitalisation within a mean of only 32 weeks of treatment versus placebo [13]. The dawn of a new era of therapeutic options for what was until then an untreatable disease arrived on May 18, 2014, with the simultaneous publication of phase III studies of two very different disease modification compounds, pirfenidone and nintedanib, which both share antifibrotic properties and have highly pleiotropic mechanisms of action, suggestive of a need in IPF to address multiple redundant wound-healing pathways in order to control what is a complex polygenic disorder [14, 15]. Nearly all of the compounds currently in development for the treatment of IPF involve mechanisms relating to lung tissue repair, regeneration, inhibition of epithelial cell apoptosis and inhibition of collagen deposition, with little interest in an anti-inflammatory/immunosuppressing approach [2], given the unequivocal failures of such approaches in the past [13, 16]. Within these few years, long-held theories of IPF pathogenesis had been overturned.

The delight that universally accompanied the long-awaited emergence of IPF medications with some disease-modifying effects needs to be tempered against the ongoing unmet needs of these patients, who are far from cured by current antifibrotic strategies. Somewhat at odds with a more dismissive view of an immunologic and inflammatory role in IPF pathogenesis are a wealth of data that provides the smoking gun to an immunobiological role in either the initiation or progression of IPF, which remains incompletely understood and, arguably therefore, unsuccessfully addressed in treatment approaches. Such a role may be more important for

subtypes of IPF that await elucidation, though it is also plausible that the association of immunologic abnormalities with IPF are a process that is downstream from fibrosis-driven biology [2, 17]. Strongly pointing towards a chronic immune process in IPF are the replicated observations in IPF lung tissue of lymphoid aggregates, suggestive of lymphoid neogenesis [17–20]. These are found in close proximity to fibroblastic foci and are composed of mainly activated $CD3^+$ T lymphocytes and mature dendritic cells, with a subset of activated $CD20^+$ cells, with some evidence also pointing to chemokine receptor (CCR)6 expression in these infiltrates, as found on memory T cells, Th-17 cells, B cells and dendritic cells [18, 20] (Fig. 6.2). These aggregates were seen in increasing numbers in IPF explants versus less advanced IPF lung surgical biopsy specimens, suggestive of a sustained role for such lymphoid tissue in progressive IPF [19]. The picture is confused however by the observation that these tertiary lymphoid structures (TLS) contain non-proliferating and non-apoptotic mature $CD45RO^+$ T and B cells [18, 19], which has led to a hypothesis of these cells homing to the lung from the systemic circulation, although data is lacking to support such an origin [19].

Another difficulty in dismissing an important role for the immune system in IPF lies in the repeated observation of areas of histopathologic UIP and non-specific interstitial pneumonia (NSIP) in the same patient when biopsies are obtained from

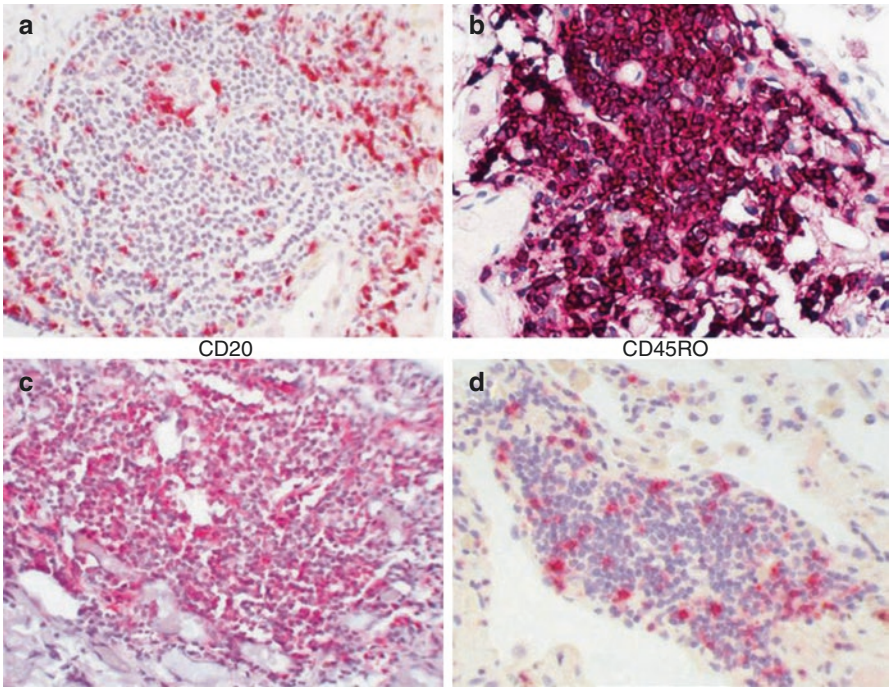


Fig. 6.2 Idiopathic pulmonary fibrosis inflammatory infiltrates. All photomicrographs show the tissue stained with Fast Red and haematoxylin counterstain. (**a**–**f**) The photos have a magnification of ×200. (**g**, **h**) The photos have a magnification of ×400. (From Ref. [20])

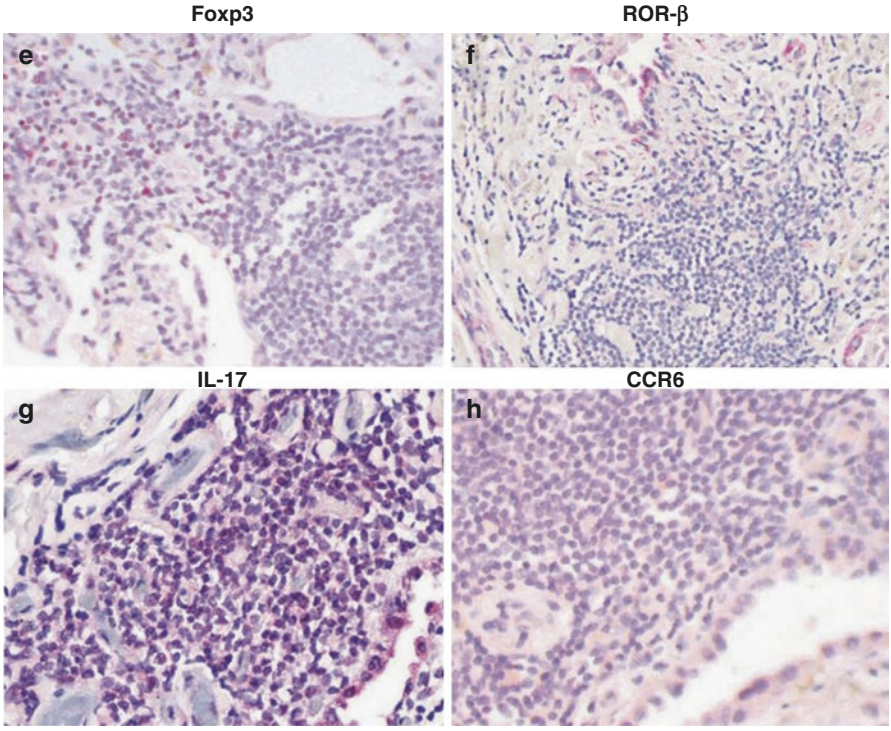


Fig. 6.2 (continued)

different lobar locations, a phenomenon thought to occur in 13–26% of cases [21, 22]. NSIP can have varying degrees of alveolar wall inflammation by predominantly lymphocytes and plasma cells in addition to fibrosis and has a better prognosis than UIP, but individuals with discordant UIP and NSIP on their multiple biopsies have a poor prognosis similar to those with concordant UIP on multiple biopsies [21, 22]. In support of an endotypic difference among the two diseases, NSIP fibroblasts appear to behave more like normal fibroblasts than is seen in IPF fibroblasts, where the latter exhibit greater contractility and secrete greater amounts of fibronectin and TGF-β1 [23]. There is a great need to further improve our understanding of the potential for an evolution of NSIP into fibrotic NSIP and later into UIP, as immunomodulatory therapy for NSIP, a putative early treatment strategy for IPF, demonstrates some efficacy versus being ineffective in UIP [24]. Some have suggested that a greater understanding of rheumatoid arthritis-associated interstitial lung disease, where the undoubtedly inflammatory disease of rheumatoid arthritis can result in either an NSIP or UIP pattern, offers a good model for gaining further insight into the pathogenesis of both of these related interstitial pneumonias [25]. Lending support of such a model, a recent study that established and validated a role for a biomarker index of three plasma molecules, MMP-7, surfactant protein D and osteopontin in discriminating IPF from alternative interstitial lung diseases (adjusted

area under the curve of 0.766, excluding RA-ILD), could not distinguish IPF from RA-ILD [26]. Of interest in such an IPF model is the shared risk factor of chronic tobacco smoke exposure in both idiopathic and RA-associated UIP. In recent times, the contributory role of immune mediators and inflammatory cells have once again gained more acceptance in schemata of IPF pathogenesis, though far more questions than answers are found [27]. The remainder of this chapter will discuss the evidence that implicates a variety of inflammatory and immunologic processes in contributing to the pathogenesis of IPF.

Innate Immunity and Altered Host Defence Mechanisms

In IPF, a repetitive cycle of local micro-injury to ageing alveolar epithelium by various factors and processes including cigarette smoke, environmental exposures, microbial colonisation/infection, microaspiration, endoplasmic reticulum stress and oxidative stress is believed to underpin the development of disease, with resultant aberrant wound healing [1]. A prototypic example of how such a diverse array of stressors can mediate tissue injury via innate immune mechanisms is the Toll-like receptor family of pattern-recognition receptors that recognise pathogen-associated molecular patterns (PAMPs) from microbes or danger-associated molecular patterns (DAMPs) from damaged tissues (Fig. 6.3) [28]. In the case of IPF, a front-line cell in this process is the type II alveolar epithelial (AEC2) cell, a pulmonary form of stem cell capable of long-term self-renewal, and in IPF, the majority of such cells exhibit evidence of apoptosis [29, 30]. In healthy innate immune systems, these AEC2 cells are recognised and phagocytosed in a non-inflammatory process known as efferocytosis [31]. Critical to the regulation of lung-injury response is the interaction of the evolutionarily conserved danger recognition receptor termed Toll-like receptor (TLR) 4 with the DAMP known as hyaluronan, a glycosaminoglycan that maintains structural integrity of the lung extracellular matrix but which is elevated in BAL fluid in IPF patients where it correlates with disease severity [32, 33]. A widely used model of experimental IPF is the use of the cancer chemotherapy agent bleomycin, instilled into mice to bring about oxidative DNA damage, cell death of alveolar macrophages and airway epithelial cells with ensuing fibrosis. In a bleomycin-induced lung fibrosis model where organoids were created from highly purified AEC2 cells, the hyaluronan-TLR4 axis was shown to play a key role in lung stem cell renewal, and perturbation of this axis by deletion of the hyaluronan synthase 2 (HAS2) enzyme led to worsened fibrosis. The same authors also demonstrated that AEC2 cells from IPF patients studied in organoid cultures had reduced HAS2 and hyaluronan expression and reduced renewal capacity (See Fig. 6.4) [33, 34].

A familial form of IPF has been linked to damaged AEC2 cells associated with a mutation in the surfactant protein C gene [35]. Ineffective repair of damaged alveolar epithelium leading to pulmonary fibrosis is supported by the observations made in a transgenic mouse model expressing human diphtheria toxin receptor on AEC2

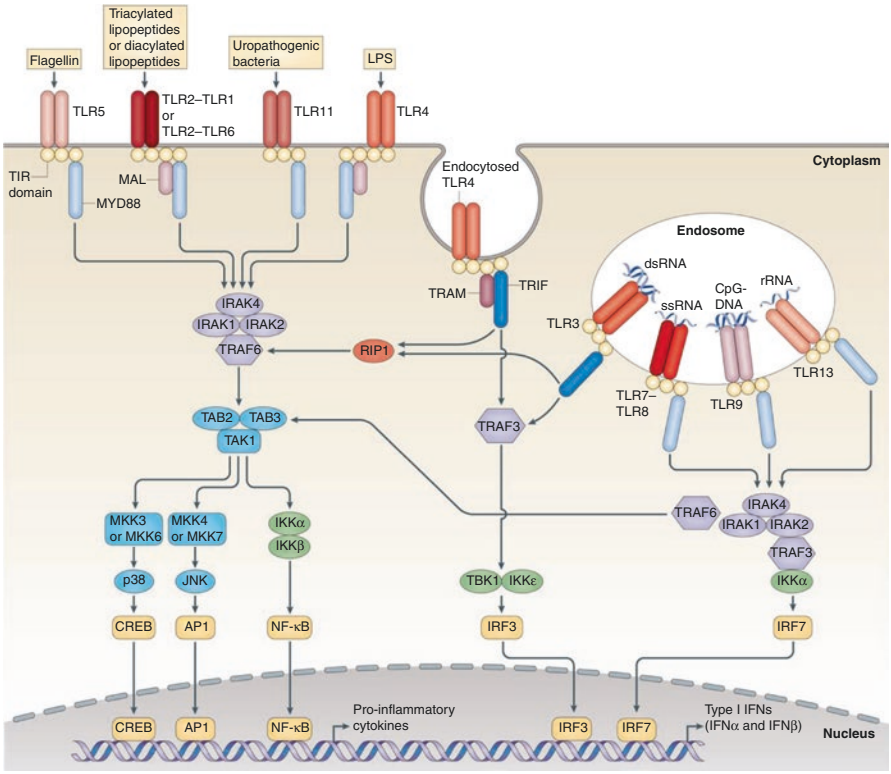


Fig. 6.3 A detailed knowledge of how mammalian Toll-like receptors (TLRs) signal has developed over the past 15 years. TLR5, TLR11, TLR4, and the heterodimers of TLR2-TLR1 or TLR2-TLR6 bind to their respective ligands at the cell surface, whereas TLR3, TLR7-TLR8, TLR 9 and TLR 13 localise to the endosomes, where they sense microbial and host-derived nucleic acids. TLR4 localises at both the plasma membrane and the endosomes. TLR signalling is initiated by ligand-induced dimerisation of receptors. Following this, the Toll-IL-1-resistance (TIR) domains of TLRs engage TIR domain-containing adaptor proteins (either myeloid differentiation primary-response protein 88 (MYD88) and MYD88-adaptor-like protein (MAL) or TIR domain-containing adaptor protein inducing IFNβ (TRIF) and TRIF-related adaptor molecule (TRAM)). TLR4 moves from the plasma membrane to the endosomes in order to switch signalling from MYD88 to TRIF. Engagement of the signalling adaptor molecules stimulates downstream signalling pathways that involve interactions between IL-1R-associated kinases (IRAKs) and the adaptor molecules TNF receptor-associated factors (TRAFs), and that lead to the activation of the mitogen-activated protein kinases (MAPKs) JUN N-terminal kinase (JNK) and p38, and to the activation of transcription factors. Two important families of transcription factors that are activated downstream of TLR signalling are nuclear factor-κB (NF-κB) and the interferon-regulatory factors (IRFs), but other transcription factors, such as cyclic AMP-responsive element-binding protein (CREB) and activator protein 1 (AP1), are also important. A major consequence of TLR signalling is the induction of pro-inflammatory cytokines, and in the case of the endosomal TLRs, the induction of type I interferon (IFN). dsRNA double-stranded RNA, IKK inhibitor of NF-κB kinase, LPS lipopolysaccharide, MKK MAP kinase kinase, RIP1 receptor-interacting protein 1, rRNA ribosomal RNA, ssRNA single-stranded RNA, TAB TAK1-binding protein, TAK TGFβ-activated kinase, TBK1 TANK-binding kinase 1. (From Ref. [28])

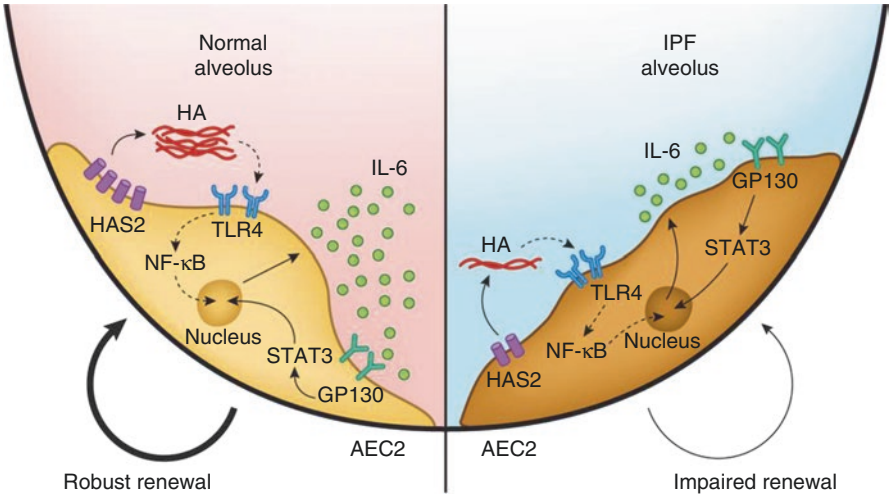


Fig. 6.4 In healthy AEC2 cells, regenerative capacity is maintained by hyaluronan synthase 2 (HAS2)-mediated production of HA, which interacts with Toll-like receptor 4 (TLR4). This in turn results in the expression of IL-6 (either indirectly or directly). In IPF-derived AEC2 cells, HAS2 expression is reduced, which causes decreased regenerative and impaired response to injury. NF-κB nuclear factor-κB, STAT3 signal transducer and activator of transcription 3, GP130 glycoprotein 130, AEC2 type 2 alveolar epithelial cell. (From Ref. [33])

cells, where administration of diphtheria toxin to these animals resulted in AEC2 cell injury and pulmonary fibrosis [36]. Among the more developed compounds currently being evaluated as investigational new drugs for IPF is a small molecule BMS-986020 antagonising a lysophosphatidic acid receptor (LPA1) in an effort to inhibit the airway epithelial cell apoptosis observed in IPF, among its many other mechanisms of action (Phase II Trial number: NCT01766817) [2, 37].

Based on the fact that gut commensal bacteria are known to influence stem cell renewal in intestinal epithelium through TLR4 interactions with microbiome components, it is plausible that the lung microbiome could influence alveolar epithelial homeostasis that is perturbed in IPF [33]. MUC5B is a gel-forming mucin that constitutes a major component of airway mucus, and along with MUC1 is the most highly expressed mucin gene in distal human airways [38]. It normally plays a key role in innate defence of airway epithelial mucosa but is overexpressed in IPF lungs [39]. A large-scale genome-wide association study [40] of idiopathic interstitial pneumonia (IIP) cases (mostly IPF) versus controls revealed common genetic variations associated with risk for IIP, including a T/G SNP in the MUC5B promoter (rs35705950), a region which has been identified previously as a risk locus for IIP [39]. A meta-analysis has since shown that the MUC5B rs35705950 polymorphism confers susceptibility to IPF in those of European or Asian genetic ancestry, and the same SNP is associated with progression of subclinical interstitial lung abnormalities on serial CT scans, though conversely also associated with improved survival in IPF [41–43]. A recent systems biology study incorporating a novel modified aptamer

technology to study proteomic differences among blood of 60 IPF subjects and 21 normal subjects pointed to a host defence defect in IPF versus normals, with greatest enrichment, among all downregulated proteins, for those governing host defence, potentially indicative of attempts in IPF to restrict airway epithelial damage and initiate reparative processes [44] (Fig. 6.5). In addition to the MUC5B SNP, other polymorphisms in genes related to epithelial integrity and host defence have been

a

Enrichment downregulated proteome								
Category	Term	Count	%	Fold enrichment	Bonferroni	BH	FDR	Kappa
GO BP	GO:0006952~defense response	25	22	4.87	2.25E–07	2.25E–07	2.56E–07	1.00
GO BP	GO:0006916~anti-apoptosis	14	12	8.14	2.14E–05	1.07E–05	2.43E–05	
GO BP	GO:0006955~immune response	23	20	3.99	5.93E–05	1.98E–05	6.74E–05	0.47
GO MF	GO:0005125~cytokine activity	13	11	8.09	1.76E–05	1.76E–05	8.02E–05	
GO BP	GO:0009611~response to wounding	20	17	4.52	9.25E–05	2.31E–05	1.05E–04	0.59
GO BP	GO:0032101~regulation of response to external stimulus	12	10	9.04	1.15E–04	2.30E–05	1.31E–04	
GO BP	GO:0042127~regulation of cell proliferation	24	21	3.65	1.36E–04	2.27E–05	1.55E–04	
GO BP	GO:0042981~regulation of apoptosis	24	21	3.57	2.01E–04	2.87E–05	2.28E–04	
GO BP	GO:0043067~regulation of progeammed cell death	24	21	3.54	2.40E–04	3.00E–05	2.72E–04	
GO BP	GO:0010941~regulation of cell death	24	21	3.53	2.56E–04	2.84E–05	2.91E–04	

b

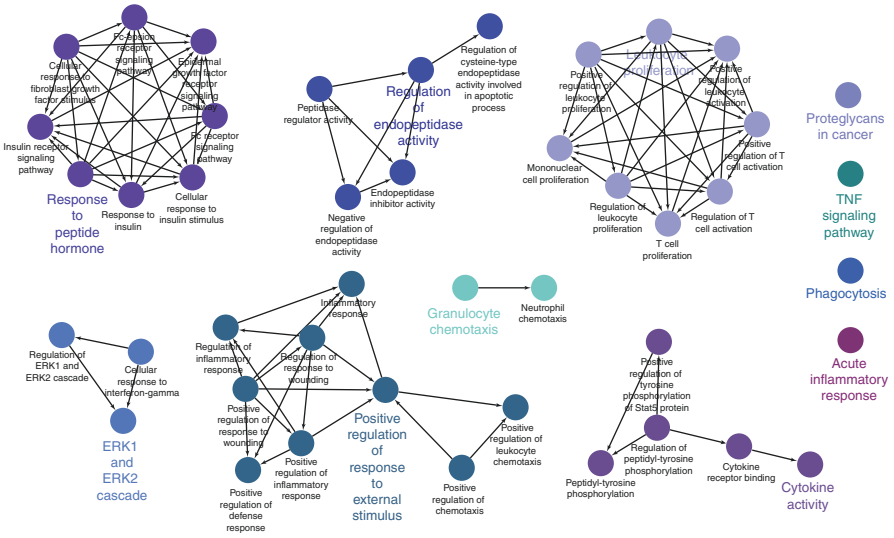


Fig. 6.5 Enrichment and network analysis of the downregulated IPF plasma proteome. (**a**) DAVID enrichment analysis was employed to select the most significantly enriched terms within the sample of downregulated proteins ($n = 116$). Bonferroni-corrected P value, BH P value and FDRs are reported. Kappa statistics reporting similarity to most significant term (low > 0.25, moderate 0.25–0.5, high 0.5–0.75, very high 0.75–1). (**b**) ClueGO visualisation and analysis of biological role (GO, Kegg pathways) was undertaken. GO terms are mapped in clusters by Kappa statistics. [Hexagon = Kegg pathway, Ellipse = Gene ontology term, arrow depicts direction of association]. The major overview term (smallest P value within cluster) is depicted in colour. Node size depicts Bonferroni-corrected P value < 0.0005 for all terms reported. Further details can be found in online supplement. (From Ref. [44])

identified as predisposing to IPF, such as the TOLLIP rs5743890 polymorphism [39, 40, 43, 45]. TOLLIP, a key modulator of innate immune responses, activates MYD88-dependent NF-κB to regulate TLR signalling and also antagonises TGF-β signalling, in addition to roles in intracellular trafficking via SMAD7 and a role in governing antigen-specific proliferation of T cells and/or B cells [46–48]. TOLLIP is also one of many gene regions that exhibit differential hypomethylation of CpG islands in IPF lung tissues compared to control lungs assessed by CpG island microarray [49]. In a discovery genome-wide association study and subsequent independent replication case-control studies with over a thousand IPF patients and over 1200 control subjects, three TOLLIP SNPs were among a handful of SNPs that remained significantly associated with IPF susceptibility, including one SNP that was also associated with IPF mortality (rs5743890), with these polymorphisms regulating TOLLIP gene expression levels in IPF [45].

The links between Toll-like signalling and IPF pathogenesis have further grown in recent years with the observation that the functional TLR3 polymorphism rs3775291, which results in defective NF-κB and IRF3 activation, is associated with increased mortality risk and accelerated decline in FVC in patients with IPF [50]. The same authors examined human IPF fibroblasts that were wild-type, heterozygous or homozygous for the rs3775291 mutation in addition to utilising a murine bleomycin model of lung fibrosis that included TLR3 knockout (TLR3−/−) mice and demonstrated defective fibroproliferative responses and impaired interferon gamma responses in the fibroblasts with alleles for rs3775291 and worsened lung fibrosis and mortality in TLR3−/− mice [50].

Independent groups have shown associations among the lung microbiome and IPF [51–54]. Han and co-investigators had obtained BAL fluid from 55 subjects with moderately severe IPF in the prospectively recruited COMET-IPF study and, in a manner not pre-specified, carried out subsequent bacterial 16 ribosomal RNA pyrosequencing to characterise the microbiome, with IPF progression defined as a composite of deteriorating pulmonary function tests (relative decrease in FVC or DLCO of ≥10% or ≥15%, respectively), death, acute exacerbation or lung transplantation. Using principal components analysis, they showed significant associations of IPF progression with increased relative abundance of a *Streptococcus* operational taxonomic unit (OTU) and a *Staphylococcus* OTU [51]. The same group more recently explored host immune responses in a given lung microbiome context using paired samples of peripheral blood mononuclear cell (PBMC) gene expression profiles and their BAL microbiome data, in addition to lung fibroblasts cultured from transbronchial biopsies, and found that immune response pathways including NOD-like receptor, TLR and RIG-like receptor signalling pathways were downregulated in association with worse progression-free survival (PFS). Their data showed that lung microbes with increased abundance and decreased community diversity were associated with decreased PBMC transcriptomic expression of immune pathways and shorter PFS. This study also provided data to support the idea that host-microbiome interactions might influence immune-mediated resident fibroblast responsiveness to TLR9 stimulation using CpG oligodeoxynucleotide [53].

Herpesviruses, a highly prevalent group of viruses, have frequently been found to be associated with the much rarer entity of IPF, suggesting a possible gene-environment interaction, with plausibility coming from the known life-long latency in the host that follows infection, potentially leading to a reactivation phenomenon in old age as a potential aetiologic trigger in susceptible individuals [55]. Among the evidence for this is the study from Kropski et al. that evaluated 75 asymptomatic at-risk first-degree relatives of FIP patients alongside 12 sporadic IPF patients and 27 healthy control subjects, which found a 14% prevalence of early interstitial lung abnormalities on high-resolution chest CT scanning and over 35% with abnormalities such as peribronchiolar and interstitial fibrosis on transbronchial biopsies in the at-risk subjects. In this study, quantitative polymerase chain reaction was used on DNA isolated from cell-free BAL supernatant and demonstrated lowest quantities of herpesviruses in normals, intermediate quantities in the at-risk subjects and highest copies of herpesviruses per millilitre of BAL fluid in those with IPF, suggestive of ongoing viral replication in those with and at risk of a UIP lung disease and compatible with a greater burden of virus mediating a greater extent of airway epithelial cell injury. For at-risk subjects, a correlation was seen among endoplasmic reticulum (ER) stress markers and herpesvirus antigens using immunohistochemical analysis of transbronchial biopsies, consistent with a mechanism of virus-mediated epithelial cell injury [9]. None of these studies, though suggestive, can prove a causal link between microbes and IPF but could support the hypothesis that dysbiosis plays a role in IPF pathogenesis, if, for example, host defence proteins are being downregulated in a given microbiomic context.

Chemotactic Cytokines

Leukocyte infiltration is a universally recognised hallmark of inflammation. Once recruited to lung tissues, leukocytes can contribute to the pathogenesis of chronic inflammation and promote fibrogenesis via the elaboration of various cytokines. Maintenance of leukocyte recruitment during inflammation requires the cell surfaces to express adhesion molecules and the production of chemotactic molecules termed chemokines [56, 57]. Chemokines can be subdivided into four families—CXC, CC, C and CXXXC—and these function as potent chemotactic factors for a variety of cell types including neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells, NK cells and T and B lymphocytes (Table 6.1). The members of the four chemokine families exhibit approximately 20–40% homology [58]. Chemokines are elaborated from an array of cells, including monocytes, neutrophils, alveolar macrophages, eosinophils, mast cells, T and B lymphocytes, NK cells, platelets and various structural cells, including keratinocytes, epithelial cells, mesangial cells, hepatocytes, fibroblasts, mesothelial cells, smooth muscle cells and endothelial cells. The ability of both immune and nonimmune cells to produce these chemokines supports the contention that such cytokines may play a pivotal role in orchestrating chronic inflammation [59].

Table 6.1 The four families of human chemokines: C, CC, CXC and CXXXC [57]

Systemic name	Human ligand name
The C chemokines	
XCL1	Lymphotactin
XCL2	SCM-1β
The CC chemokines	
CCL1	I-309
CCL2	Monocyte chemotactic protein-1 (MCP-1)
CCL3	Macrophage inflammatory protein-1 alpha (MIP-1α)
CCL4	Macrophage inflammatory protein-1 beta (MIP-1β)
CCL5	Regulated on activation normal T-cell expressed and secreted (RANTES)
CCL7	Monocyte chemotactic protein-3 (MCP-3)
CCL8	Monocyte chemotactic protein-2 (MCP-2)
CCL9	Macrophage inflammatory protein-1 delta (MIP-1δ)
CCL11	Eotaxin
CCL13	Monocyte chemotactic protein-4 (MCP-4)
CCL14	HCC-1
CCL15	HCC-2
CCL16	HCC-4
CCL17	Thymus and activation-regulated chemokine (TARC)
CCL18	DC-CK-1
CCL19	Macrophage inflammatory protein-3 beta (MIP-3β)
CCL20	Macrophage inflammatory protein-3 alpha (MIP-3α)
CCL21	6Ckine
CCL22	MDC
CCL23	MPIF-1
CCL24	MPIF-2
CCL25	TECK
CCL26	Eotaxin-3
CCL27	CTACK
CCL28	MEC
The CXC chemokines	
CXCL1	Growth-related oncogene alpha (GRO-α)
CXCL2	Growth-related oncogene beta (GRO-β)
CXCL3	Growth-related oncogene gamma (GRO-γ)
CXCL4	Platelet factor-4 (PF4)
CXCL5	Epithelial neutrophil-activating protein-78 (ENA-78)
CXCL6	Granulocyte chemotactic protein-2 (GCP-2)
CXCL7	Neutrophil-activating protein-2 (NAP-2)
CXCL8	Interleukin-8 (IL-8)
CXCL9	Monokine induced by interferon-γ (MIG)
CXCL10	Interferon-γ-inducible protein (IP-10)
CXCL11	Interferon-inducible T-cell alpha chemoattractant (ITAC)

(continued)

Table 6.1 (continued)

Systemic name	Human ligand name
CXCL12	Stromal cell-derived factor-1 (SDF-1)
CXCL13	B-cell-attracting chemokine-1 (BCA-1)
CXCL14	BRAK/bolekine
CXCL16	
The CXXXC chemokine	
CXC3CL1	Fractalkine

CXC Chemokines and Their Receptors

Within the CXC chemokine family, there are subdivisions on the basis of a structure/function domain reflecting the presence or absence of three amino acid residues (Glu-Leu-Arg; ELR motif) that are located before the first cysteine amino acid residue in the primary structure of these cytokines. CXC chemokines that are ELR positive are chemoattractants for neutrophils and have potent angiogenic activities. The ELR negative CXC chemokines are highly induced by interferons, are chemoattractants for mononuclear cells, and inhibit angiogenesis [60].

Seven CXC chemokine receptors have been identified, which are G protein-coupled receptors. CXCR1 and CXCR2 receptors are found on neutrophils, T lymphocytes, monocytes/macrophages, eosinophils, basophils, keratinocytes and mast cells and endothelial cells, and these bind to ELR+ chemokines [61]. CXCR3 is expressed on activated T lymphocytes and HUMVECs and is the receptor for CXCL9, CXCL10 and CXCL11. The CXCL12 receptor is CXCR4 and is the cofactor for lymphotropic HIV-1, and in contrast to CXCR3, CXCR4 appears to be expressed on unactivated T-lymphocytes [61]. There are other chemokine receptors that bind chemokines without a subsequent signal-coupling event. The DARC receptor binds both CXC and CC chemokines without apparent signal coupling. This receptor, first discovered on human erythrocytes, is thought to represent a reservoir for chemokines, binding pro-inflammatory chemokines when concentration levels are high during tissue inflammation and releasing them when chemokine levels are lower [62, 63]. A second nonsignalling chemokine receptor is the D6 receptor, which binds several CC chemokines with high affinity, including CCL2, CCL4, CCL5 and CCL7^{-} [64].

The Role of CXC Chemokines in Pulmonary Fibrosis

IPF is characterised by the progressive deposition of collagen within the interstitium and subsequent destruction of lung tissue [10, 12, 65]. While the mechanisms of cellular injury and the role of classic inflammatory cells remain unclear, CXCL8 is significantly elevated in IPF, as compared with either normal or sarcoidosis patients, and correlates with BALF presence of neutrophils. The alveolar macrophage is an important cellular source of CXCL8 in IPF [66]. In addition, BALF levels of CXCL8 in IPF

may correlate with a worse prognosis [67]. More recently, CXCL13, which mediates B-cell trafficking in concert with its cognate receptor CXCR5 and is implicated in the pathogenesis of several immunologic disorders, was studied in the lung and plasma from IPF, COPD and control subjects. By way of biomarker utility, plasma CXCL13 was shown to be higher in IPF and highest in IPF complicated by pulmonary arterial hypertension or acute exacerbations. Interestingly, longitudinal measures of the chemokine over time (yearly) showing a relative rise of at least 50% from an earlier value were predictive of respiratory failure. The specificity of the biomarker to IPF was supported by less predictive abilities of the biomarker in COPD subjects [68].

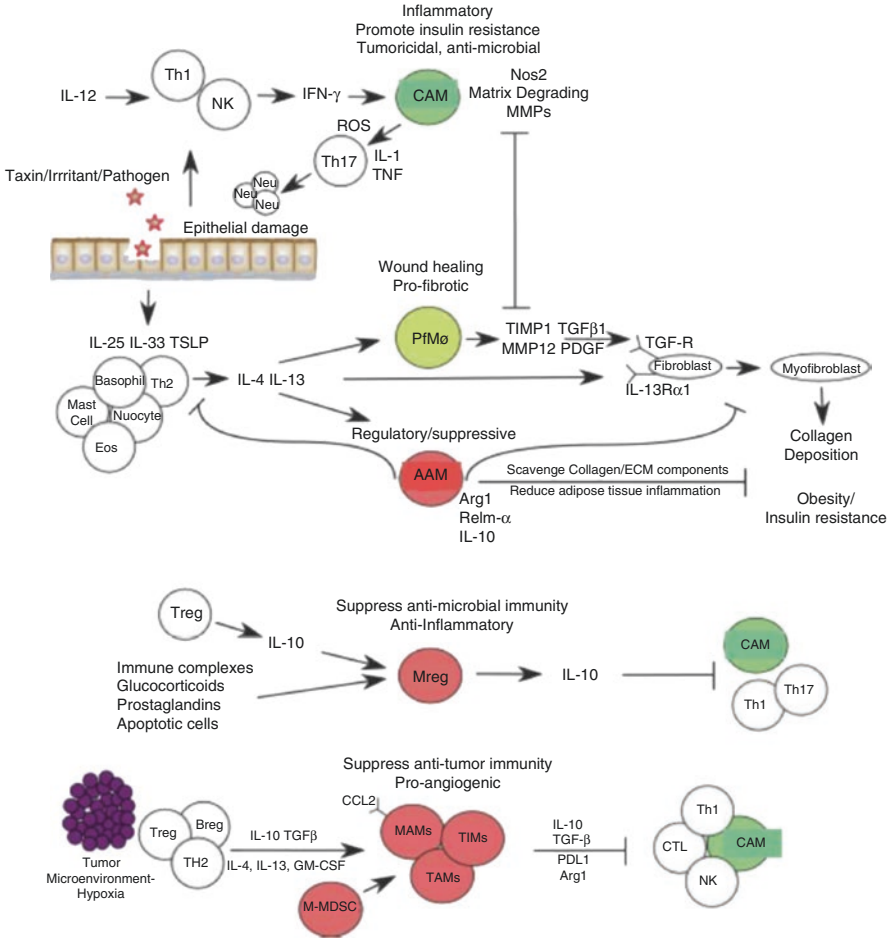
Vascular Remodelling in Pulmonary Fibrosis: The Role of CXC Chemokines

The first to identify neovascularisation in IPF was Turner-Warwick in 1963, who demonstrated extensive neovascularisation within areas of pulmonary fibrosis, with anastomoses between the systemic and pulmonary microvasculature [69]. Further evidence of neovascularisation during the pathogenesis of pulmonary fibrosis has been uncovered in the bleomycin model of pulmonary fibrosis [70]. An imbalance in the levels of angiogenic chemokines (CXCL5, CXCL8), as compared with angiostatic chemokines (CXCL9, CXCL10, CXCL11), favouring net angiogenesis has been demonstrated in animal models but additionally in tissue specimens from patients with IPF [71]. Renzoni et al. have shown evidence of vascular remodelling in both IPF and fibrosing alveolitis associated with systemic sclerosis [72]. Cosgrove et al. demonstrated a relative absence of vessels in the fibroblastic foci of IPF, providing further support for the concept of vascular remodelling in IPF. They also noted significant vascularity in the areas of fibrosis around the fibroblastic foci, with numerous abnormal vessels in the regions of severe architectural distortion [73]. These findings are similar to those of Renzoni and support the concept of regional heterogeneity of vascularity in IPF. This heterogeneity is an intuitive feature, as usual interstitial pneumonia, which is the pathological description of IPF, is defined by its regional and temporal heterogeneity [65].

CXCL14 is another CXC chemokine family member, known to be involved in the trafficking of various inflammatory mononuclear cells including immature dendritic cells, and can antagonise CXCL12-CXCR4 interactions [74–76]. Its expression in lung epithelium is modestly upregulated in healthy smokers and even more so in COPD and lung adenocarcinoma [77]. CXCL14 is also a potent inhibitor of angiogenesis, and recently it has been demonstrated to be elevated in IPF lung tissue at the RNA and protein level and in blood, where it is postulated to have a role as a biomarker of Hedgehog signalling [75, 78]. With the availability of effective IPF therapeutic agents, there is now interest in clarifying the mechanisms of action of these agents, and as a relevant example, nintedanib is known to inhibit tumour angiogenesis in lungs by acting on endothelial cells, pericytes and smooth muscle cells, though the role for nintedanib-mediated angiogenesis regulation in IPF awaits further study [79, 80].

Macrophages

Macrophages, highly plastic and diverse types of cell which arise from monocyte lineage as part of the mononuclear phagocytic system, are important for host defence including antimicrobial activities while also having a recognised role in wound healing and fibrogenesis through the production and release of chemokines capable of recruiting inflammatory cells and leading to the proliferation and activation of collagen-secreting myofibroblasts (Fig. 6.6). While much of the data linking macrophages to IPF pathogenesis has centred on the use of imperfect models of lung fibrosis such as the murine bleomycin model, such models provide important insights that can be extended by supportive human biospecimen data, and the plausibility of a macrophage role in IPF is suggested by various findings, not least of all



the expansion of alveolar macrophages in BAL fluid in response to chronic smoking, an IPF risk factor, and in IPF itself [81–83]. Before the vast diversity of cells and functionality within the mononuclear phagocytic system was better appreciated, working classifications were arrived at, such as term M1 or classically activated macrophages ("inflammatory phenotype"), to describe macrophages that activate immune defences (e.g. TNF-α, IL-1, IL-6, ROS, NOS2) in response to pathogens or tissue injury that elicit Th1 inflammation. In contrast, M2 or alternatively activated macrophages are found in response to type II inflammation (e.g. IL-4, IL-13) and mediate wound healing and fibrosis among other reparative and homeostatic effects that can be subverted by recurring insults [84]. The complexity of macrophage involvement in airway epithelial homeostasis is apparent from the work of Cao and colleagues, who extended their previous discovery of a pulmonary vascular niche (involving a platelet-derived CXCL12 homolog called SDF1 which primes pulmonary capillary endothelial cells, or PCECs) that drives alveolar regeneration in mice, by studying this niche in models of lung fibrosis [85, 86]. They identified a population of perivascular macrophages that interact with PCECs and perivascular fibroblasts following repetitive lung injury, to obstruct normal lung

Fig. 6.6 Macrophages exhibiting unique activation profiles regulate disease progression and resolution. Macrophages are highly plastic cells that adopt a variety of activation states in response to stimuli found in the local milieu. During pathogen invasion or following tissue injury, local tissue macrophages often adopt an activated or "inflammatory phenotype". These cells are commonly called "classically activated" macrophages (CAMs), because they were the first activated macrophage population to receive a formal definition. These macrophages are activated by IFN-γ and/or following Toll-like receptor engagement, leading to the activation of the NF-kβ and Stat1 signalling pathways, which in turn increases production of reactive oxygen and nitrogen species and pro-inflammatory cytokines like TNF-α, IL-1 and IL-6 that enhance anti-microbial and anti-tumour immunity, but may also contribute to the development of insulin resistance and diet-induced obesity. Epithelial-derived alarmins and the type-2 cytokines IL4 and IL13, in contrast, result in an "alternative" state of macrophage activation that has been associated with wound healing, fibrosis, insulin sensitivity and immunoregulatory functions. These wound-healing, pro-angiogenic and pro-fibrotic macrophages (PfMø) express TGF-β1, PDGF, VEGF, WNT ligands and various matrix metalloproteinases that regulate myofibroblast activation and deposition of extracellular matrix components. Alternatively activated macrophages (AAMs) also express a variety of immunoregulatory proteins like arginase-1 (*Arg1*), Relm-alpha (*Retn1a*), *Pd12* and *I110* that regulate the magnitude and duration of immune responses. Therefore, in contrast to CAMs that activate immune defences, AAMs are typically involved in the suppression of immunity and re-establishment of homeostasis. Although type-2 cytokines are important inducers of suppressive or immunoregulatory macrophages, it is now clear that several additional mechanisms can also contribute to the activation of macrophages with immunoregulatory activity. Indeed, IL10-producing Tregs, Fc gamma receptor engagement, engulfment of apoptotic cells and prostaglandins have also been shown to preferentially increase the numbers of regulatory macrophages (Mregs) that suppress inflammation and inhibit anti-microbial and anti-tumour defences. The tumour microenvironment itself also promotes the recruitment and activation of immune inhibitory cells, including those of the mononuclear phagocytic series such as myeloid-derived suppressor cells (MDSCs), tumour-infiltrating macrophages (TIMs), tumour-associated macrophages (TAMs) and metastasis-associated macrophages (MAMs) that promote angiogenesis and tumour growth, while suppressing anti-tumour immunity. (From Ref. [84])

regeneration and contribute to pulmonary fibrosis by suppressing PCEC-derived CXCR7 expression. Loss of CXCR7 on PCECs leads to recruitment of vascular endothelial growth factor 1 (VEGFR1)-expressing perivascular macrophages that stimulate Wnt/β-catenin-dependent upregulation of Notch ligand Jagged 1, with pro-fibrotic sequelae [85].

Abnormal persistence of pulmonary macrophages has also been found to have pro-fibrotic potential. The homeostasis of such cells is regulated in part by mitophagy (a type of autophagy with selective engulfment of dysfunctional mitochondria by autophagosomes), a quality-control process that can be switched on by mitochondrial reactive oxygen species [87]. Larson-Casey and co-workers implicated AKT1, one of the family of three serine/threonine protein kinases called AKT that ordinarily regulates cell survival, proliferation and differentiation, in the mitochondrial ROS generation and macrophage dysfunction that can lead to impaired mitophagy with resultant apoptosis resistance and the development of pulmonary fibrosis versus controls, employing a bleomycin murine model with conditional deletion of *Akt1* in macrophages. The authors additionally showed the alveolar macrophages obtained from IPF patients had evidence of increased mitophagy and resistance to apoptosis, consistent with a mechanistic role for these processes in IPF [88]. Another member of the AKT family, AKT2, has been shown to be necessary for bleomycin-induced pulmonary fibrosis and inflammation, and in the fibrosis-resistant *Akt2*−/− mice, adoptive transfer of wild-type macrophages restored the fibrosis in a process that also involved macrophage-specific production of TGF-β1 and IL-13, raising interest in AKT2 as a potential therapeutic target for IPF [82].

IPF is characterised by high expression of the protein chitinase 3-like 1(CHI3L1 or YKL-40; the mouse homolog is Brp39*)* in the lung and in the circulation [89, 90]. CHI3L1 has been found to augment expression of the alternative macrophage activation marker CD206 in response to IL-13, and CD206+ macrophages are present at increased levels in IPF lungs [91]. CHI3L1 also tracks with CCL18 expression, another marker of alternatively activated macrophages [90]. Zhou et al. also showed that CHI3L1 exerts context-specific effects in IPF, with translational data showing a potential inhibitory effect (low CHI3L1 levels) on lung injury in the bleomycin-induced mouse model injury phase, while also showing an apparent augmentation of fibrogenesis (with high CHI3L1 levels) during the fibrotic phase in these animals. A YKL-40 transgenic mouse model was used to show an increased collagen, macrophage and lymphocyte accumulation in the lungs of the YKL-40-upregulated mice in response to bleomycin administration, with M2 markers markedly increased in lung tissue. The $CD206^{+}$ macrophages in the transgenic YKL-40 mice showed in vitro evidence of stimulating fibroblasts to proliferate (but not transform into myofibroblasts). When total lung macrophages were depleted in the transgenic mice by liposomal clodronate, there was a significant reduction in bleomycin-induced pulmonary fibrosis [90].

Subsets of circulating monocytes have been identified in efforts to simplify the complexities of the mononuclear phagocytic system, including an “inflammatory monocyte” which highly expresses Ly6C, among other cell surface markers, that is recruited from the circulation in response to injury or infection [84]. In a study that used multiple in vivo depletional strategies and adoptive transfer techniques, circu-

lating Ly6C^{hi} monocytes were shown in separate models of pulmonary fibrosis to facilitate progression of the fibrosis with evidence also provided from human IPF BAL samples of markedly increased expression of the M2/alternatively activated marker CD163 on IPF alveolar macrophages vs control subjects [92]. More recently, an atypical monocyte has been characterised that shares features of a granulocyte (bi-lobed segmented nuclear shape and many cytoplasmic granules) and has been termed segregated-nucleus-containing atypical monocytes (SatM), bearing the marker signature of Ceacam1+Msr1+Ly6C-F4/80-Mac1+, and appears to be critical for fibrosis. The cells are regulated by CCAAT/enhancer binding protein β (C/EBPβ), and *Cebpb−/−* chimaeric mice, lacking in SatM cells, were found to be protected from bleomycin-induced fibrosis, but not bleomycin-induced inflammation, and adoptive transfer of SatM into *Cebpb−/−* mice restored fibrosis susceptibility [93]. The lack of participation of this cell type in general inflammatory responses sets it apart from other monocytes and emphasises the redundancy of the simplistic M1/M2 classification, as multiple distinct phenotypes with disorder-specific behaviour are now being identified, creating an imperative for better understanding of how monocyte/macrophage biology pertains to IPF pathogenesis [93, 94].

Neutrophils

The increased numbers of neutrophils in IPF lungs versus normals has been described for decades, including a tendency for the cells to persist over time [5, 95]. Since the widespread adoption of idiopathic interstitial pneumonia classification with a stricter definition of IPF, it has become apparent that neutrophilic infiltrates are rare in IPF compared to the extent of fibrotic changes, with minimal interstitial inflammation usually evident on histopathologic inspection, and usually more mononuclear cells than neutrophils [5, 10, 20]. The mild degree of inflammation observed histopathologically in the UIP lesion of IPF is composed of mainly small lymphocytes, with scattered plasma cells, and occasional neutrophils and eosinophils. The location of the inflammation tends to be mainly in areas of collagen deposition or honeycomb change and is rare to be seen in otherwise structurally normal alveolar septa, and in contrast with historical opinion of IPF pathogenesis, the presence of severe inflammation now leads pathologists to suspect an alternative diagnosis other than UIP [10]. The neutrophil remains an important target in fibrotic disorders, including IPF, with evidence that the cells are implicated in bleomycin-induced pulmonary fibrosis, where resistance to the fibrotic process is observed among neutrophil elastase-knockout mice [96]. Though originally approved in Japan for ARDS therapy, the neutrophil elastase inhibitor sivelestat appeared to increase the long-termmortality rate in mechanically ventilated patients with acute lung injury and is not being developed for ILD [97].

A more successful therapy, pirfenidone, possesses antifibrotic and antioxidant properties but also has anti-inflammatory effects, with the precise mechanism of action in IPF still unclear [2]. It is possible that acute effects ascribed to the drug may be relevant to the observed lower rate of IPF acute exacerbations with pirfeni-

done. When rats are challenged with LPS, their BAL neutrophilia induced by LPS is inhibited in a dose-dependent manner by pretreatment with pirfenidone [98]. Among the other pleiotropic capabilities of pirfenidone is the ability to inhibit TNF-α secreted and cell-associated levels, although only at supratherapeutic doses in animal models, and the pulmonary anti-inflammatory activity of the drug has been shown to occur independently of TNF-α inhibition [98, 99]. There is also evidence of it having an inhibitory effect on other Th1 inflammatory mediators including IL-1, IL-6, IL-8 and IL-12 [100]. Much is still to be learned regarding the extent of redundancy of pirfenidone's many effects in mediating its benefit in IPF.

Balestro and colleagues took an interesting approach to exploring hypothetical factors involved in IPF progression by performing pathologic quantification of cells from the explanted lung in slow progressors (annual decline in % predicted FVC <10%, $n = 48$) and rapid progressors (annual decline in % predicted FVC ≥10%, $n = 25$) who underwent lung transplantation for IPF. Morphometric analysis showed the rapid progressors had a higher quantity of $CD45^+$ leukocytes/mm [2] than the slow progressors ($p = 0.01$), with both innate (neutrophils $p = 0.02$ and macrophages $p = 0.04$) andadaptive ($CD4^+$ $p = 0.01$, $CD8^+$ $p = 0.005$ and B cells $p = 0.003$) inflammatory cells expanded in numbers in rapid versus slow progressors [101]. It can be argued that although such an approach has the limitation of looking at the final pathway (consequences, not causes) of the disease, the "end stage" cannot account for the observed differences among rapid and slow progressors. In contrast, an earlier, smaller study that defined slow progressors by >24 months of symptoms before first presentation, and rapid progressors by <6 months of symptoms before first presentation, had 8 open lung biopsy cases from "rapid" progressors and 27 from "slow" progressors, with a semi-quantitative approach used to define various histopathologic parameters, including extent of interstitial inflammation, with no discriminative ability found among rapid versus slow progressors using histopathology at the time of IPF diagnosis, or using BAL cell profile in rapid versus slow progressors [102].

An intriguing new role for the neutrophil in mediating age-related pulmonary fibrosis, and hence of potential relevance to IPF and/or acute exacerbations of IPF, is a process termed NETosis, whereby activated neutrophils release their chromatin as neutrophil extracellular traps (NETs) [103]. These traps/NETs are composed of filaments of decondensed chromatin which extrudes from the dying neutrophil and are covered in granular proteins including antimicrobial peptides that can entrap pathogens [104]. A potentially protective role for such NETs is offset by the potential for tissue damage and inflammation from inappropriate NET release however, as has been demonstrated in a mouse model of transfusion-related acute lung injury, with NETs appearing in the lung microvasculature [105]. There is in vitro evidence that in response to exposure to fibrogenic agents including cigarette smoke extract and bleomycin, NET-derived components promote the differentiation of human lung fibroblasts into a myofibroblast phenotype and ex vivo evidence of NETs in close proximity to alpha-smooth muscle actin-expressing fibroblasts obtained from NSIP lung biopsies [106]. NETosis appears to be dependent on the citrullination of specific arginine residues on histone tails catalysed by the enzyme peptidylarginine

deiminase 4 (PADI4). Using a padi4-deficient (*padi4−/−*) mouse model, Martinod et al. found that the incidence of age-induced pulmonary fibrosis was reduced (although not completely prevented) in *padi4−/−* mice than in wild-type mice. PADI4 is known to be highly expressed in inflammatory cellsand weakly expressed in lung tissue, leading the authors to surmise that reduced neutrophil NETosis is likely responsible for the antifibrotic effect, supported by the observation that neutrophils were primed for NETosis (as defined by a high percentage of citrullinated histone H3-positive neutrophils) in aged wild-type mice but not in aged *padi4−/−* mice [107]. It is timely now for work to target PADI4 as a lung protection strategy in acute exacerbations of IPF.

Adaptive Immunity

There is increasing awareness of roles for adaptive immunity in IPF, potentially in initiation and/or disease progression. As mentioned earlier, lymphoid aggregates are a recognised pathologic feature of IPF lesions and in most if not all other disease settings are usually pathognomic for the presence of chronic immune responses [18, 19, 108]. From an immunity standpoint, there is a predominance of T cells in BAL fluid and lung tissue from IPF patient, with $CD3^+$ T lymphocytes and mature dendritic cells found in the vicinity of fibroblastic foci and regions of high collagen deposition [17–19, 68]. The aggregates also display $CD20^+$ B lymphocytes, which form cohesive clusters in the centre of these aggregates (Fig. 6.7). In contrast with COPD or idiopathic pulmonary arterial hypertension, these tertiary lymphoid structures have non-proliferating and non-apoptotic features and therefore are likely to have already been activated when recruited to the lymphoid aggregate lesions [18].

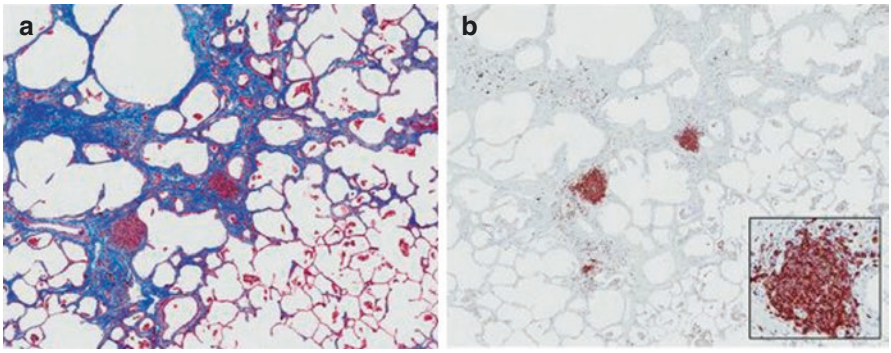


Fig. 6.7 Accumulation of $CD20^+$ B-cell aggregates in the lung tissue of IPF patients around areas of pulmonary fibrosis that are normally absent in healthy lungs. (**a**) Masson's trichrome stain of the lung tissue of an IPF patient. (**b**) Immunohistochemical stain of $CD20^+$ B cells in a serial section of the same tissue. The $CD20^+$ aggregates accumulate in areas where there is fibrosis (blue areas in **a**). (From Ref. [110])

The tertiary lymphoid structures include mature dendritic cells, and because it is known that activated T cells within the lung retain competency in effector cytokine production, it is plausible that chronic pulmonary inflammation could result from reactivation of memory T cells by maturing dendritic cells within IPF lymphoid aggregates [18, 109, 110].

In a study of 53 IPF patients' surgical lung biopsies, multivariate analyses showed that increasing fibroblastic foci scores were independently associated with greater declines in FVC and DL_{CO} at 6 and 12 months of follow-up, but unexpectedly at the time of this study, increasing interstitial mononuclear cell infiltrates were also independently associated with lung function decline, though only at 6 months, leading the authors to postulate that such active inflammation could have a role early in the development of fibrosis, or represent an epiphenomenon related to fibroblastic activity [111].

In further support of immune mechanisms in IPF beyond the lung compartment, circulating T lymphocytes are abnormally activated in IPF versus normal and exhibit biased CD4 T-cell receptor β-chain variable (TCRBV) repertoires relating to oligoclonal proliferations that indicate the presence of cellular immune responses to antigens in IPF [112]. This does not occur in health, where T lymphocytes do not react to anatomically accessible self-antigens [113]. A prevalent feature of many chronic adaptive immune response states is that repeated cycles of antigen-induced proliferation will lead to the loss of cell surface CD28 expression in T lymphocytes [114]. Gilani and colleagues have demonstrated a similar form of marked differentiation of circulating $CD4^+$ T cells in IPF with striking downregulation of CD28. Furthermore, these $CD4^+$ $CD28^-$ cells had discordant expression of various activation and cytotoxic markers versus control cells and were also demonstrated in IPF lung tissues and associated with poor clinical outcomes [115].

Interleukin 13 and its receptors have received attention as a potential inflammatory target in IPF, given its secretion from Th2 lymphocytes, epithelial cells, innate lymphoid cells-2 and macrophages and the recognition that IL-13 stimulates fibroblast proliferation and extracellular matrix synthesis by inducing TGF-β, platelet-derived growth factor, connective tissue growth factor, collagen 1 and fibronectin production [95, 116]. Pulmonary tissue fibroblast cell lines from IPF patients exhibit the highest expression of IL-13 receptor alpha 1 and IL-13 receptor alpha 2 compared to similar cell lines from other idiopathic interstitial pneumonia patients and normals, and the proliferation of such IPF fibroblasts was inhibited by a chimeric protein of human IL-13 and a truncated version of *Pseudomonas* exotoxin [117].

There is controversy attached to a potential role for T-cell co-stimulatory cells in regulating lung fibrosis, with discordant regulatory effects identified in inducible T-cell co-stimulator (ICOS) depending on tissue compartment and species under study [44, 118]. One study of IPF subjects utilised a discovery cohort ($n = 45$) and a separate replication cohort ($n = 75$) to validate a PBMC gene expression profile and found deceased expression of "the costimulatory signal during T-cell activation" Biocarta pathway in those who had a shorter transplant-free survival, with a putative four-gene biomarker of ICOS, CD28, lCK and ITK proving most predictive

of such an adverse course, and the proteins likely to arise from CD4+CD28+ T cells. The biomarker showed an area under the (receiver operating characteristic) curve of 78.5% at 2.4 months for predicting death and lung transplant in the replication cohort, representing a two- to fourfold increased risk of patients dying of IPF or having a lung transplant [118]. Another study, which lacked a validation cohort, showed an ostensibly opposing direction of expression for ICOS (i.e. upregulation) in IPF versus normals. The authors speculated that in light of the known secretion of ICOS by activated T lymphocytes in IPF [119], there could be a correlation of a loss of ICOS expression on cells with elevated plasma ICOS levels and reduced transcription [44].

The IPF therapy nintedanib was first developed as an anti-angiogenic anti-cancer drug and functions as an ATP-competitive inhibitor of fibroblast growth factor receptor (FGFR)-1 and vascular endothelial growth factor receptor (VEGFR)-2. Its ability to inhibit platelet-derived growth factor receptor (PDGFR)-α and PDGFR-β led to its therapeutic evaluation in IPF [80], and these mechanisms that diminish fibroblast-/myofibroblast-mediated fibrogenesis are likely to be responsible for the observed benefit of the drug in IPF. Nintedanib has also been shown in animal model systems to possess potent anti-inflammatory effects [120]. In a bleomycin-induced mouse model of lung fibrosis, lymphocyte counts in BAL fluid were significantly lowered irrespective of the nintedanib dose delivered, in addition to reductions of the pro-inflammatory cytokine IL-1β in lung tissue, while in another in vivo model of silica-induced lung fibrosis, the injured mice that received nintedanib exhibited reductions in both neutrophils and lymphocytes, but not in macrophages in BAL, in addition to reduced lung tissue levels of IL-1β and another pro-inflammatory cytokine IL-6 [120].

Autoimmunity and IPF

The observations by independent investigators of lymphoid aggregates in perifibrotic lung tissue coupled with various autoantibodies in serum have led to a theory in IPF of a breakdown in immunological self-tolerance to antigens derived from injured and ageing airway epithelial cells [17–20, 110] (Fig. 6.8). The earliest descriptions of autoantibodies in what we now call IPF were hypothesised and described in the pre-pathological-standardisation era, when IPF/CFA included other IIP entities such as DIP and NSIP, with reactive IgG autoantibodies (molecular weight 70–90 kDa) identified in CFA patients against lung alveolar lining cells and DNA topoisomerase II α [121–123]. Nonetheless, idiopathic UIP is recognised to have a multiplicity of associations with autoantibodies, as outlined in Table 6.1 [124–136]. Both blood and BAL fluid of IPF patients have decreased CD4+ CD25+ FOXP3+ regulatory T cells or Tregs versus healthy controls and may be central to IPF pathogenesis, given their key role in the generation of immunologic tolerance which is a checkpoint to autoantibody production [137].

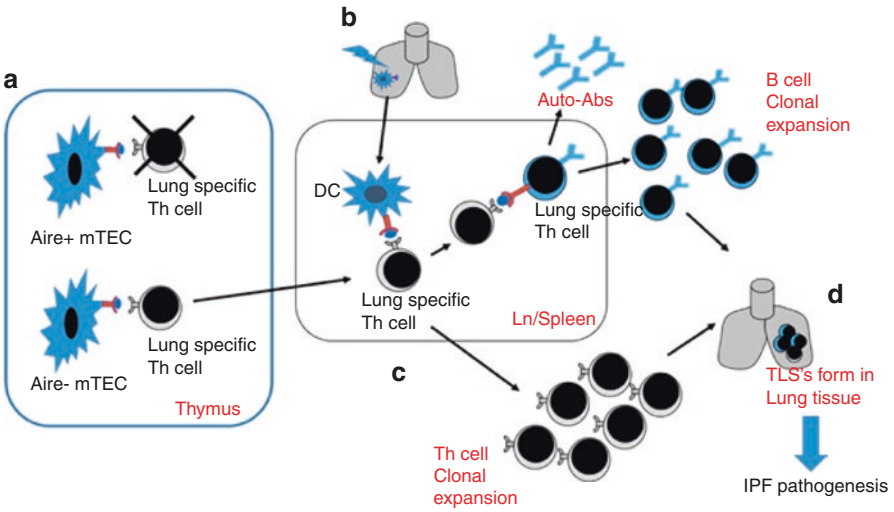


Fig. 6.8 Model of disease pathogenesis of IPF due to breakdown in self-tolerance to lung-specific protein antigens. (**a**) In the thymus, AIRE$^+$ mTECs can present self-antigens to developing thymocytes and self-reactive T cells are eliminated by apoptosis. In patients with APS-1 with mutations in which the *Aire* gene is faulty, mTECs fail to eliminate lung-specific T cells, and they complete maturation in the thymus and migrate to the periphery. (**b**) In response to injury in the lung, dendritic cells (DCs) can pick up and process lung-specific Ag and migrate to regional lymph nodes or spleen to present Ag to lung-specific Th cells. (**c**) The activated Th cells can provide help to Ag-specific B cells and both undergo clonal expansion, and Ag-specific B cells can mature as plasma cells and secrete autoantibodies into the blood. (**d**) Autoreactive T and B cells migrate to the lung to form TLSs, but they typically lack proliferating B cells and apoptotic cells in these sites, which are hallmarks of active germinal centres [9, 12, 44]. Due to chronic tissue damage, fibrosis develops and leads to IPF pathogenesis. (From Ref. [110])

Some of these associations of autoantibodies with IPF are plausibly pathogenic due to high expression of the target antigen in lung parenchymal tissues and/or have been linked to functional decline in IPF or other poor outcomes. For example, periplakin, a component of desmosomes but also strongly expressed in bronchial and alveolar epithelium, is one such target autoantigen, with circulating autoantibodies directed against it over-represented in the serum of IPF subjects compared to CTD-ILD, COPD or healthy subjects and associated with worse physiologic restriction and gas exchange on pulmonary function testing [126].

Type V collagen, a relatively less abundant collagen of pulmonary interstitial tissues compared to the major collagen in the lung, type I collagen, is ordinarily sequestered within fibrils of type I collagen but can become exposed to immune processes arising from lung remodelling of IPF, with subsequent development of anti-collagen V antibodies. This increase in type V collagen in IPF lung is associated with extent of fibrosis and predicts survival [138]. Interest is beginning to rise again in immunotherapies for IPF, exemplified by the knowledge that circulating autoantibodies against type V collagen are found in approximately 40% of patients with IPF [130], with even higher prevalence (60%) in IPF of anti-collagen V reac-

tive T cells using a trans-vivo delayed-type hypersensitivity test [139]. Nebulised type V collagen given in a murine bleomycin-induced fibrosis model prevented further collagen deposition and fibrosis by suppressing TGF-β superfamily of genes [130]. A subsequent proof-of-concept phase I study using oral immunotherapy with bovine type V collagen given once daily for 6 months to IPF patients (n = 30) showed a suggestion of stabilisation of the IPF-progression marker MMP7, and a decrease in C1q binding, consistent with a potential immunological effect of therapy on anti-collagen V antibody binding and activity, when the lowest-dose cohort was compared to the highest-dose cohort [140].

Also illustrative of the case for autoimmune dysregulation in IPF progression is the identification of anti-heat shock protein (HSP)70 humoral and cellular autoreactivity found in 30/122 (25%) IPF subjects versus 5/60 (3%) control subjects and found to be associated with HLA allele biases, significantly worse FVC and a worse 1-year survival of 40 ± 10% versus 80 ± 5% in controls (hazard ratio = 42; 95% confidence interval = 2.0–8.6; $p < 0.0001$) [127]. These antibodies were seen in non-IPF ILD patients also but not linked to clinical progression in such patients. In contrast, the circulating autoantibodies widely obtained for clinical use in connective tissue disorders (including antinuclear antibody, extractible nuclear antigens such as Jo-1, etc.) have been shown to be no more frequently found in IPF subjects (22% prevalence) versus healthy control subjects (21%) and to be associated with a more favourable survival in IPF [141]. Others have found a positive serologic test rate in IPF subjects lacking clinical features of connective tissue disease, of 29% [142]. The IPF subjects in these latter studies are distinct from subjects that are now studied under the emerging label of interstitial pneumonia with autoimmune features (IPAF), where criteria from both serologic domains and clinical domains of connective tissue disorders would need to accompany the presence of ostensibly idiopathic UIP in order to be considered IPAF [143].

Recently, a study employing whole-proteomic analysis (>7900 proteins) of 45 ILD tissues (including IPF) in addition to fibrotic scleroderma skin samples and suitable controls for both organ types identified the most significant common factor among different idiopathic ILD and skin fibrosis samples to be a protein MZB1, localised to a terminally differentiated, antibody-producing tissue resident plasma B-cell phenotype, $MZB1^{+}/CD38^{+}/CD138^{+}/CD27^{+}/CD45/CD20^{-}$, in both lung and skin fibrosis at high prevalence. These MZB1 plasma B cells were quite dispersed beyond tertiary lymphoid structures, though with a perivascular abundance, and levels correlated positively with tissue immunoglobulin G levels and DLCO, consistent with a common involvement of antibody-mediated autoimmunity in pulmonary and non-pulmonary fibrosis [136].

Other Immunologically Active Cells

Fibrocytes are bone marrow-derived mesenchymal cells of monocyte origin that have features of both macrophages and fibroblasts and found in circulating blood as

well as sites of tissue fibrosis in a variety of injured organs including the lungs, where they have been postulated to be recruited through a CXCR4/CXCL12 axis [144–147]. It has been established that chronic inflammatory stimuli mediate differentiation, trafficking and accumulation of fibrocytes in autoimmune conditions characterised by the additional presence of fibrosis, such as asthma with chronic airflow obstruction due to subepithelial basement membrane fibrosis, or scleroderma, and several potential roles for the cell have been postulated in chronic inflammatory disease states based on observations to date (Fig. 6.9) [144]. Fibrocytes

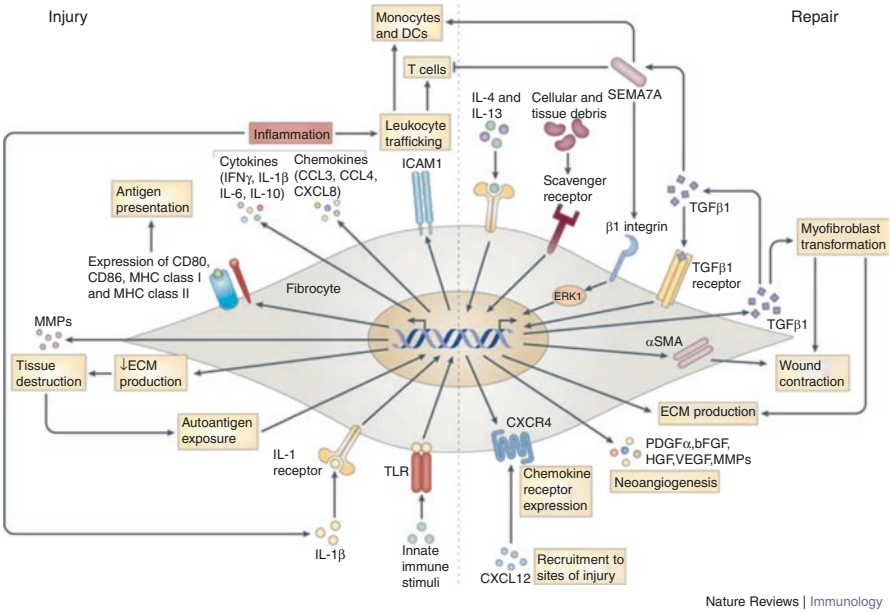


Fig. 6.9 Using autoimmunity as a model, the possible roles of fibrocytes are proposed. In the setting of autoantigen exposure or acute injury, or following stimulation by interleukin-1β (IL-1β), serum factors and innate immune stimuli, fibrocytes adopt a pro-inflammatory phenotype characterised by the secretion of interferon-γ (IFNγ), IL-6, IL-8, CC-chemokine ligand 3 (CCL3) and CCL4. Leukocyte trafficking is enhanced through the expression of intercellular adhesion molecule 1 (ICAM1). Production of extracellular matrix (ECM) components is decreased, and antigen-presenting capabilities are increased by the expression of CD80, CD86 and MHC class I and II molecules. Tissue destruction may be increased by the expression of matrix metalloproteinases (MMPs). As the local milieu begins to favour repair and remodelling (or perhaps concurrently with ongoing injury in the right biological context), fibrocytes adopt a more reparative phenotype. In this setting, transforming growth factor-β1 (TGFβ1) stimulates fibrocyte development through non-canonical pathways mediated by semaphorin 7A (SEMA7A) and β1 integrin, although other TGFβ1-mediated signalling pathways may also be involved. SEMA7A could activate monocytes and dendritic cells (DCs) while dampening T-cell responses. ECM production is also stimulated by T helper 2 (T_H2) cell cytokines (such as IL-4 and IL-13), as well as by exposure to apoptotic cells and cellular debris. Myofibroblast transformation is promoted by TGFβ1. Platelet-derived growth factor-α (PDGFα), IL-10, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF) support neoangiogenesis, and recruitment to sites of injury is promoted by the expression of chemokine receptors such as CXC-chemokine receptor 4 (CXCR4). αSMA α-smooth muscle actin, CXCL CXC-chemokine ligand, ERK extracellular signal-regulated kinase, TLR Toll-like receptor. (From Ref. [144])

gained increased attention in IPF with the finding of significantly higher levels of circulating CD45+, collagen 1+ fibrocytes in stable IPF patients versus ARDS or healthy control subjects, and in this study the levels of circulating fibrocytes increased further during acute exacerbations of IPF, in addition to being associated with worse survival [148]. Further proof for this biomarker principle came in a study of patients with Hermansky-Pudlak syndrome, a group of autosomal recessive disorders that result in oculocutaneous albinism, and (in the case of genotypes HPS-1, HPS-2 and HPS-4) development of interstitial pneumonia of a UIP-like pathology that can be slowed by pirfenidone [149, 150]. In a cross-sectional analysis of 66 patients with Hermansky-Pudlak syndrome and 12 age-matched controls, circulating fibrocytes (CD45+, Col1+) and activated fibrocytes (also positive for α–smooth muscle actin) were markedly elevated in certain subjects with HPS who had ILD but not in ILD-free HPS or controls. They also followed patients with longitudinal fibrocyte estimations and showed episodic spikes in levels of fibrocytes that strongly associated with death from pulmonary fibrosis [151]. Due to conflicting studies regarding whether or not these cells can differentiate in vivo into myofibroblasts, it is perhaps more likely that fibrocytes are contributing to fibrogenesis through paracrine signalling, potentially influencing other inflammatory cells or resident lung cells in the vicinity such as fibroblasts, endothelial or airway epithelial cells [147, 152, 153]. A proposed model of the potential role for fibrocytes in tissue injury, repair and remodelling is shown in Fig. 6.10 [144]. A key issue that has muddied the waters in the elucidation of fibrocyte pathobiology is the lack of consistency across investigative laboratories as to what cell marker sets should be used to define fibrocytes and other technical factors that may affect reproducibility of findings [153].

Inflammation and Acute Exacerbations of IPF

The natural history of IPF can be interspersed by an acute, clinically significant respiratory deterioration characterised by evidence of new, widespread alveolar abnormalities, termed an acute exacerbation [154]. There is uncertainty as to the aetiology of these deadly exacerbations, but the prevailing view is that acute factors known to cause acute lung injury, such as microbial infection, microaspiration or mechanical lung stretch, interact with chronic factors including the upregulated population of fibroblasts and dysfunctional epithelial cells, to bring about the widespread acute lung injury that typifies these exacerbations, with hyaline membrane formation and interstitial oedema, in addition to a variable presence of neutrophils [154, 155]. In the acute exacerbation of IPF setting, there has long been a theory that viruses in particular play a key aetiologic role. In contrast, a study of 43 patients who were experiencing an acute exacerbation found that the majority of such IPF subjects appeared to have no evidence of viral infection when BALF and serum were subjected to multiplex PCR, pan-viral microarray and high-throughput cDNA sequencing for viruses. A significant minority, 28%, had evidence of torque teno virus and significantly more so than in stable controls, with a similar rate of this virus also found in acute lung injury controls [156]. The ability of nintedanib and

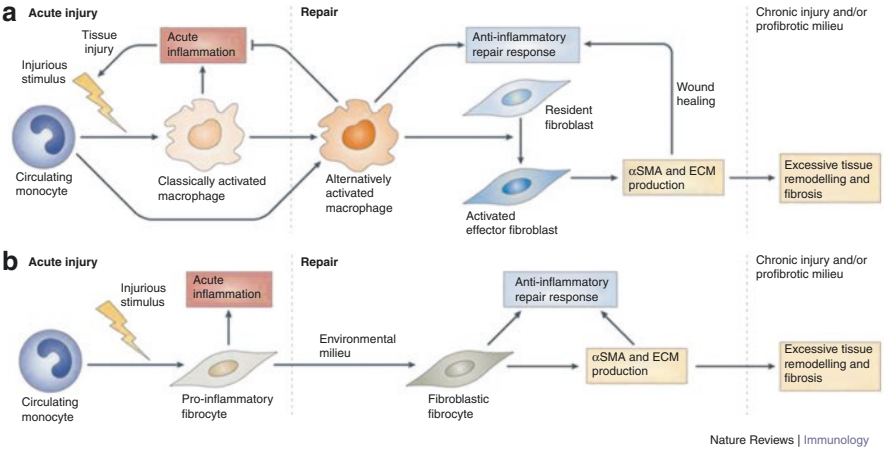


Fig. 6.10 (**a**) Current models suggest that in response to injurious stimuli, classically activated macrophages infiltrate diseased organs and mediate a programme of acute inflammation. As injury ceases and repair begins, the macrophage phenotype shifts towards that of alternative activation to dampen inflammation and promote repair. These macrophages stimulate resident fibroblasts to adopt an activated effector state characterised by the expression of α-smooth muscle actin (αSMA) and enhanced extracellular matrix (ECM) production. In the setting of severe or persistent injury, or a profibrotic milieu, this response shifts towards excessive remodelling and fibrosis. (**b**) This model of many cells acting together is contradicted by the finding that fibrocytes have properties of both macrophages and fibroblasts. Thus, an alternative model of repair is proposed in which fibrocytes traffic to injured organs, where they participate in the inflammatory events that are also attributed to macrophages. As damage subsides, fibrocytes respond to local cues to downregulate their inflammatory responses and adopt a fibroblastic phenotype to promote repair and, in some pathological conditions, remodelling and fibrosis. (From Ref. [144])

pirfenidone to favourably impact on the incidence of acute exacerbations of IPF may relate to some or all of purported mechanisms of action of these agents, including potentially their anti-inflammatory effects. Pirfenidone also appears to have a beneficial effect on respiratory-related hospitalisations in IPF patients, events that are more common than purely acute exacerbations of IPF, and, for example, included pneumonia events [157].

The prognosis for IPF acute exacerbations, which is poor, has been evaluated alongside putative serum biomarkers, one of which is anti-heat shock protein 70 autoantibody level. In a study of 122 IPF patients and 60 controls, anti-HSP70 IgG autoantibodies were found in 25% and 3%, respectively, and in IPF the autoantibody was associated with IPF CD4 T-lymphocyte and monocyte autoreactivity, greater FVC reduction and a shorter 1-year survival [127]. A small trial of 11 critically ill IPF subjects with acute exacerbation, 7 of whom had autoantibodies against HEp-2 cells, investigated outcomes following treatment with rituximab and therapeutic plasma exchanges and in some cases the further addition of intravenous immunoglobulin. An intention-to-treat analysis (including the two withdrawals prior to treatment) showed a 1-year survival advantage versus 20 historical controls

(controls from within 2 years prior to the experimental therapy enrolment) of 39% versus 0% (and 46 ± 15% versus 0% for 11 treated subjects vs controls), although the lack of a prospective control group and potential confounding limit interpretation of the results [158].

Future and Ongoing Work

The pleiotropic effects and substantial redundancy that constitute the various immunomodulatory pathways implicated to date in IPF make for great difficulties in reductionist approaches to deciphering cause or effect of a given pathway target molecule. It is impossible with our present knowledge to conclusively state whether or not the indisputably present immune dysfunction of IPF is a primary cause, a cause of progressive disease, or a secondary response such as immunosenescence, or a phenomenon downstream of more pathogenic initial injuries in ageing lungs. There is a distinct possibility that immune-mediated IPF endotypes have been overlooked in studies of unselected immunomodulatory therapies for IPF to date [159, 160]. Interest is now increasing again in therapies that attempt to address immune or inflammatory mechanisms in IPF. From a microbiomic perspective, there are at least two clinical trials planned or underway to evaluate the impact of co-trimoxazole or doxycycline on IPF-relevant clinical outcomes in selected patients with IPF (Clean-up IPF Trial, ClinicalTrials.gov identifier NCT02759120 and EME-TIPAC, EudraCT number 2014-004058-32) [159]. Based on a recent methodologically flawed but thought-provoking retrospective multicohort analysis of 11 IPF patients treated with the well-tolerated mycophenolate modafinil (MMF, a potent inhibitor of lymphocyte purine synthesis and lymphocyte proliferation) who seemed to have a weak signal towards reduced FVC decline and reduced mortality compared to 30 IPF patients receiving other historically ineffective/harmful therapies or no therapies, the authors suggested a future trial of combination therapy of a licenced IPF antifibrotic agent and MMF, in a justifiable bid to better address inflammatory endotypes missed by current antifibrotic agents [161]. Efforts to modulate neutrophil function offer new promise in fibrotic disorders, including IPF. Inhibitors of NETosis (e.g. a PADI4 inhibitor) could plausibly offer hope as a therapy in the setting of acute exacerbations of IPF, where neutrophilia is a known feature [94]. An ongoing trial is examining the role of rituximab as a B-lymphocyte depletion strategy for the reduction of autoantibodies implicated in IPF, in the hope that clinical benefits will also be apparent, including the effect on acute exacerbations (ClinicalTrials.gov trial identifier: NCT01969409). Recent developments in the re-engineering of chimeric antigen receptor T cells specific for autoantigen-producing B cells, as a means of targeting therapy for autoimmune disease, offer an intriguing new tool to deplete autoreactive B-cell clones, while conserving normal adaptive immune processes, and an appropriate design may have utility in an autoimmune-mediated IPF [162]. Through investigative approaches such as those outlined above, it may yet prove possible to

Table 6.2 Putative autoantibodies of relevance to idiopathic pulmonary fibrosis

Name of autoantigen	Comment	References
Annexin 1	The most abundant annexin in mammalian lung, expressed in alveolar epithelial type II cells and macrophages. Identified in acute exacerbations of IPF	[129]
BPIFB1	12% of IPF patients had autoantibodies against BPIFB1	[125]
Collagen V	Linked to fibrosis extent and survival	[130]
Cytokeratin 8	Epithelial cell cytoskeleton filament. Antibody complexes found in 29% of IPF patients	[131]
Cytokeratin 18	Detected in sera of IPF patients	[132]
Cytokeratin 19	Detected in sera of IPF	[133]
HSP70	Associated with poor prognosis in IPF	[127]
Interleukin-1a	Associated with rapidly progressive IPF	[128]
KCNRG	A bronchial epithelial antigen	[134]
LPLUNC1	A vomeromodulin-like protein expressed in human bronchiolar epithelium. Linked with pulmonary fibrosis of autoimmune polyglandular syndrome type I	[124]
MZB1	Prevalent across ILD types and skin fibrosis, localised to B lymphocytes	[136]
Periplakin	Found in IPF serum and BALF. Associated with more severe disease	[126]
Vimentin	Autoantibody levels linked to serious adverse outcomes in two separate IPF cohorts	[135]

exploit immune and inflammatory pathways in IPF that have been partially deciphered but that have thus far eluded successful therapeutic intervention (Table 6.2).

References

1. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. Lancet. 2017;389(10082):1941–52.
2. Spagnolo P, Wells AU, Collard HR. Pharmacological treatment of idiopathic pulmonary fibrosis: an update. Drug Discov Today. 2015;20(5):514–24.
3. Agostini C, Semenzato G. Immunology of idiopathic pulmonary fibrosis. Curr Opin Pulm Med. 1996;2(5):364–9.
4. Martinez FJ, Collard HR, Pardo A, et al. Idiopathic pulmonary fibrosis. Nat Rev Dis Primers. 2017;3:17074.
5. Hunninghake GW, Gadek JE, Lawley TJ, Crystal RG. Mechanisms of neutrophil accumulation in the lungs of patients with idiopathic pulmonary fibrosis. J Clin Invest. 1981;68(1):259–69.
6. Reynolds HY, Fulmer JD, Kazmierowski JA, Roberts WC, Frank MM, Crystal RG. Analysis of cellular and protein content of broncho-alveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. J Clin Invest. 1977;59(1):165–75.
7. Dall'Aglio PP, Pesci A, Bertorelli G, Brianti E, Scarpa S. Study of immune complexes in bronchoalveolar lavage fluids. Respiration. 1988;54(Suppl 1):36–41.

8. Bitterman PB, Rennard SI, Keogh BA, Wewers MD, Adelberg S, Crystal RG. Familial idiopathic pulmonary fibrosis. Evidence of lung inflammation in unaffected family members. N Engl J Med. 1986;314(21):1343–7.
9. Kropski JA, Pritchett JM, Zoz DF, et al. Extensive phenotyping of individuals at risk for familial interstitial pneumonia reveals clues to the pathogenesis of interstitial lung disease. Am J Respir Crit Care Med. 2015;191(4):417–26.
10. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. Am J Respir Crit Care Med. 1998;157(4 Pt 1):1301–15.
11. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med. 2000;161(2 Pt 1):646–64.
12. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013;188(6):733–48.
13. Idiopathic Pulmonary Fibrosis Clinical Research N, Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 2012;366(21):1968–77.
14. King TE Jr, Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2083–92.
15. Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2071–82.
16. Douglas WW, Ryu JH, Schroeder DR. Idiopathic pulmonary fibrosis: impact of oxygen and colchicine, prednisone, or no therapy on survival. Am J Respir Crit Care Med. 2000;161(4 Pt 1):1172–8.
17. DePianto DJ, Chandriani S, Abbas AR, et al. Heterogeneous gene expression signatures correspond to distinct lung pathologies and biomarkers of disease severity in idiopathic pulmonary fibrosis. Thorax. 2015;70(1):48–56.
18. Marchal-Somme J, Uzunhan Y, Marchand-Adam S, et al. Cutting edge: nonproliferating mature immune cells form a novel type of organized lymphoid structure in idiopathic pulmonary fibrosis. J Immunol. 2006;176(10):5735–9.
19. Todd NW, Scheraga RG, Galvin JR, et al. Lymphocyte aggregates persist and accumulate in the lungs of patients with idiopathic pulmonary fibrosis. J Inflamm Res. 2013;6:63–70.
20. Nuovo GJ, Hagood JS, Magro CM, et al. The distribution of immunomodulatory cells in the lungs of patients with idiopathic pulmonary fibrosis. Mod Pathol. 2012;25(3):416–33.
21. Flaherty KR, Travis WD, Colby TV, et al. Histopathologic variability in usual and nonspecific interstitial pneumonias. Am J Respir Crit Care Med. 2001;164(9):1722–7.
22. Monaghan H, Wells AU, Colby TV, du Bois RM, Hansell DM, Nicholson AG. Prognostic implications of histologic patterns in multiple surgical lung biopsies from patients with idiopathic interstitial pneumonias. Chest. 2004;125(2):522–6.
23. Miki H, Mio T, Nagai S, et al. Fibroblast contractility: usual interstitial pneumonia and nonspecific interstitial pneumonia. Am J Respir Crit Care Med. 2000;162(6):2259–64.
24. Strieter RM. Con: inflammatory mechanisms are not a minor component of the pathogenesis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2002;165(9):1206–7. discussion 7-8
25. Gifford AH, Matsuoka M, Ghoda LY, Homer RJ, Enelow RI. Chronic inflammation and lung fibrosis: pleotropic syndromes but limited distinct phenotypes. Mucosal Immunol. 2012;5(5):480–4.
26. White ES, Xia M, Murray S, 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):1242–51.
27. Richeldi L, Collard HR, du Bois RM, et al. Mapping the future for pulmonary fibrosis: report from the 17th international colloquium on lung and airway fibrosis. Eur Respir J. 2013;42(1):230–8.

28. O'Neill LA, Golenbock D, Bowie AG. The history of toll-like receptors – redefining innate immunity. Nat Rev Immunol. 2013;13(6):453–60.
29. Plataki M, Koutsopoulos AV, Darivianaki K, Delides G, Siafakas NM, Bouros D. Expression of apoptotic and antiapoptotic markers in epithelial cells in idiopathic pulmonary fibrosis. Chest. 2005;127(1):266–74.
30. Desai TJ, Brownfield DG, Krasnow MA. Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature. 2014;507(7491):190–4.
31. Ellson CD, Dunmore R, Hogaboam CM, Sleeman MA, Murray LA. Danger-associated molecular patterns and danger signals in idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol. 2014;51(2):163–8.
32. Bjermer L, Lundgren R, Hallgren R. Hyaluronan and type III procollagen peptide concentrations in bronchoalveolar lavage fluid in idiopathic pulmonary fibrosis. Thorax. 1989;44(2):126–31.
33. Mercer PF, Chambers RC. Innate immune signaling and stem cell renewal in idiopathic pulmonary fibrosis. Nat Med. 2016;22(11):1210–2.
34. Liang J, Zhang Y, Xie T, et al. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. Nat Med. 2016;22(11):1285–93.
35. Thomas AQ, Lane K, Phillips J 3rd, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med. 2002;165(9):1322–8.
36. Sisson TH, Mendez M, Choi K, et al. Targeted injury of type II alveolar epithelial cells induces pulmonary fibrosis. Am J Respir Crit Care Med. 2010;181(3):254–63.
37. Cao P, Aoki Y, Badri L, et al. Autocrine lysophosphatidic acid signaling activates beta-catenin and promotes lung allograft fibrosis. J Clin Invest. 2017;127(4):1517–30.
38. Hackett NR, Butler MW, Shaykhiev R, et al. RNA-Seq quantification of the human small airway epithelium transcriptome. BMC Genomics. 2012;13:82.
39. Seibold MA, Wise AL, Speer MC, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. N Engl J Med. 2011;364(16):1503–12.
40. Fingerlin TE, Murphy E, Zhang W, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. Nat Genet. 2013;45(6):613–20.
41. Lee MG, Lee YH. A meta-analysis examining the association between the MUC5B rs35705950 T/G polymorphism and susceptibility to idiopathic pulmonary fibrosis. Inflamm Res. 2015;64(6):463–70.
42. Araki T, Putman RK, Hatabu H, et al. Development and progression of interstitial lung abnormalities in the Framingham Heart Study. Am J Respir Crit Care Med. 2016;194(12):1514–22.
43. Peljto AL, Zhang Y, Fingerlin TE, et al. Association between the MUC5B promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. JAMA. 2013;309(21):2232–9.
44. O'Dwyer DN, Norman KC, Xia M, et al. The peripheral blood proteome signature of idiopathic pulmonary fibrosis is distinct from normal and is associated with novel immunological processes. Sci Rep. 2017;7:46560.
45. Noth I, Zhang Y, Ma SF, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. Lancet Respir Med. 2013;1(4):309–17.
46. Bulut Y, Faure E, Thomas L, Equils O, Arditi M. Cooperation of toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and Borrelia burgdorferi outer surface protein a lipoprotein: role of toll-interacting protein and IL-1 receptor signaling molecules in toll-like receptor 2 signaling. J Immunol. 2001;167(2):987–94.
47. Zhu L, Wang L, Luo X, et al. Tollip, an intracellular trafficking protein, is a novel modulator of the transforming growth factor-beta signaling pathway. J Biol Chem. 2012;287(47):39653–63.
48. Kimman TG, Banus S, Reijmerink N, et al. Association of interacting genes in the toll-like receptor signaling pathway and the antibody response to pertussis vaccination. PLoS One. 2008;3(11):e3665.

49. Rabinovich EI, Kapetanaki MG, Steinfeld I, et al. Global methylation patterns in idiopathic pulmonary fibrosis. PLoS One. 2012;7(4):e33770.
50. O'Dwyer DN, Armstrong ME, Trujillo G, 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.
51. Han MK, Zhou Y, Murray S, et al. Lung microbiome and disease progression in idiopathic pulmonary fibrosis: an analysis of the COMET study. Lancet Respir Med. 2014;2(7):548–56.
52. Molyneaux PL, Willis-Owen SAG, Cox MJ, et al. Host-microbial interactions in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2017;195(12):1640–50.
53. Huang Y, Ma SF, Espindola MS, et al. Microbes associate with host innate immune response in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2017;196:208–19.
54. Molyneaux PL, Cox MJ, Willis-Owen SA, et al. The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2014;190(8):906–13.
55. Moore BB, Moore TA. Viruses in idiopathic pulmonary fibrosis. Etiology and exacerbation. Ann Am Thorac Soc. 2015;12(Suppl 2):S186–92.
56. Thelen M, Stein JV. How chemokines invite leukocytes to dance. Nat Immunol. 2008;9(9):953–9.
57. Butler MWMP, Keane MP. Chemokines, Adipokines, and growth factors in the lung. In: Grippi JAE MA, Fishman JA, Kotloff RM, Pack AI, Senior RM, editors. Fishman's pulmonary diseases and disorders. 5th ed. New York: McGraw-Hill Medical; 2015. p. 327–40.
58. Strieter RM. Interleukin-8: a very important chemokine of the human airway epithelium. Am J Phys Lung Cell Mol Phys. 2002;283(4):L688–9.
59. Koelink PJ, Overbeek SA, Braber S, et al. Targeting chemokine receptors in chronic inflammatory diseases: an extensive review. Pharmacol Ther. 2012;133(1):1–18.
60. Strieter RM, Polverini PJ, Kunkel SL, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem. 1995;270(45):27348–57.
61. Strieter RM, Burdick MD, Mestas J, Gomperts B, Keane MP, Belperio JA. Cancer CXC chemokine networks and tumour angiogenesis. Eur J Cancer. 2006;42(6):768–78.
62. Dawson TC, Lentsch AB, Wang Z, et al. Exaggerated response to endotoxin in mice lacking the Duffy antigen/receptor for chemokines (DARC). Blood. 2000;96(5):1681–4.
63. Lee JS, Wurfel MM, Matute-Bello G, et al. The Duffy antigen modifies systemic and local tissue chemokine responses following lipopolysaccharide stimulation. J Immunol. 2006;177(11):8086–94.
64. Rot A. In situ binding assay for studying chemokine interactions with endothelial cells. J Immunol Methods. 2003;273(1–2):63–71.
65. Raghu G, Collard HR, Egan JJ, et al. 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.
66. Mukaida N. Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. Am J Phys Lung Cell Mol Phys. 2003;284(4):L566–77.
67. Southcott AM, Jones KP, Li D, et al. Interleukin-8. Differential expression in lone fibrosing alveolitis and systemic sclerosis. Am J Respir Crit Care Med. 1995;151(5):1604–12.
68. Vuga LJ, Tedrow JR, Pandit KV, 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.
69. Turner-Warwick M. Precapillary systemic-pulmonary anastomoses. Thorax. 1963;18:225–37.
70. Keane MP, Belperio JA, Arenberg DA, et al. IFN-gamma-inducible protein-10 attenuates bleomycin-induced pulmonary fibrosis via inhibition of angiogenesis. J Immunol. 1999;163(10):5686–92.
71. Keane MP, Belperio JA, Burdick MD, Lynch JP, Fishbein MC, Strieter RM. ENA-78 is an important angiogenic factor in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2001;164(12):2239–42.
72. Renzoni EA. Neovascularization in idiopathic pulmonary fibrosis: too much or too little? Am J Respir Crit Care Med. 2004;169(11):1179–80.

73. Cosgrove GP, Brown KK, Schiemann WP, et al. Pigment epithelium-derived factor in idiopathic pulmonary fibrosis: a role in aberrant angiogenesis. Am J Respir Crit Care Med. 2004;170(3):242–51.
74. Salogni L, Musso T, Bosisio D, et al. Activin A induces dendritic cell migration through the polarized release of CXC chemokine ligands 12 and 14. Blood. 2009;113(23):5848–56.
75. Shellenberger TD, Wang M, Gujrati M, et al. BRAK/CXCL14 is a potent inhibitor of angiogenesis and a chemotactic factor for immature dendritic cells. Cancer Res. 2004;64(22):8262–70.
76. Tanegashima K, Suzuki K, Nakayama Y, et al. CXCL14 is a natural inhibitor of the CXCL12-CXCR4 signaling axis. FEBS Lett. 2013;587(12):1731–5.
77. Shaykhiev R, Sackrowitz R, Fukui T, et al. Smoking-induced CXCL14 expression in the human airway epithelium links chronic obstructive pulmonary disease to lung cancer. Am J Respir Cell Mol Biol. 2013;49(3):418–25.
78. Jia G, Chandriani S, Abbas AR, et al. CXCL14 is a candidate biomarker for hedgehog signalling in idiopathic pulmonary fibrosis. Thorax. 2017;72:780–7.
79. Hilberg F, Roth GJ, Krssak M, et al. BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. Cancer Res. 2008;68(12):4774–82.
80. Wollin L, Wex E, Pautsch A, et al. Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. Eur Respir J. 2015;45(5):1434–45.
81. Butler MW, Hackett NR, Salit J, et al. Glutathione S-transferase copy number variation alters lung gene expression. Eur Respir J. 2011;38(1):15–28.
82. Nie Y, Sun L, Wu Y, et al. AKT2 regulates pulmonary inflammation and fibrosis via modulating macrophage activation. J Immunol. 2017;198(11):4470–80.
83. Schupp JC, Binder H, Jager B, et al. Macrophage activation in acute exacerbation of idiopathic pulmonary fibrosis. PLoS One. 2015;10(1):e0116775.
84. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature. 2013;496(7446):445–55.
85. Cao Z, Lis R, Ginsberg M, et al. Targeting of the pulmonary capillary vascular niche promotes lung alveolar repair and ameliorates fibrosis. Nat Med. 2016;22(2):154–62.
86. Rafii S, Cao Z, Lis R, et al. Platelet-derived SDF-1 primes the pulmonary capillary vascular niche to drive lung alveolar regeneration. Nat Cell Biol. 2015;17(2):123–36.
87. Chen Y, Azad MB, Gibson SB. Superoxide is the major reactive oxygen species regulating autophagy. Cell Death Differ. 2009;16(7):1040–52.
88. Larson-Casey JL, Deshane JS, Ryan AJ, Thannickal VJ, Carter AB. Macrophage Akt1 kinase-mediated mitophagy modulates apoptosis resistance and pulmonary fibrosis. Immunity. 2016;44(3):582–96.
89. Korthagen NM, van Moorsel CH, Barlo NP, et al. Serum and BALF YKL-40 levels are predictors of survival in idiopathic pulmonary fibrosis. Respir Med. 2011;105(1):106–13.
90. Zhou Y, Peng H, Sun H, et al. Chitinase 3-like 1 suppresses injury and promotes fibroproliferative responses in mammalian lung fibrosis. Sci Transl Med. 2014;6(240):240ra76.
91. Lee CG, Hartl D, Lee GR, et al. Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. J Exp Med. 2009;206(5):1149–66.
92. Gibbons MA, MacKinnon AC, Ramachandran P, et al. Ly6Chi monocytes direct alternatively activated profibrotic macrophage regulation of lung fibrosis. Am J Respir Crit Care Med. 2011;184(5):569–81.
93. Satoh T, Nakagawa K, Sugihara F, et al. Identification of an atypical monocyte and committed progenitor involved in fibrosis. Nature. 2017;541(7635):96–101.
94. Dimmeler S, Zeiher AM. Netting insights into fibrosis. N Engl J Med. 2017;376(15):1475–7.
95. Wick G, Grundtman C, Mayerl C, et al. The immunology of fibrosis. Annu Rev Immunol. 2013;31:107–35.
96. Chua F, Dunsmore SE, Clingen PH, et al. Mice lacking neutrophil elastase are resistant to bleomycin-induced pulmonary fibrosis. Am J Pathol. 2007;170(1):65–74.
97. Zeiher BG, Artigas A, Vincent JL, et al. Neutrophil elastase inhibition in acute lung injury: results of the STRIVE study. Crit Care Med. 2004;32(8):1695–702.

98. Spond J, Case N, Chapman RW, et al. Inhibition of experimental acute pulmonary inflammation by pirfenidone. Pulm Pharmacol Ther. 2003;16(4):207–14.
99. Nakazato H, Oku H, Yamane S, Tsuruta Y, Suzuki R. A novel anti-fibrotic agent pirfenidone suppresses tumor necrosis factor-alpha at the translational level. Eur J Pharmacol. 2002;446(1–3):177–85.
100. Ahidjo BA, Maiga MC, Ihms EA, et al. The antifibrotic drug pirfenidone promotes pulmonary cavitation and drug resistance in a mouse model of chronic tuberculosis. JCI Insight. 2016;1(14):e86017.
101. Balestro E, Calabrese F, Turato G, et al. Immune inflammation and disease progression in idiopathic pulmonary fibrosis. PLoS One. 2016;11(5):e0154516.
102. Selman M, Carrillo G, Estrada A, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. PLoS One. 2007;2(5):e482.
103. Viby NE, Isidor MS, Buggeskov KB, Poulsen SS, Hansen JB, Kissow H. Glucagon-like peptide-1 (GLP-1) reduces mortality and improves lung function in a model of experimental obstructive lung disease in female mice. Endocrinology. 2013;154(12):4503–11.
104. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303(5663):1532–5.
105. Caudrillier A, Kessenbrock K, Gilliss BM, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. J Clin Invest. 2012;122(7):2661–71.
106. Chrysanthopoulou A, Mitroulis I, Apostolidou E, et al. Neutrophil extracellular traps promote differentiation and function of fibroblasts. J Pathol. 2014;233(3):294–307.
107. Martinod K, Witsch T, Erpenbeck L, et al. Peptidylarginine deiminase 4 promotes age-related organ fibrosis. J Exp Med. 2017;214(2):439–58.
108. Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. Nat Rev Immunol. 2006;6(3):205–17.
109. Harris NL, Watt V, Ronchese F, Le Gros G. Differential T cell function and fate in lymph node and nonlymphoid tissues. J Exp Med. 2002;195(3):317–26.
110. Hoyne GF, Elliott H, Mutsaers SE, Prele CM. Idiopathic pulmonary fibrosis and a role for autoimmunity. Immunol Cell Biol. 2017;95:577–83.
111. Nicholson AG, Fulford LG, Colby TV, du Bois RM, Hansell DM, Wells AU. The relationship between individual histologic features and disease progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2002;166(2):173–7.
112. Feghali-Bostwick CA, Tsai CG, Valentine VG, et al. Cellular and humoral autoreactivity in idiopathic pulmonary fibrosis. J Immunol. 2007;179(4):2592–9.
113. Monaco C, Andreakos E, Kiriakidis S, Feldmann M, Paleolog E. T-cell-mediated signalling in immune, inflammatory and angiogenic processes: the cascade of events leading to inflammatory diseases. Curr Drug Targets Inflamm Allergy. 2004;3(1):35–42.
114. Fasth AE, Dastmalchi M, Rahbar A, et al. T cell infiltrates in the muscles of patients with dermatomyositis and polymyositis are dominated by CD28null T cells. J Immunol. 2009;183(7):4792–9.
115. Gilani SR, Vuga LJ, Lindell KO, et al. CD28 down-regulation on circulating CD4 T-cells is associated with poor prognoses of patients with idiopathic pulmonary fibrosis. PLoS One. 2010;5(1):e8959.
116. Passalacqua G, Mincarini M, Colombo D, et al. IL-13 and idiopathic pulmonary fibrosis: possible links and new therapeutic strategies. Pulm Pharmacol Ther. 2017;45:95–100.
117. Jakubzick C, Choi ES, Carpenter KJ, et al. Human pulmonary fibroblasts exhibit altered interleukin-4 and interleukin-13 receptor subunit expression in idiopathic interstitial pneumonia. Am J Pathol. 2004;164(6):1989–2001.
118. Herazo-Maya JD, Noth I, Duncan SR, et al. Peripheral blood mononuclear cell gene expression profiles predict poor outcome in idiopathic pulmonary fibrosis. Sci Transl Med. 2013;5(205):205ra136.
119. Ashley SL, Xia M, Murray S, et al. Six-SOMAmer index relating to immune, protease and Angiogenic functions predicts progression in IPF. PLoS One. 2016;11(8):e0159878.

120. Wollin L, Maillet I, Quesniaux V, Holweg A, Ryffel B. Antifibrotic and anti-inflammatory activity of the tyrosine kinase inhibitor nintedanib in experimental models of lung fibrosis. J Pharmacol Exp Ther. 2014;349(2):209–20.
121. Wallace WA, Roberts SN, Caldwell H, et al. Circulating antibodies to lung protein(s) in patients with cryptogenic fibrosing alveolitis. Thorax. 1994;49(3):218–24.
122. Meliconi R, Bestagno M, Sturani C, et al. Autoantibodies to DNA topoisomerase II in cryptogenic fibrosing alveolitis and connective tissue disease. Clin Exp Immunol. 1989;76(2):184–9.
123. Turner-Warwick M, Doniach D. Auto-antibody studies in interstitial pulmonary fibrosis. Br Med J. 1965;1(5439):886–91.
124. Shum AK, DeVoss J, Tan CL, et al. Identification of an autoantigen demonstrates a link between interstitial lung disease and a defect in central tolerance. Sci Transl Med. 2009;1(9):9ra20.
125. Shum AK, Alimohammadi M, Tan CL, et al. BPIFB1 is a lung-specific autoantigen associated with interstitial lung disease. Sci Transl Med. 2013;5(206):206ra139.
126. Taille C, Grootenboer-Mignot S, Boursier C, et al. Identification of periplakin as a new target for autoreactivity in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;183(6):759–66.
127. Kahloon RA, Xue J, Bhargava A, 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.
128. Ogushi F, Tani K, Endo T, et al. Autoantibodies to IL-1 alpha in sera from rapidly progressive idiopathic pulmonary fibrosis. J Med Investig. 2001;48(3–4):181–9.
129. Kurosu K, Takiguchi Y, Okada O, et al. Identification of annexin 1 as a novel autoantigen in acute exacerbation of idiopathic pulmonary fibrosis. J Immunol. 2008;181(1):756–67.
130. Vittal R, Mickler EA, Fisher AJ, et al. Type V collagen induced tolerance suppresses collagen deposition, TGF-beta and associated transcripts in pulmonary fibrosis. PLoS One. 2013;8(10):e76451.
131. Dobashi N, Fujita J, Ohtsuki Y, et al. Circulating cytokeratin 8:anti-cytokeratin 8 antibody immune complexes in sera of patients with pulmonary fibrosis. Respiration. 2000;67(4):397–401.
132. Dobashi N, Fujita J, Murota M, et al. Elevation of anti-cytokeratin 18 antibody and circulating cytokeratin 18: anti-cytokeratin 18 antibody immune complexes in sera of patients with idiopathic pulmonary fibrosis. Lung. 2000;178(3):171–9.
133. Fujita J, Dobashi N, Ohtsuki Y, et al. Elevation of anti-cytokeratin 19 antibody in sera of the patients with idiopathic pulmonary fibrosis and pulmonary fibrosis associated with collagen vascular disorders. Lung. 1999;177(5):311–9.
134. Alimohammadi M, Dubois N, Skoldberg F, et al. Pulmonary autoimmunity as a feature of autoimmune polyendocrine syndrome type 1 and identification of KCNRG as a bronchial autoantigen. Proc Natl Acad Sci U S A. 2009;106(11):4396–401.
135. Yang Y, Fujita J, Bandoh S, et al. Detection of antivimentin antibody in sera of patients with idiopathic pulmonary fibrosis and non-specific interstitial pneumonia. Clin Exp Immunol. 2002;128(1):169–74.
136. Schiller HB, Mayr CH, Leuschner G, et al. Deep proteome profiling reveals common prevalence of MZB1-positive plasma B cells in human lung and skin fibrosis. Am J Respir Crit Care Med. 2017;196:1298–310.
137. Kotsianidis I, Nakou E, Bouchliou I, et al. Global impairment of CD4+CD25+FOXP3+ regulatory T cells in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;179(12):1121–30.
138. Parra ER, Teodoro WR, Velosa AP, de Oliveira CC, Yoshinari NH, Capelozzi VL. Interstitial and vascular type V collagen morphologic disorganization in usual interstitial pneumonia. J Histochem Cytochem. 2006;54(12):1315–25.
139. Bobadilla JL, Love RB, Jankowska-Gan E, et al. Th-17, monokines, collagen type V, and primary graft dysfunction in lung transplantation. Am J Respir Crit Care Med. 2008;177(6):660–8.

140. Wilkes DS, Chew T, Flaherty KR, et al. Oral immunotherapy with type V collagen in idiopathic pulmonary fibrosis. Eur Respir J. 2015;45(5):1393–402.
141. Lee JS, Kim EJ, Lynch KL, et al. Prevalence and clinical significance of circulating autoantibodies in idiopathic pulmonary fibrosis. Respir Med. 2013;107(2):249–55.
142. Moua T, Maldonado F, Decker PA, Daniels CE, Ryu JH. Frequency and implication of autoimmune serologies in idiopathic pulmonary fibrosis. Mayo Clin Proc. 2014;89(3):319–26.
143. Fischer A, Antoniou KM, Brown KK, et al. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. Eur Respir J. 2015;46(4):976–87.
144. Reilkoff RA, Bucala R, Herzog EL. Fibrocytes: emerging effector cells in chronic inflammation. Nat Rev Immunol. 2011;11(6):427–35.
145. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med. 1994;1(1):71–81.
146. Song JS, Kang CM, Kang HH, et al. Inhibitory effect of CXC chemokine receptor 4 antagonist AMD3100 on bleomycin induced murine pulmonary fibrosis. Exp Mol Med. 2010;42(6):465–72.
147. Phillips RJ, Burdick MD, Hong K, et al. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. J Clin Invest. 2004;114(3):438–46.
148. Moeller A, Gilpin SE, Ask K, 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.
149. Suzuki T, Li W, Zhang Q, et al. Hermansky-Pudlak syndrome is caused by mutations in HPS4, the human homolog of the mouse light-ear gene. Nat Genet. 2002;30(3):321–4.
150. Gahl WA, Brantly M, Troendle J, et al. Effect of pirfenidone on the pulmonary fibrosis of Hermansky-Pudlak syndrome. Mol Genet Metab. 2002;76(3):234–42.
151. Trimble A, Gochuico BR, Markello TC, et al. Circulating fibrocytes as biomarker of prognosis in Hermansky-Pudlak syndrome. Am J Respir Crit Care Med. 2014;190(12):1395–401.
152. Kleaveland KR, Velikoff M, Yang J, et al. Fibrocytes are not an essential source of type I collagen during lung fibrosis. J Immunol. 2014;193(10):5229–39.
153. Moore BB, Kolb M. Fibrocytes and progression of fibrotic lung disease. Ready for showtime? Am J Respir Crit Care Med. 2014;190(12):1338–9.
154. Collard HR, Ryerson CJ, Corte TJ, et al. Acute exacerbation of idiopathic pulmonary fibrosis. An international working group report. Am J Respir Crit Care Med. 2016;194(3):265–75.
155. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J. 2011;37(2):356–63.
156. Wootton SC, Kim DS, Kondoh Y, et al. Viral infection in acute exacerbation of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;183(12):1698–702.
157. Ley B, Swigris J, Day BM, et al. Pirfenidone reduces respiratory-related hospitalizations in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2017;196:756–61.
158. Donahoe M, Valentine VG, Chien N, et al. Autoantibody-targeted treatments for acute exacerbations of idiopathic pulmonary fibrosis. PLoS One. 2015;10(6):e0127771.
159. Brownell R, Kaminski N, Woodruff PG, et al. Precision medicine: the new frontier in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2016;193(11):1213–8.
160. Maher TM. Precision medicine in idiopathic pulmonary fibrosis. QJM. 2016;109(9):585–7.
161. Nambiar AM, Anzueto AR, Peters JI. Effectiveness and safety of mycophenolate mofetil in idiopathic pulmonary fibrosis. PLoS One. 2017;12(4):e0176312.
162. Ellebrecht CT, Bhoj VG, Nace A, et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science. 2016;353(6295):179–84.

Chapter 7
Mechanisms of Fibrosis in IPF

Nathan Sandbo

Abbreviations

AEC	Alveolar epithelial cell
BAL	Bronchoalveolar lavage
BMPs	Bone morphogenetic proteins
cav-1	Caveolin-1
Col I	Collagen I
CTGF	Connective tissue growth factor
DAMPs	Danger-associated molecular patterns
ECM	Extracellular matrix
ED-A	Extra type III domain A
ELMOD2	ELMO domain containing 2
EMT	Epithelial to mesenchymal transition
ER	Endoplasmic reticulum
GFP	Green fluorescent protein
GWAS	Genome-wide association study
HLA	Human leukocyte antigen
hTERT	Human telomerase reverse transcriptase
IL-1β	Interleukin-1β
IL-13	Interleukin-13
IL-17A	Interleukin-17A
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-8/CXCL8	Interleukin-8
IPF	Idiopathic pulmonary fibrosis
LAP	Latency-associated peptide

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LOXL2	Lysyl oxidase 2
LPA	Lysophosphatidic acid
LTBP-1	Latent binding protein-1
MCP-1	Monocyte chemoattractant protein-1
MIP-1α	Macrophage inflammatory protein-1α
MMPs	Matrix metalloproteinases
MUC5B	Mucin 5B gene
PAI-1	Plasminogen activator inhibitor-1
PAI-2	Plasminogen activator inhibitor-2
PAMPs	Pathogen-associated molecular patterns
PDGF	Platelet-derived growth factor
PEDF	Pigment epithelium-derived factor
PGE2	Prostaglandin E2
PI3K	Phosphoinositide 3-kinase
PINK1	Putative kinase 1
PRA	Proteinase-activated receptors
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PTGER2	Prostaglandin E2 receptor
RAGE	Receptor for advanced glycation end products
SASP	Senescence-associated secretory phenotype
SNP	Single nucleotide polymorphism
SP-C	Surfactant protein C
TERC	RNA component of telomerase
TGF-β	Transforming growth factor-β
TGF-βR1	TGF-β type I receptor
TGF-βR2	TGF-β type II receptor
TIMPs	Tissue inhibitors of matrix metalloproteinases
TNF-α	Tumor necrosis factor-α
TOLLIP	Toll-interacting protein
UIP	Usual interstitial pneumonia
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor
WISP	WNT-induced signaling protein
α-SMA	α-Isoform of smooth muscle actin

Introduction

The normal reparative response to tissue injury involves the orchestrated involvement of multiple cell types under the influence of a myriad of autocrine, paracrine, and inflammatory mediators, with a goal of reestablishing tissue integrity and barrier function. Wound-healing in the adult human does not fully recapitulate embryologic developmental patterning, resulting in the formation of a scar at the site of injury [1, 2]. Resolution of the reparative response is important to preserve existing

normal tissue architecture and involves the tight spatiotemporal regulation of signaling involved in wound healing [3]. Fibrosis, characterized by excessive extracellular matrix accumulation and disruption of normal tissue architecture, can occur as a result of chronic injury, chronic inflammation, or dysregulation of the normal reparative process within a tissue bed.

In the lung the alveolar walls are formed by delicately apposed monolayers of alveolar epithelial cells and endothelial cells that are separated only by their respective basement membranes [4]. This delicate architecture forms the primary gas exchanging interface of the lung, allowing rapid diffusion of oxygen and carbon dioxide between the alveolar airspace and the alveolar capillary blood. The surrounding supporting interstitial spaces of the lung are comprised of a fine network of fibrillar proteins (collagens, fibronectin, elastin) in composite with hydrated glycosaminoglycans [5]. In pulmonary fibrosis, there is a dramatic disruption of this intricate structural organization with expansion of the connective tissue compartment of the lung due to accumulation of matrix components, associated alveolar obliteration and collapse, and progressive distortion of normal lung architecture [6]. These changes result in disturbances in gas exchange and, when progressive, respiratory failure and death.

IPF is one of several disorders of the lung characterized by aberrant tissue fibrosis. In contrast to several other forms of pulmonary fibrosis, such as the fibroproliferative phase of acute respiratory distress syndrome (diffuse alveolar epithelial cell injury), or fibrotic sarcoidosis (exuberant granulomatous inflammation), the underlying etiology of the fibrotic response has historically not been immediately clinically apparent in IPF, hence its name. However, aging is one of the single most important demographic risk factors for the development of IPF, making it a prototypical aging-related disorder. More recently, several genetic alterations have been identified that increase the risk for development of the disease. Understanding how age-related changes in cell function intersects with underlying genetic susceptibilities and exogenous insults to drive recurrent injury and the non-resolving wound-healing response may be getting us closer to understanding the etiology of this disorder.

The histopathology of IPF is defined by the usual interstitial pneumonia (UIP) pattern [7], which is characterized by spatial variegation of the fibrotic process [6, 8, 9] with normal-appearing areas of the lung adjacent to areas characterized by severe scarring and architectural distortion and the presence of microscopic honeycombing [6, 7].

Staining for early collagen forms that are indicative of collagen synthesis reveals that active, synthetic fibroblasts are present in clusters near the air-tissue interface, termed fibroblastic foci [6]. The presence of these spatially discreet foci of “activated” fibroblasts in juxtaposition to areas of “old” scar containing fewer fibroblasts and more mature collagen along with normal-appearing alveoli suggests an indolent but progressive process. The presence of these various stages of fibrosis within the same pathologic specimen is termed “temporal heterogeneity” and is a required diagnostic element of the UIP pattern [7]. While areas of scarring may contain a

mild, mixed inflammatory infiltrate as part of the UIP pattern, it does not predominate when compared to the fibrotic reaction.

The distinctive lesions of IPF, which are the fibroblastic foci, lend some insight into the underlying biology mediating this disorder. The fibroblastic foci are the site of "new fibrosis" with fibroblasts most proximal to the airspace demonstrating the greatest amount of collagen synthesis. Basal lamina remnants appear on the interstitial side of the fibroblastic focus, suggesting that this structure has developed within the previously intact airspace [6]. Supporting this concept, foci are often associated with a poorly adherent, hyperplastic epithelial cell layer on their luminal (airspace) side along with areas of epithelial sloughing. These structures form a reticulated network of fibrosis throughout the lung and are thought to represent the "leading edge" of new fibrosis [10]. The numbers of these structures present on surgical lung biopsy correlate with survival [11, 12], consistent with this role in disease progression.

An Overview of the Current and Evolving Model of IPF Pathogenesis

Given that several forms of pulmonary fibrosis are the result of a robust inflammatory response [13], it is not surprising that historically, IPF was originally viewed as a disorder primarily characterized by an early, macrophage-mediated alveolitis, with resultant progressive tissue fibrosis [14, 15]. The development of more precise classification schemes for the idiopathic interstitial pneumonias resulted in an improved appreciation of the lack of extensive inflammation in the histopathology of IPF [7] and called into question the role of inflammation in the disease process. It is now clear that broad inhibition of immune function using corticosteroids and azathioprine does not positively affect disease progression and patient outcomes [16–18]. Thus, the concept of IPF as a product of a robust, disordered inflammatory response has been supplanted by the current concept of IPF as a disorder characterized by repetitive alveolar epithelial cell injury and an aberrant, non-resolving wound-healing response [19, 20].

The temporal relationships of the key pathogenic events of IPF are largely inferred from the knowledge gained from several decades of investigations into the mechanisms of epithelial cell injury and the reparative response of cells and tissues [2, 21, 22]. This work from animal models of pulmonary fibrosis and correlative studies in IPF lung specimens has now been enriched by recent systems biology (genomic, transcriptomic, proteomic, etc.) studies that had significantly increased our understanding of IPF, leading to the current model of disease pathogenesis.

IPF pathogenesis is characterized by repetitive injury to AEC leading to apoptosis and disruption of the AEC layer. The risk for repetitive AEC injury in any one individual may be modified by genetic factors, age-related changes in AEC biology, and exogenous exposures. Dysregulated telomere biology, aging-related changes,

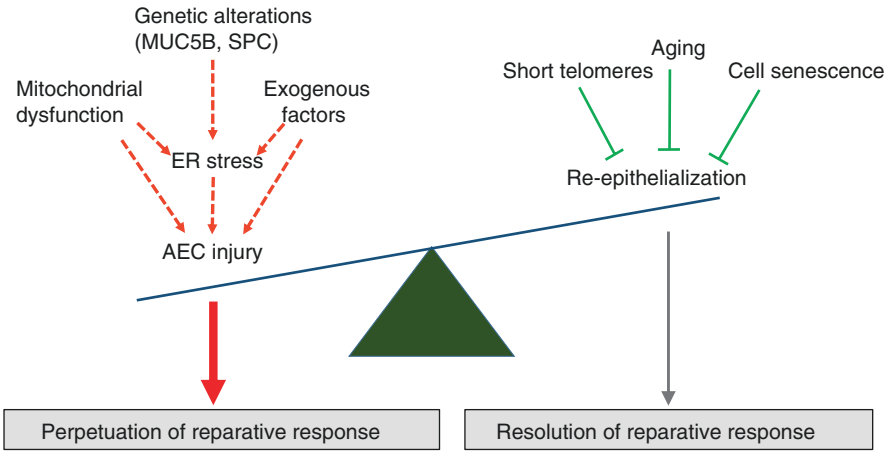

Fig. 7.1 Factors contributing to AEC injury and failure of reepithelialization in IPF. Reepithelialization is essential for resolution of the wound-healing response. Genetic susceptibility, aging-related changes in mitochondrial function, and repetitive exposure to exogenous factors all predispose to the development of UPR, ER stress, and ultimately AEC injury (apoptosis and dropout). In IPF, normal reepithelialization is hindered by telomere dysfunction, aging-related factors, and AEC senescence. Lack of reepithelialization (and loss of homeostatic AEC-derived signals) results in perpetuation of the wound-healing response

and the development of cell senescence interact to inhibit the reestablishment of a normal AEC barrier (reepithelialization), thereby perpetuating the aberrant wound-healing response (Fig. 7.1). AEC-derived signals play an important role in the potentiation of the wound-healing response via secretion of profibrotic cytokines (especially transforming growth factor-β [TGF-β]), chemokines, and proteases that trigger the recruitment and activation of inflammatory cells and fibroblasts. Local elaboration of matrix metalloproteinases results in disruption of the basal lamina of the alveolus. AEC injury is accompanied by the formation of a serum-derived, fibrinous exudate, which serves as a provisional matrix analogous to that of dermal wounds [23]. Chemokines and serum-derived factors present in the provisional matrix lead to the influx and expansion of local mesenchymal cell progenitor populations from the local interstitium, and there is also recruitment of circulating cell populations and acquisition of aberrant epithelial cell phenotypes that may play a role in the perpetuation of the fibrotic response. Activation of fibroblasts by TGF-β results in a highly contractile and synthetic phenotype, termed the myofibroblast, which serves as the primary effector cell for matrix production and tissue remodeling. Myofibroblast activation and tissue remodeling persist in IPF, possibly due to failure to reestablish normal epithelialization plus aberrant behavior of the myofibroblasts, which may be related to age-related cellular senescence, epigenetic reprogramming, or matrix-driven propagation of the fibrotic response. Progressive matrix deposition and remodeling ensue, resulting in a severely disordered tissue architecture (honeycombing) and organ dysfunction (Fig. 7.2).

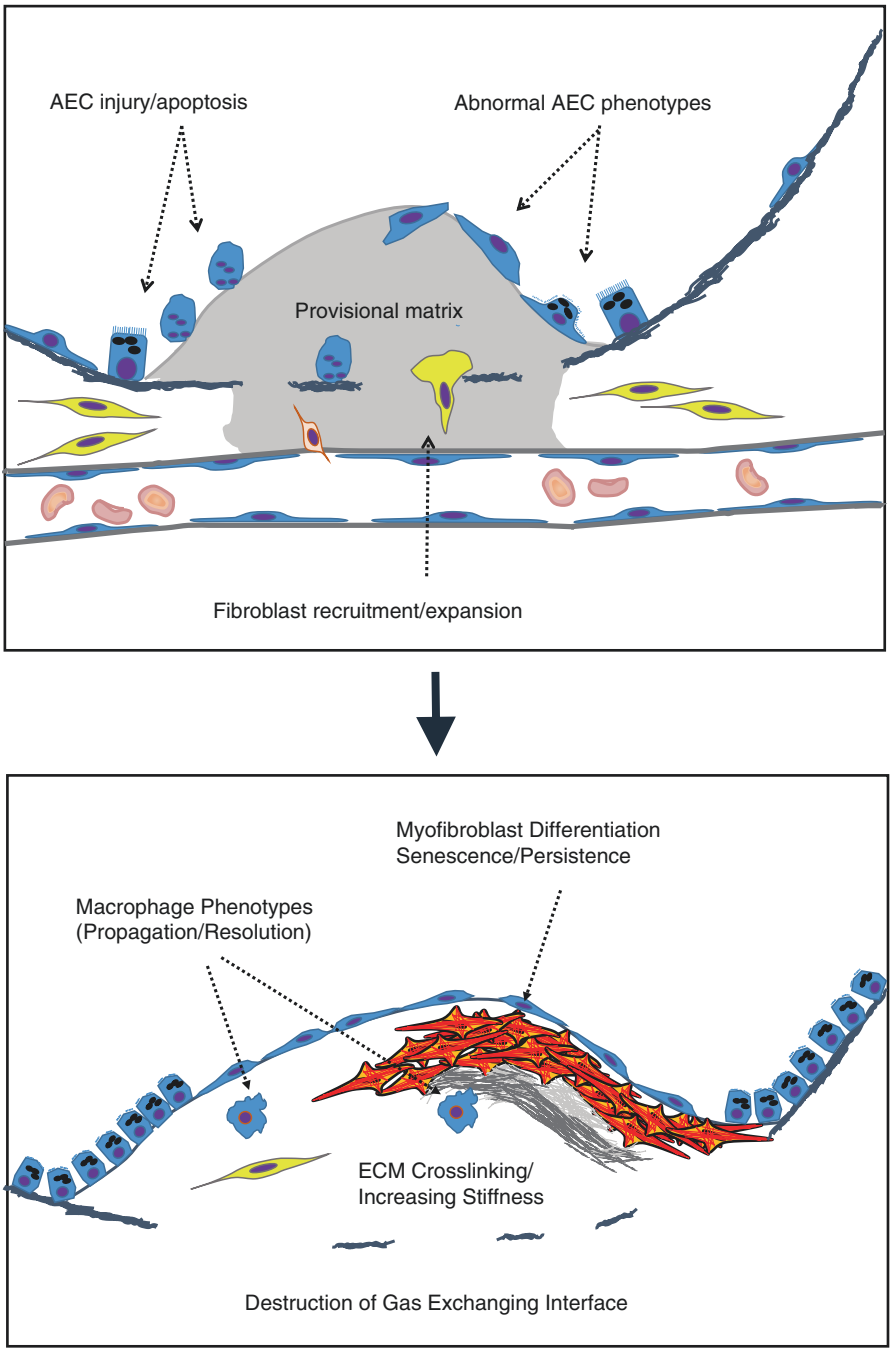
AEC injury/apoptosis
Abnormal AEC phenotypes
Provisional matrix
Fibroblast recruitment/expansion
Myofibroblast Differentiation
Senescence/Persistence
Macrophage Phenotypes
(Propagation/Resolution)
ECM Crosslinking/
Increasing Stiffness
Destruction of Gas Exchanging Interface

Fig. 7.2 Model of fibroblast focus formation. *Upper panel:* Epithelial cell injury leads to apoptosis and AEC dropout, resulting in a denuded basement membrane. There is an attempt to reepithelialize the airspace, but multiple aberrant AEC phenotypes emerge that lack proper homeostatic function and elaborate profibrotic mediators. Disruption of the normal basement membrane (broken lines) and alterations in alveolar capillary permeability result in the elaboration of a serum-derived exudate (provisional matrix) within the alveolar airspace. Localized progenitor populations expand the fibroblast population, while circulating bone marrow-derived cells also populate the wound. *Lower panel:* In response to TGF-β, matrix cues, and other soluble mediators, fibroblasts differentiate into (myo)fibroblasts and elaborate and incorporate abundant ECM. ECM deposition and cross-linking stiffen the matrix. Increases in tissue stiffness promote further TGF-β signaling, myofibroblast differentiation, and apoptosis resistance, perpetuating the fibrotic response. Cell-intrinsic or acquired metabolic derangements and senescence factors also perpetuate the fibrotic response. Progressive fibrosis emanating from this lesion results in obliteration of the adjacent capillaries, prevents gas exchange, and ultimately results in macroscopic architectural distortion and honeycombing of the lung

Several novel concepts that build upon this conceptual framework have emerged in recent years and are discussed in greater detail later in the chapter. Repetitive epithelial cell injury may be triggered by a combination of genetic and age-related factors that lead to increased susceptibility to alveolar epithelial cell stress, coupled with a "second hit" of exogenous "triggers" such as tobacco smoke, gastroesophageal reflux, changes in the local bacterial microbiome, or indolent viral replication that result in epithelial injury [24–26]. Global analysis of gene expression and non-coding microRNA in human subjects with IPF have demonstrated that signals associated with embryologic development and TGF-β-associated signals comprise a significant portion of the reparative gene expression response in humans with IPF [27]. These pathways are linked to the epithelial cell responses to injury, repair of the disrupted alveolar cell layer, and myofibroblast activation in IPF. How differences between the developmental and reparative response in these signaling pathways lead to the propagation of fibrosis remains an area of investigation [28], but given the strong link between aging and the development of IPF, it is likely that cell-intrinsic changes with age modify these responses.

Finally, remodeled, fibrotic matrix is not merely the end result of the fibrotic response. Biomechanical features of the matrix environment, such as its stiffness, are an independent determinant of fibroblast response and fibrotic progression, suggesting a new mechanism of aberrant cell behavior/function in IPF. In total, these mechanisms result in a mutually reinforcing cycle of fibrotic signaling, leading to non-resolving tissue fibrosis. The subsequent sections explore these concepts in detail.

Alveolar Epithelial Cell Injury and Failure of Normal Reepithelialization

Alveolar epithelial cell injury The normal alveolar epithelial lining of the lung is comprised of two types of epithelial cells, type I and type II AEC, forming a single-

cell thick layer. Type I cells are flat, highly specialized cells whose membrane comprises the bulk of the alveolar-capillary interface in normal lung tissue [29]. Type II cells have a cuboidal morphology, with intracellular lamellar bodies. Type II cells secrete surfactant proteins, retain proliferative capacity, and are responsible for the regeneration of epithelium after injury [30], including trans-differentiation to type I cells [31]. In IPF, alveolar epithelial cell morphology is severely deranged, with overt epithelial cell necrosis and denudation of the capillary basement membrane [32], as well as extensive type II pneumocyte apoptosis [33]. Alveolar spaces that have been disrupted by extensive fibrotic changes in IPF lungs are lined with numerous, hyperplastic type II pneumocytes, cuboidal epithelial cells that may be derived from the adjacent bronchiolar lining cells [34], and abnormal-appearing, elongated epithelial cells [35]. The presence of these abnormal epithelial phenotypes in areas that normally contain predominately type I epithelial cells is suggestive of a failure of normal reepithelialization after injury [36].

While AEC injury could be consequent to an ongoing, aberrant fibrotic response, several lines of evidence suggest that AEC injury may be a primary driver of the non-resolving reparative response. For example, in IPF alveolar epithelial cell apoptosis is found in areas without significant interstitial fibrosis, suggesting that this process may be a primary inciting factor [33].

Genetic susceptibility may predispose to AEC injury and failure of reepithelialization Further support for the concept of AEC-driven fibrosis comes from studies that have identified genetic alterations that confer susceptibility to AEC injury and apoptosis and promote aberrant AEC function. Several rare mutations in surfactant protein C (SP-C), a protein produced by the type II AEC cells, have been identified in patients with the familial form of pulmonary fibrosis [37, 38], which can have an identical histopathology to sporadic IPF. These mutations result in misfolding and altered processing of SP-C by type II AEC cells, leading to deficient expression and secretion, ER stress, and apoptosis [38–40]. Mice with germline deletion of SP-C develop interstitial lung disease as adults [41], suggesting a causal relationship for disordered SP-C biology. Rare mutations in surfactant protein A2 have also been identified in patients with familial pulmonary fibrosis (FPF) and in rare cases of sporadic IPF [42, 43]. These mutations result in a similar defect in protein stability, defective secretion, and a subsequent increase in ER stress-associated signaling [44].

The interaction of aging with AEC injury may be a very important etiologic factor in the development of IPF. Telomeres are multi-protein structures that cap the end of chromosomes and prevent their degradation. Chromosomal telomere shortening occurs with cell division and aging and is associated with the development of cell senescence and susceptibility to apoptosis [45]. Telomerase is present in progenitor cells, where it counteracts telomere shortening, thereby preserving proliferative potential [46]. Diseases of disrupted telomere homeostasis such as dyskeratosis congenita are characterized by short telomeres, premature graying, bone marrow failure, and the development of pulmonary fibrosis [47]. Several mutations in the two components of telomerase, telomerase reverse transcriptase

(hTERT) and the RNA component of telomerase (TERC) [48, 49], have been identified in patients with FPF. When compared to age-matched family members without the mutation, family members with TERT and TERC loss-of-function mutations had shorter telomeres and increased risk for the development of pulmonary fibrosis [48]. The presence of these mutations was associated with a penetrance of pulmonary fibrosis of 40% in the affected individuals [50]. However, the original mutations identified were only identified in a small percentage of patients with sporadic IPF [51]. Subsequent studies of patients with FPF have identified mutations in several additional proteins associated with telomere homeostasis (RTEL, PARN, dyskerin, and TINF2) [52–56].

The concept of telomere length-dependent susceptibility to alveolar epithelial injury and the development of pulmonary fibrosis are supported by the identification of short telomeres as an independent risk factor for the development of the sporadic IPF [57]. A genome-wide association study (GWAS) has identified a common single nucleotide polymorphism (SNP) in the hTERT gene that confers risk for the development of IPF [58], and whole exome sequencing of a cohort of sporadic IPF patients found mutations in TERT, RTEL1, and PARN that may be responsible for 11% of IPF [50].

Linkage analysis of a cohort of Finnish families with FPF identified the gene ELMO domain containing 2 (ELMOD2) [59, 60] as a candidate gene associated with the development of pulmonary fibrosis. ELMOD2, which is normally expressed in epithelial cells and macrophages of the lung, had significantly decreased expression in lungs of IPF patients. ELMOD2 may play a role in the response of epithelial cells and macrophages to viral infection [59], potentially linking an environmental and genetic trigger in this disorder.

Two large GWAS investigations have identified a SNP in the promoter region of the mucin 5B gene (MUC5B) that is strongly associated with the development of familial and sporadic forms of pulmonary fibrosis [61, 62]. The minor (risk conferring) allele is present in 34–37.5% of IPF cases and 9–11% of controls. The presence of homozygosity for the minor allele confers a 10- to 20-fold increase in the risk for developing IPF. MUC5B is present at increased levels in fibrotic areas of IPF lungs, and the mutant allele for this gene is associated with significantly increased expression of MUC5B in lungs of subjects without pulmonary fibrosis when compared to counterparts homozygous for the wild-type allele. This suggests that the discovered SNP results in alterations in gene expression that may contribute to the development of IPF.

GWAS studies have also identified additional polymorphisms that are associated with IPF in the genes of desmoplakin and dipeptidyl peptidase 9 [63], which are involved in epithelial function, and Toll-interacting protein (TOLLIP), which is involved in innate immune responses [64].

Exogenous factors Several exogenous agents that could trigger alveolar epithelial injury are associated with the development of IPF. Gastroesophageal reflux disease is present in up to 90% of patients with IPF [65, 66], and co-existing treatment with proton pump inhibitors has been associated with longer patient survival [67, 68].

Exposure to cigarette smoke is a powerful risk factor for the development of both IPF and FPF [69, 70], and approximately 70% of IPF patients are current or former cigarette smokers [71]. Workplace exposures are less robustly linked [72] but may contribute in a cohort of IPF patients. There is evidence of microsatellite instability in the DNA from IPF lungs [73] which suggests that somatic mutations due to exogenous exposures could account for acquired genetic risk and increase susceptibility to injury and aberrant reparative responses.

Several viruses that are trophic for the lung epithelium have been identified in IPF lungs [74] with the family of herpes viruses having the strongest association. A high prevalence of herpes virus DNA has been identified in the AECs and immune cells of the IPF lung [75–77]. The presence of herpes viral antigens has also been associated with signs of ER stress in the AECs [78, 79], suggesting a possible mechanism of injury triggered by viral infection. Finally, alterations in the bacterial microbiome of the lung are associated with IPF and its progression [80, 81].

These observations provide conceptual evidence that intrinsic epithelial defects may render the epithelial cell susceptible to repetitive injury, possibly from the environmental factors listed above, which could lead to perpetuation of the wound-healing response.

This concept has been experimentally demonstrated by targeted injury to type II AECs in mice via transgenic expression of SP-C-driven diphtheria toxin receptor expression followed by intraperitoneal diphtheria toxin administration. Changes in AEC gene expression and function were present in the transgenic animals, and repeated exposure to diphtheria toxin resulted in the development of alveolar interstitial fibrosis without induction of inflammation [82].

Several potential mechanisms likely account for the development of fibrosis in response to AEC injury. These include failure of reepithelialization with AEC dropout and loss of homeostatic signaling, acquisition of aberrant epithelial phenotypes (including senescence-associated secretory phenotype (SASP) and epithelial-mesenchymal transition (EMT)), and endoplasmic reticulum stress and elaboration of AEC-derived profibrotic soluble mediators (Fig. 7.1).

Failure of normal alveolar reepithelialization Self-limited lung injury is characterized by regeneration of the alveolar epithelium and reestablishment of the normal alveolar epithelial cell layer via proliferation of type II AEC and subsequent transdifferentiation to type I AECs [83–86]. Additional epithelial progenitor populations may contribute to this process [87]. Reepithelialization reestablishes the normal homeostatic function of the epithelium and promotes resolution of the reparative response. IPF is characterized by a failure of reepithelialization with the development of a disordered epithelial layer characterized by proliferation of bronchiolar basilar epithelial cells exhibiting signs of epithelial stress and atypia [88], along with the presence of AECs that exhibit an abnormal, intermediate phenotype with traits of type I and type II cells [36].

Experiments performed in an ex vivo model of hyperoxia-mediated AEC injury support the importance of reestablishing a normal alveolar epithelial cell layer in

regulating fibrotic progression. Lungs that exhibit decreased rates of epithelial cell proliferation develop fibrosis, while lungs that rapidly reepithelialize revert to normal [83]. Similarly, utilizing diphtheria toxin-mediated depletion of airway progenitor (Clara) cells, Perl and colleagues [89] demonstrated that chronic depletion of Clara cells results in incomplete and aberrant reepithelialization of the bronchiolar airway and the development of peribronchiolar fibrosis, while acute depletion, which presumably leaves a reserve of Clara cell progenitors, results in normal reepithelialization and did not lead to fibrosis. Similarly, a fibrotic response results from daily administration of diphtheria toxin to injure SP-C expressing type II AEC in mice [82]. In contrast, repetitive injury to type II AEC every 2 weeks, which allows for recovery of cell populations, did not result in fibrosis [86]. These results suggest that allowing reepithelialization to occur may inhibit the development of fibrosis.

The receptor for advanced glycation end products (RAGE) is a transmembrane receptor that is a specific marker for differentiated type I epithelial cells [90]. The expression of RAGE in type I cells likely plays a role in their differentiation and homeostasis by promoting cell spreading and attachment to the basement membrane [91, 92]. IPF lungs demonstrate abnormally low expression of RAGE [92, 93] that is consistent with the presence of disrupted reepithelialization. Dysfunctional RAGE expression may also play a role in mediating the fibrotic process, however, as RAGE-null mice develop more severe experimental pulmonary fibrosis and spontaneously develop fibrotic-like lesions as they age [93].

Aging is likely an important contributor to deficient reepithelialization. During aging, somatic cells progressively lose telomere length [45, 94]. Loss of telomeres in progenitor populations of AEC contributes to cellular senescence, apoptosis, and diminished replicative capacity, thereby contributing to stem cell exhaustion [95].

Acquisition of aberrant epithelial cell phenotypes In addition to the morphologic alterations in AEC cells visible on histologic specimens from IPF lung, unbiased, single-cell transcriptional profiling of AECs from normal and IPF lung revealed an alteration in AEC phenotypes in IPF, with frequent co-expression of type I AEC, type II AEC, and conducting airway cell markers. This suggested that indeterminate or transitional epithelial phenotypes are common in IPF [96]. Several aberrant AEC phenotypes have been experimentally characterized and are discussed below.

Epithelial-mesenchymal transition (EMT) On a morphologic basis, AECs are present in IPF that have a flattened morphology, which may represent pro-migratory phenotypes that are attempting to reepithelialize the alveolar space after injury [35]. This morphology is similar to epithelial cells that are undergoing EMT. EMT is the process by which epithelial cells lose attributes of full epithelial differentiation (cuboidal shape, apical-basal polarization, cell-cell contacts, epithelial gene repertoire) and take on attributes of mesenchymal cell lineages (spindle morphology, loss of cell contacts, mesenchymal gene expression). EMT is accompanied by the loss of several epithelial markers such as E-cadherin, the acquisition of mesenchymal

markers N-cadherin and vimentin, and the upregulation of transcription factors implicated in EMT, such as Twist, SNAI1 (Snail), and SNAI2 (Slug) [97]. EMT is critical for gastrulation during embryogenesis [98], and epithelial cells that have undergone EMT have an augmented ability to metastasize [99]. Several forms of tissue injury and repair demonstrate the presence of EMT as part of their pathogenesis [100], and deletion of snail protects from the development of hepatic fibrosis [101], suggesting a mechanistic role in the propagation of tissue fibrosis. Tissue sections from established models of experimental pulmonary fibrosis, such as the bleomycin model [102], also demonstrate evidence of EMT [103–105], and lung tissue from patients with IPF demonstrates increased expression of Twist and Snail, suggesting the presence of EMT-associated signaling in human IPF and co-localization of epithelial and mesenchymal proteins within the same cell [105–108]. Single-cell sequencing of AECs also identified a population of cells that displayed co-expression of mesenchymal and AEC lineage markers [96]. These data suggest that EMT and associated signaling is present in IPF, and this may be the source of significant profibrotic signals.

Regulation of EMT during development is mediated, in part, by family members of the transforming growth factor-β superfamily of cytokines [98], which includes TGF-β1, TGF-β2, TGF-β3, and bone morphogenetic proteins (BMPs). TGF-β/BMP balance is important in the development of the mesodermal/epithelial compartment during development and regulates EMT [109, 110]. TGF-β induces EMT in both developmental and fibrotic contexts [111] and is a potent inducer of EMT in ex vivo epithelial cell cultures [112], although cell-contact and integrin-mediated signaling can modify this response [113, 114]. Several BMPs are implicated in the reverse process of mesenchymal to epithelial transition and can antagonize TGF-β-dependent signaling. Interestingly, the expression of two of these BMPs, BMP-2 and BMP-4, is altered in IPF [28], and the inhibitor of BMP signaling, gremlin, is increased in IPF lungs [115], implicating dysregulated TGF-β/BMP signaling balance in the pathogenesis of the disorder.

Senescence-associated secretory phenotype (SASP) Aging can intersect with injury to alter AEC phenotype via the induction of cell senescence. Whereas cell senescence plays an important embryologic and antineoplastic role in health, acquisition of a hypoproliferative, secretory phenotype SASP may be deleterious in certain contexts of tissue repair. Induction of lung injury results in the induction of cell senescence [116], aged mice have increased numbers of senescent cells in their lungs [117], and AEC in IPF lungs display markers of senescence [118, 119]. The SASP is associated with the elaboration of profibrotic mediators and matrix proteins that may perpetuate the fibrotic response [120]. Senescent cells are prone to persist in remodeled tissue, thereby contributing to the lack of resolution of the wound-healing response. Thus, these cells may be an attractive target for antifibrotic therapies.

Endoplasmic reticulum (ER) stress and disrupted proteostasis Type II AECs are highly metabolically active cells that continuously secrete proteins, including

surfactant proteins, into the alveolar space. To maintain this high level of secretory function, highly developed protein processing machinery is required. The ER is the subcellular site of initial posttranslational processing of secreted proteins. When an imbalance exists between the ability of the ER to sufficiently process the requisite amount of proteins to maintain cell homeostasis, there is activation of the unfolded protein response (UPR), and under certain conditions activation of pro-apoptotic pathways may occur. This pathway can be activated by the expression of misfolded surfactant proteins that are implicated in familial forms of pulmonary fibrosis [44, 121]. ER stress and chronic aggregation of misfolded proteins are present in AEC of lungs from patients with sporadic IPF, which is independent of known genetic defects [78]. Chaperone proteins serve an important function in protein folding in the ER. Loss of HSP70, an important chaperone protein, has been observed in IPF lung and may contribute to the activation of the UPR [122]. The ER stress markers, ATF4, ATF6, and CHOP, are preferentially localized to the epithelial cells of patients with sporadic IPF, in contrast to normal lungs or lungs with chronic obstructive pulmonary disease [123]. These changes are often localized to areas with significant fibrosis and co-localize with markers of apoptosis, suggesting a role in this process [123]. Furthermore, ER stress and UPR activation can also drive AECs toward a more mesenchymal morphology and gene expression repertoire (EMT) [124].

Aging contributes to the development of ER stress [125], potentially increasing the susceptibility to repetitive epithelial cell injury. A significant percentage of proteins are misfolded under normal conditions [126], and aging results in derangements in proteostasis with increases in misfolded proteins and oxidative damage. This leads to the accumulation of misfolded proteins with activation of the UPR.

With increases in misfolded proteins under conditions of cellular stress and with aging, the cell may try to compensate via catabolism and clearance of these proteins. Clearance of misfolded proteins occurs via the cell-regulated process of autophagy. Autophagy is a homeostatic function of the cell that allows for the degradation of proteins and thereby participates in maintaining proteostatic balance. Autophagy is a highly regulated process that leads to the formation of a specialized subcellular complex called the autophagosome. Unfortunately, aging is associated with deficient autophagy [127], and the IPF lung has evidence of abnormal autophagy [128, 129]. Aged mice have deficient autophagic responses to lung injury with a disproportionate targeting of mitochondria for autophagy (mitophagy), which can result in a further decrease in metabolic fitness [130]. Profibrotic signaling emanating from TGF-β inhibits autophagy via signaling through mTORC1, beclin1, and LC3 [131, 132], and genetically targeting a key component of autophagosome formation (ATG4B) results in accentuation of the fibrotic response in mice [133].

In addition to disproportionate loss of mitochondria due to mitophagy, aged AEC have increased amounts of dysmorphic and dysfunctional mitochondria [134]. Broad measures of mitochondrial fitness, such as ATP production, are also decreased with age. Mitochondrial dysfunction in aged animals is associated with increases in oxidative stress [135] and can lead to apoptosis of type II AEC [136]. Loss of sirtuin-3, a primary mitochondrial deacetylase that regulates mitochondrial integrity, results in increased AEC mitochondrial DNA damage and apoptosis [137].

As with aged cells, AECs in IPF lung have an increase in enlarged and dysmorphic mitochondria, which is associated with increased ER stress. In aged and IPF type II AEC, there is a decrease in the expression of protein phosphatase and tensin homolog-induced putative kinase 1 (PINK1), a kinase involved in the maintenance of mitochondrial homeostasis [134]. Loss of PINK1 in mice is associated with the induction of AEC ER stress and apoptosis, along with the development of spontaneous pulmonary fibrosis [134, 138].

Loss of homeostatic signaling and epithelial-mesenchymal cross talk Type II AECs maintain normal alveolar homeostasis via the production of surfactant, the regulation of fluid balance, and the interaction with other structural cells of the alveolus [139, 140]. Under normal conditions, AECs have an inhibitory effect on fibroblasts [141]. Thus, AEC dropout and failure to normally reepithelialize the airspace in IPF may lead to loss of inhibitory signaling from the AEC to the mesenchyme. One potential mediator of mesenchymal inhibition is prostaglandin E2 (PGE2). PGE2 is a product of cyclooxygenase and prostaglandin E synthases that is produced by local alveolar epithelial cells, monocytes, and other structural cells of the lung [142, 143]. PGE2 has shown to have an inhibitory effect on fibroblast proliferation [144, 145], migration [146], and collagen synthesis [147, 148]. In IPF, levels of PGE2 are decreased in bronchoalveolar lavage (BAL) [149], and EP2 prostaglandin receptor expression and signaling in fibroblasts are diminished [150]. Thus, AEC injury may result in the loss of the PGE2 production by AECs, leading to fibroblast activation during pulmonary fibrosis.

The WNT/β-catenin signaling pathway has been implicated in mediating altered epithelial cell function during lung injury and fibrosis. WNT/β-catenin signaling mediates branching morphogenesis during lung development and the maintenance of progenitor cells [151]. WNT proteins are secreted glycoproteins that can signal in a paracrine or autocrine fashion through their receptors (Frizzled proteins) and co-receptors (LRPs) to stabilize β-catenin, leading to its nuclear translocation. In the adult lung, WNT/β-catenin signaling is involved in epithelial cell proliferation, differentiation, and cell-cell adhesion in the lung [151, 152]. A common finding from recent unbiased gene expression screens of lung tissue from patients with IPF is that many developmental pathways are upregulated, including markers of the WNT/β-catenin pathway [28, 153–155]. The WNT genes, WNT2 and WNT5a, and the WNT receptors, Frizzled 7 and Frizzled 10, are increased in the lungs of patients with IPF [28, 153, 156]. Patients with IPF have increases in nuclear localization of β-catenin in the hyperplastic epithelium adjacent to fibrotic lesions [157] as well as increased phosphorylation of the Wnt/LRP receptors, suggesting activation of this pathway [158]. Consistent with the role of this pathway in pulmonary fibrosis, several WNT-/β-catenin-dependent genes are upregulated in IPF [154, 155, 159], and disruption of signaling via the WNT target gene, WNT-induced signaling protein (WISP), inhibited both markers of EMT and the development of fibrosis in response to bleomycin [159].

Finally, AECs are an important source of profibrotic mediators that can signal to the surrounding mesenchyme resulting in fibroblast recruitment and induction of

matrix production [36]. Several profibrotic growth factors are localized to the epithelial cells in IPF including TGF-β1 [160, 161], platelet-derived growth factor (PDGF) [162], monocyte chemoattractant protein-1 (MCP-1) [163], connective tissue growth factor (CTGF) [164], endothelin-1 [165], and tumor necrosis factor-α (TNF-α) [160, 166, 167]. AECs are also the source of several matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) that are implicated in IPF pathogenesis [168]. Experimentally, the induction of ER stress in AEC is associated with increased secretion of TGF-β [169], linking aberrant signaling from ER stress with a mechanism by which fibrotic responses can be promoted.

The Provisional Matrix and Coagulant Balance

The fibroblastic foci of UIP are found on the luminal side of the alveolar basement membrane in association with disruptions in the basement membrane [6]. These structures are morphologically analogous to the fibroblast collections that organize fibrinous alveolar exudates during the fibroproliferative phase of acute lung injury and the Masson bodies of organizing pneumonia. IPF lungs demonstrate evidence of endothelial injury, with swelling of endothelial cells and reduplication of the endothelial cell capillary basement membrane [170] and increased trans-endothelial permeability [171]. Interestingly, the degree of capillary permeability in IPF also correlates with prognosis [171, 172]. Animal models of pulmonary fibrosis indicate that vascular leak may be an important driver of the fibrotic response [173]. These observations suggest that the initial injury to the alveolar epithelial cell layer in IPF is accompanied by the exudation of serum-derived factors into the alveolar airspace to form the provisional matrix [6, 174]. Alveolar epithelial cells and macrophages express tissue factor [175, 176], which interacts with coagulation factors present in the alveolar exudate and activates the extrinsic coagulation pathway. Activation of the coagulation cascade results in the generation of thrombin, and subsequent thrombin-mediated conversion of serum-derived fibrinogen to fibrin forms the provisional matrix [177]. The provisional matrix also contains serum-derived fibronectin [6, 170] and growth factors, such as PDGF, facilitating subsequent fibroblast recruitment, migration, and matrix organization [178] (Fig. 7.2).

Stabilization of the nascent fibrin-containing provisional matrix in healing wounds would be predicted to require the presence of an increased procoagulant balance, as normal lung tissue expresses proteases such as the plasminogen activator, urokinase, that promote local fibrinolysis [179]. Immunohistochemical staining of IPF lungs demonstrates the deposition of fibrin localized in the alveolar space in areas adjacent to the epithelial cell layer [180], and BAL samples from patients with IPF demonstrate increased levels of plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2) and a reduction in urokinase activity [176, 181, 182], suggesting the presence of increased procoagulant balance.

Increased procoagulant activity also contributes to profibrotic signaling via the entrapment of serum-derived mediators present within the provisional matrix, forming a reservoir of growth factors that can be activated as the provisional matrix is remodeled [183]. The importance of procoagulant signaling is supported by studies in experimental models of pulmonary fibrosis. PAI-1-deficient mice are protected from the development of fibrosis, and the fibrotic response is potentiated by transgenic overexpression of PAI-1 [184].

Products of activation of the coagulation cascade, such as thrombin, also act as growth factors for fibroblasts. Thrombin is produced from the conversion of prothrombin to thrombin by Factor Va and Factor Xa and can signal through proteinase-activated receptors (PAR) found on epithelial cells and fibroblasts in the lung. Thrombin signaling occurs via proteolytic activation of its high-affinity receptor, PAR-1, leading to the expression of profibrotic cytokines, activation of TGF-β, and myofibroblast differentiation [177]. Germline deletion of the PAR1 receptor is protective against the development of bleomycin-induced pulmonary fibrosis [185]. Other coagulation proteinases may play a role in coagulation-dependent signaling as well. Factor X co-localizes to the alveolar epithelia of IPF lungs and can signal via PAR-1 [186]. Factor VIIa is also found in abundance on tissue biopsies from IPF lung, and in combination with tissue factor, Factor VIIa can mediate PAR-2-dependent proliferation of fibroblasts [187].

Despite the robust evidence supporting a key role for coagulation balance in the pathogenesis of fibrosis, a recent, large randomized clinical trial of systemic anticoagulation with warfarin for patients with IPF did not show a benefit, and the trial was terminated before completion due to increased deaths in the treatment arm [188]. Nonetheless, pharmacotherapy directed at specific coagulation cascade targets and coagulation-associated signaling remains a potential strategy for therapy.

Myofibroblasts: Effector Cells of Fibrosis

Concept of the myofibroblast The primary effector cell for connective tissue remodeling is the myofibroblast, a mechanically active, matrix-producing mesenchymal cell with distinct morphologic features that differ from normal resident fibroblasts. Myofibroblasts are characterized by the presence of large, bundled microfilaments and enlarged focal adhesions [189], and myofibroblast differentiation has been historically defined by the expression of both contractile proteins, such as the α-isoform of smooth muscle actin (α-SMA), and matrix proteins, such as collagens and the extra type III domain A (ED-A) splice isoform of fibronectin [190]. Myofibroblasts expressing α-SMA are not thought to be present in the normal tissue of the lung, although niche populations of microfilament containing α-SMA(−) myofibroblasts have been identified [191]. In contrast, α-SMA(+) myofibroblasts are invariably found in granulation tissue of wounds [192] and in scarring diseases that occur in other organs [193, 194]. Myofibroblasts act as central mediators of connective tissue remodeling via their production of matrix proteins,

pro- and anti-proteinase proteins, and modulation of matrix organization and tension [189, 195, 196]. Their presence in the lung is associated with the formation of a dense collagen matrix and progression of pulmonary fibrosis [197].

Origins of myofibroblasts The potential origins of myofibroblasts are diverse with several cellular precursors implicated in the expansion of the myofibroblast population during tissue fibrosis [189]. These include the resident fibroblasts of the alveolar interstitium, mesenchymal progenitor populations [198], alveolar epithelial cells that have undergone EMT, and circulating, bone marrow-derived progenitors that are termed "fibrocytes" [199].

Fibrocytes are circulating progenitor cells that express the hematopoietic surface antigens, CD34 and CD45, along with the fibroblast-associated proteins such as collagen I (Col I), collagen III, and collagen IV [200]. The cells were originally identified in a model of dermal wound healing [201] and are derived from bone marrow precursors [202]. Subsequently, studies using chimeric mice and bone marrow precursors tagged with green fluorescing protein (GFP) demonstrated the accumulation of GFP+, Col I+ cells in their lungs after the induction of bleomycin-induced pulmonary fibrosis [200, 203]. Fibrocytes express the chemokine receptor CXCR4, and fibrocyte recruitment to the lung is dependent on the CXCR4 receptor ligand, CXCL12 [204]. Several other studies in murine models of pulmonary fibrosis have demonstrated that circulating fibrocytes can express additional fibroblast-associated markers (S100A, vimentin, α-SMA) in association with their recruitment to the lung [104, 200, 204, 205]. However, conflicting data exist as to the potential of these cells to contribute to the myofibroblast (α-SMA expressing) population in vivo, with several studies demonstrating no evidence of an α-SMA+ fibrocyte population during experimental fibrosis [203, 206] and an inability of fibrocytes to express α-SMA [207]. Regardless of the ability of fibrocytes to become "fully differentiated" myofibroblasts, they may promote fibrosis via other paracrine effects, such as the production of profibrotic cytokines [208]. Fibrocytes and elevations in CXCL12 are present in the blood of patients with IPF [209] as well as in ex vivo preparations of lung specimens from patients with IPF [210]. Elevations in circulating fibrocytes are a marker of disease progression in human IPF [211], and neutralizing antibodies against CXCL12 ameliorate bleomycin-induced pulmonary fibrosis [204].

An additional hypothesized source of myofibroblasts is through the process of epithelial-mesenchymal transition. As previously discussed, substantial evidence supports the presence of aberrant epithelial signaling, including EMT-associated signaling, in IPF and experimental pulmonary fibrosis [108, 212]. Additionally, lineage marking techniques that can broadly label distal airway and AECs during gestation provide evidence that epithelial cells can express markers of mesenchymal cells during experimental lung fibrosis [103, 104]. In contrast, a more restricted lineage marking strategy of adult type II AEC cells or terminal bronchial epithelial cells found that no α-SMA+ cell population derives from these epithelial lineages in the bleomycin model of pulmonary fibrosis [85]. Additionally, in vitro work has demonstrated limitations in the ability of lung epithelial cells to contribute to the

collagen organization that comprises a stiff, remodeled matrix [213]. Discrepancies between these studies could be explained by technical differences in marking techniques or the presence of a discrete epithelial progenitor population that evaded lineage marking in the adult murine lung that could differentiate into type II cells or undergo EMT directly in response to injury [214]. Recent evidence supports the existence of such a population [215]. However, single-cell sequencing of IPF and normal lung found several abnormal epithelial cell populations in the lung, but no evidence of a population strongly co-expressing epithelial and myofibroblast markers was observed [96, 216]. Thus, while EMT-associated signaling programs are present in pulmonary fibrosis and appear to mediate important profibrotic cross talk between the epithelial and mesenchymal compartments, it is unlikely that epithelial-derived cells are a significant contributor to the contractile and matrix-producing cells of the parenchyma in pulmonary fibrosis.

These data suggest that the resident mesenchymal precursor cell population within the lung remains a predominant source of myofibroblasts during tissue fibrosis. The resident mesenchymal precursor population is a mixed population of several different mesenchymal cell subtypes important for the normal homeostatic maintenance and turnover of the lung connective tissue scaffold, and this cell population can proliferate and expand in response to injury [85, 140, 191, 217]. Upon exposure to profibrotic signals, such as cell, serum, or matrix-derived TGF-β, these cells can differentiate into myofibroblasts [218].

Upon expansion in response to pulmonary injury and fibrosis, the fibroblast population exhibits significant heterogeneity with the presence of several different subphenotypes [219, 220]. Myofibroblasts are defined by the expression of α-SMA and collagen production, but a significant subset lacks the cell surface marker Thy-1 [221], which correlates with a more fibrotic myofibroblast phenotype [222]. Xia and colleagues were able to isolate mesenchymal cell progenitors that shared features with mesenchymal stem cells but had retained differences in profibrotic features [198]. These profibrotic features were associated with the expression of the calcium-binding protein S100A4 [223]. Single-cell sequencing-based characterization of fibroblast populations in murine models of pulmonary fibrosis also demonstrates heterogeneity in the mesenchymal cell population that contributes to the fibrotic milieu [224, 225].

Aberrant fibroblast behavior Deranged fibroblast biology likely plays an important role in the propagation of pulmonary fibrosis by enabling a disproportionate and non-resolving fibrotic response to epithelial injury. Populations of lung fibroblasts isolated from patients with IPF demonstrate differences in global gene expression [226], proliferative capacity [46, 227], resistance to apoptosis [228], anchorage-independent growth [229], and deficits in translational control [230] when compared to normal lung fibroblasts.

The putative mechanisms mediating some of these disordered functions have begun to be elucidated. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a lipid/protein phosphatase that can act as a tumor suppressor via inhibition of phosphoinositide 3-kinase (PI3K)/Akt signaling pathway. Levels of PTEN

are nearly absent in the fibroblastic foci of IPF lungs and in ex vivo IPF fibroblast cultures, which stands in contrast to normal lung tissue and fibroblasts [227, 231]. Disordered PTEN activity in IPF fibroblasts conveys an abnormal proliferative response to polymerized collagen matrices via increases in PI3K/Akt signaling, and PTEN-deficient mice develop an accentuated fibroproliferative wound-healing response and more severe bleomycin-induced pulmonary fibrosis [227].

Caveolin-1 (cav-1) serves as a scaffolding protein and can inhibit the responses to growth factor signaling [232, 233]. The fibroblastic foci of IPF lungs lack cav-1 staining, and cav-1 expression by fibroblasts decreases in response to TGF-β. In contrast, overexpression of cav-1 disrupts TGF-β signaling and matrix protein induction, and its overexpression attenuates bleomycin-induced pulmonary fibrosis [234]. The loss of caveolin-1 in IPF myofibroblasts also results in decreases in PTEN expression [169].

Finally, myofibroblasts from IPF lungs manifest deficits in response to the anti-fibrotic cytokine, PGE2 [150], and the mechanism mediating PGE2 "resistance" has been linked to decreased expression of the PGE2 receptor, EP2, by IPF myofibroblasts [235].This is partially due to hypermethylation of the promoter region for the EP2 receptor, which leads to decreased EP2 expression [236].

Just as AECs demonstrate age-related changes that can contribute to the propagation of tissue fibrosis, resident lung fibroblast behavior can change with age. Age-related senescent fibroblasts are found in IPF lung, have increased secretion of inflammatory mediators, and can become resistant to apoptosis, thereby perpetuating the fibrotic response [216, 237, 238]. Aged-matched fibroblasts from IPF lung also have an observed increase in mitochondrial dysfunction, disrupted autophagy, and mitophagy [131, 238, 239].

Paracrine Mediators of Tissue Fibrosis

Growth factors TGF-β was one of the first cytokines implicated in the normal wound-healing response [240], and it plays a central role in the pathobiology of tissue fibrosis [241–243]. Patients with IPF have increased immuno-localization of TGF-β in epithelial cells, macrophages, and myofibroblasts in areas of active fibrosis (fibroblastic foci) [244, 245]. Inhibition of TGF-β signaling protects against fibrotic progression in experimental models of pulmonary fibrosis [241, 246, 247].

TGF-β is secreted as a latent protein that is dimerized and forms a complex with latent binding protein-1 (LTBP-1) via its latency-associated peptide (LAP) [248]. As part of this complex, it is tethered to matrix elements, such as fibrillin and fibronectin [249], and is unable to activate the TGF-β receptor on neighboring cells [250]. Activation of latent TGF-βs may occur via direct proteolytic cleavage by several proteinases, including MMP-2 and MMP-9, or via interactions with α_v-containing integrins [251]. In the lung $\alpha_v\beta_6$ integrins expressed on the surface of epithelial cells bind the LAP of the latent TGF-β complex and facilitate its activa-

tion by G-protein-coupled receptor agonists such as thrombin and lysophosphatidic acid [252, 253] via the generation of cell-mediated mechanical tension. The application of tension to the $\alpha_v\beta_6$ integrin releases TGF-β from the latent complex, thereby allowing it to interact with its cognate receptor complex on the surface of adjacent cells (such as fibroblasts) [254].

The TGF-β receptor complex is a heterodimer comprised of a TGF-β type I receptor (TGF-βR1) and a type II receptor (TGF-βR2) with TGF-βR1 having serine-threonine kinase activity. Upon activation, TGF-βR1 phosphorylates receptor-activated SMAD effector proteins (SMAD2 and SMAD3) resulting in association with the common mediator SMAD (SMAD4) and translocation to the nucleus with activation of SMAD target genes. Signaling via this pathway appears to be critical during fibrogenesis, as SMAD3 null mice are protected from experimental pulmonary fibrosis [241], and depletion of the high-affinity type II TGF-beta receptor in resident fibroblasts inhibits experimental pulmonary fibrosis [255].

TGF-β receptor activation also results in the activation of several noncanonical signaling pathways that promote myofibroblast differentiation and resistance to apoptosis. Activations of mitogen-associated kinase pathways [256] that include TGF-activated kinase [257], PTEN/PI3kinase/Akt [258], focal adhesion kinase [259, 260], the tyrosine kinase c-Abelson [261], the small GTPase rho/cytoskeletal-dependent signals [262, 263], oxidant-mediated signaling [264], and others have been identified as downstream targets of TGF-β. Activation of these pathways results in cell shape change and the regulation of gene programs mediating fibroblast phenotype and survival [265, 266].

Functionally, TGF-β results in pleotropic effects that promote a coordinated fibrotic response. Treatment of AECs with TGF-β can result in the induction of apoptosis or the induction of EMT, depending on the matrix substrate to which they are exposed [103, 113]. In fibroblasts, TGF-β results in myofibroblast differentiation [218], apoptosis resistance [266], and marked upregulation of the expression of matrix components [190, 267, 268]. Finally, TGF-β mediates the epigenetic regulation of gene expression via the induction of several microRNAs that mediate the fibrotic response, and these microRNAs are also differentially regulated in IPF and include mir-21 and let-7d [269, 270].

Lysophosphatidic acid (LPA) is a lipid-derived mediator that can be produced by platelets, membrane phospholipids, and lung surfactant [271], and LPA signals through several G-protein-coupled receptors to exert its biologic effects. In the context of pulmonary fibrosis, LPA appears to promote the fibrotic response via induction of epithelial cell apoptosis [272], increased endothelial cell permeability [173], and increased fibroblast migration [173, 273] and survival [272]. Elevated levels of LPA have been found in BAL fluid from patients with IPF, and LPA_1 receptor knockout mice are protected from the development of pulmonary fibrosis [173].

Multiple other growth factors including endothelin-1 [274], angiotensin II [275], PDGF [276], and transforming growth factor-α [277] have been identified as playing a role in the fibrotic response, are implicated in IPF pathogenesis, and may serve as targets for therapy.

Immune cells and inflammatory mediators Early studies in IPF lungs identified significant alteration in levels of several cytokines and chemokines typically involved in mediating the inflammatory response. Despite the lack of therapeutic benefit to broad immunosuppression in IPF, inflammatory cells and their associated signaling may still play a role in the pathobiology of IPF, potentially via the modulation of the fibrotic response. BAL neutrophilia is associated with a worse prognosis [278], and mRNA profiling of IPF monocytes reveals upregulation of several markers of macrophage activation [279]. Macrophage populations appear to be important in mediating the wound-healing response, in part by contributing soluble factors to fibroblast activation [145]. The inflammatory cytokines, TNF-α and interleukin-1β (IL-1β), are both localized to epithelial cells at sites of fibrosis in IPF [167, 280] and are released by alveolar macrophages obtained from patients with IPF or asbestosis [281]. Similarly, a downstream target of IL-1β, interleukin-17A (IL-17A), is increased in the BAL fluid of patients with IPF and mediates the fibrotic response to bleomycin in a murine model [282]. Markers of the Th2 immune response, including interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13), also have been found in increased levels in the interstitium of patients diagnosed with cryptogenic fibrosing alveolitis [283]. IL-13 plays a key role in inducing Th2 responses in the lung in chronic inflammation [284], and IL-13 levels and IL-13 receptor expression correlate with disease severity [285].

Chemokines play a role in IPF via the recruitment of monocytes, leukocytes, and fibrocytes to the injured lung, and chemokines are involved in the angiogenic remodeling that occurs in fibrotic lung disease. CCL-12 and its receptor CXCR4 are strongly implicated in fibrocyte recruitment to the lung [200] along with MCP-1 and its receptor CCR2 [286]. Macrophage inflammatory protein-1α (MIP-1α) and MCP-1 are increased in tissue and BAL [287–292] in human IPF and likely participate in macrophage recruitment, which can amplify the fibrotic response via production of profibrotic cytokines and recruitment of additional inflammatory cells via chemokines [293]. Production of CCL-18 by macrophages has also been implicated in the progression of pulmonary fibrosis, and circulating levels of CCL-18 correlate with survival in IPF [294]. Conversely, macrophages may facilitate resolution of the fibrotic response via phagocytosis of apoptotic cells and the production of matrix metalloproteinases [284]. Alternatively activated macrophages, which represent the majority of macrophages in IPF lungs, may play a role in this process, as depletion of this cell cohort attenuates the fibrotic response in bleomycin-induced pulmonary fibrosis [295].

Several unbiased assessments of genetic alterations in pulmonary fibrosis have renewed interest in derangements in innate immune signaling in IPF. TOLLIP is a key signaling component of the innate immune system and has a single nucleotide variant (rs5743890) that is associated with both susceptibility to developing IPF and worse outcome [64]. In addition, polymorphic variants in Toll-like receptor 3 and 9 have also been identified as risk factors for IPF progression [296, 297]. The innate immune system may play an important role in the response to injury via recognition of danger- and pathogen-associated molecular patterns (DAMPs and PAMPs) by

pattern recognition receptors. Alteration of the lung microbiome in IPF could be an important source of PAMPs with subsequent activation of the innate immune system and increased mortality risk [81, 298], while cell debris, collagen fragments, and mitochondrial DNA may play a role as DAMPs in the injured and fibrotic lung.

With respect to the adaptive immune system, reduction in the T-cell regulatory genes, CD28, ICOS, and the tyrosine kinases LCK and ITK is predictive of poor outcome in IPF [299, 300]. GWAS-based studies have identified an association between the human leukocyte antigen (HLA) region and the development of fibrotic idiopathic interstitial pneumonias [301]. A recent proteomic analysis of IPF lung identified the presence of a plasma B-cell population that was not seen in normal lung [302].

Despite the abundance of inflammatory mediators and immune cell types that have been implicated in IPF pathogenesis, much work remains to be done to determine how these varied pathways intersect with other components of the fibrotic process. An improved understanding of these interactions may allow for a more rational approach to targeting these pathways for therapeutic benefit in the future.

Tissue Remodeling and Failure to Resolve the Wound

Angiogenesis, the formation of new blood vessels, is an important component of the wound-healing response in several tissue beds. In dermal wounds the angiogenic response potentiates the influx of inflammatory mediators that participate in the tissue remodeling process. Insofar as the pathobiology of IPF is an extrapolation of many of the mechanisms that mediate other forms of wound healing, it would not be surprising to detect an angiogenic response. Indeed, pathologic evaluation of the IPF lung has demonstrated areas of neovascularization as well as the presence of pulmonary-systemic anastomoses that are often seen in a subpleural location [303]. Additionally, circulating levels of the angiogenic cytokines, interleukin-8 (IL-8/CXCL8) and endothelin-1, are elevated in patients with IPF compared to normal controls and correlate with disease progression [304].

However, there is significant spatial heterogeneity of neovascularization and vascular density in IPF tissue biopsies when compared to normal lungs. When carefully quantified using endothelial cell markers, the level of neovascularization present within an area of IPF lung is inversely correlated with degree of parenchymal fibrosis in that area [305–307]. Furthermore, complete vascular obliteration is often seen in areas of dense parenchymal fibrosis. Most often, areas of neovascularization are present adjacent to intact AECs, suggestive of an angiogenic response that attempts to reestablish the normal alveolar/capillary interface [306]. This suggests significant spatial heterogeneity to the angiogenic response in IPF with areas of angiogenic signaling alternating with areas defined by angiostatic signaling.

Corroborating these observations, the angiostatic cytokine, endostatin, was found to be elevated in the serum of IPF patients [308], while serum levels of vascular endothelial growth factor (VEGF) have been observed to be decreased.

Clarifying the issue significantly, it has been shown that local VEGF expression is absent in areas of dense fibrosis, while the angiostatic protein, pigment epithelium-derived factor (PEDF), which is a VEGF antagonist, has increased expression in the fibroblastic foci of IPF lungs [309]. PEDF is a TGF-β target gene, suggesting that the local environment of the fibroblastic focus is characterized by an angiostatic environment. Whether the angiostatic environment of areas of fibrosis is cause or consequence of the fibrotic response is unclear. Similarly, the role of the scattered areas of neovascularization in adjacent lung tissue remains undetermined.

Role of matrix remodeling on progression of fibrosis The normal matrix environment is maintained by the constant and tightly regulated control of cell activation, matrix production, and extracellular matrix (ECM) proteolysis in order to maintain "normal" lung architecture [5]. As tissue fibrosis proceeds, matrix organization is severely altered with increased accumulation of multiple matrix components that include extra domain-A (EDA) fibronectin, hyaluronic acid, and collagen isoforms. In response to TGF-β, other growth factors, and environmental cues, collagen synthesis is induced and secreted by fibroblasts and myofibroblasts. Collagens are secreted as a soluble promolecule that subsequently self-assembles to form insoluble collagen fibrils that are relatively resistant to degradation by proteases [168]. Studies of the collagen content of IPF lungs have demonstrated that collagen III is the primary component in areas of alveolar septal fibrosis with collagen I predominating in areas of mature fibrosis [310, 311].

Extracellular matrix turnover is tightly regulated by several families of proteinases and their respective inhibitors [22]. Matrix metalloproteinases (MMPs) comprise a family of proteinases that can target collagen and other matrix components for degradation. Given the role of these molecules in maintaining the balance of matrix molecules during normal tissue homeostasis, a defect in the balance of these factors might be expected in disorders such as IPF that are characterized by matrix accumulation. In line with this expectation, several tissue inhibitors of metalloproteinases (TIMPs) are locally expressed in pulmonary fibrosis [312], and overall collagenase inhibitory activity is elevated in IPF patients when compared to controls [313]. However, total collagenase activity is increased in IPF as well [314], and several matrix metalloproteinases including MMP-1, MMP-2, and MMP-7 have been identified as highly enriched genes in the tissue from IPF lungs [153, 315]. Interestingly, an assessment of global gene expression in IPF lungs found a strong bias toward increased protease expression, which supports a net degradative environment [316]. Given this observation, the effects of spatial localization of protease/antiprotease expression likely predominate over global assessments of protease/antiprotease "balance."

Analysis of MMP expression demonstrates the importance of spatial localization in IPF. MMP-1 is increased in IPF [153] and localizes to the alveolar epithelium [312] rather than the fibroblastic focus, where it participates in the processing of cytokines, which stands in contrast to its role in collagen fibril degradation [168]. MMP-7 (matrilysin) is a highly upregulated gene in IPF lungs when compared with

control samples, and the degree of MMP-7 elevation in BAL fluid from IPF patients correlates with survival [172]. MMP-7 also localizes to the alveolar epithelial cells [154], but it has diverse roles that are relevant to tissue remodeling, and these roles are distinct from its degrading effect on matrix proteins. In particular, MMP-7 can activate other MMPs, regulate TGF-β activation, and activate osteopontin [155, 317]. MMP-2 is a gelatinase that targets collagen IV as a substrate [318], and it is increased in the BAL fluid from IPF patients [312] and has been localized to AECs [319, 320], where it may contribute to alveolar basement membrane degradation. MMP-9 is also expressed by epithelial cells and inflammatory cells [321], has increased expression in patients with IPF [322], and has been associated with increases in endothelial permeability, neutrophil activation, and rapidly progressive disease [172, 323]. TIMPs also have differential localization with TIMP-2 predominating in the fibroblastic foci, where it may facilitate matrix stabilization and accumulation [312].

MMPs can also modify the matrix remodeling response via the cleavage of matrix proteins, which yields fragments that can act as cell signaling ligands [22]. Additionally, MMPs and TIMPs can themselves mediate profibrotic signaling via proteolytic activation of growth factors, chemokines, and shedding of membrane-associated ligands [318]. These profibrotic effects of MMPs may predominate in IPF, making inferences concerning the net effect of increased MMP expression on matrix accumulation difficult.

Matrix composition and organization plays a key role in modifying cell behavior, and dysregulation of matrix cues has been implicated in various disease states including tumor progression [324]. In the context of IPF, individual ECM components can significantly modify the response to soluble and matrix-derived mediators. For example, primary AECs cultured on fibrinogen or fibrin and treated with TGF-β will undergo EMT, while the same cells when cultured on Matrigel (collagen and laminin) and treated with TGF-β will undergo apoptosis [103]. Myofibroblast differentiation is also dependent on the presence of several matrix cues. EDA-FN is preferentially expressed in healing wounds, and its presence is required for TGF-β-induced myofibroblast differentiation [190]. Mice deficient in this isoform are protected from bleomycin-induced pulmonary fibrosis [325]. De novo expression of the matrix protein, periostin, has been implicated in the fibrotic remodeling that occurs with asthma [326]. Periostin is also highly expressed in the fibroblastic foci and serum of patients with IPF [327], and periostin-deficient mice are protected from bleomycin-induced pulmonary fibrosis [328]. Increased expression of matrix-associated proteoglycans, such as hyaluronic acid, participates in the fibrotic process, which likely occurs via recruitment of inflammatory cells and the facilitation of fibroblast migration through cognate receptors such as CD44 [329]. Thus, while the in vivo details of matrix-dependent signaling are currently lacking, it is likely that altered expression of these and other matrix components facilitate and perpetuate the fibrotic response in IPF.

Incorporation of new matrix elements is not merely a result of haphazard matrix protein accumulation but proceeds in an orderly fashion [21]. Newly synthesized fibronectin is desolubilized by integrin-mediated incorporation [106] and serves as

a scaffold for collagen and other matrix protein deposition [330]. Newly deposited collagen and elastin are cross-linked via the action of tissue transglutaminases and lysyl oxidases [331], which increases tissue stiffness. In IPF, lysyl oxidase 2 (LOXL2) is increased in the fibroblastic foci. However, directly inhibiting its activity using a monoclonal antibody did not prevent progression of IPF. Similarly, tissue transglutaminase 2 expression and activity is upregulated in IPF, and germline knockout of this protein prevents the development of experimental pulmonary fibrosis [332].

Alterations in the biomechanical characteristics of the ECM during fibrosis, such as increased tissue elasticity (stiffness), can independently modify cell behaviors and phenotype determination. Tissue stiffness is quantified by its shear modulus, which is typically determined via atomic force microscopy [333]. Careful determinations have demonstrated that normal lung tissue has a shear modulus of 0.5 kPa, whereas the median shear modulus in fibrotic lung increases to 6 kPa [334]. However, significant spatial heterogeneity of tissue stiffness exists within the fibrotic lung with uninvolved areas retaining a near normal shear modulus but areas of dense fibrosis having a shear modulus that surpasses 15 kPa.

All cell types likely sense and respond to alterations in the biomechanical features of the matrix [335]. The development of tension across a healing wound modifies myofibroblast differentiation [336, 337], and release of this tension leads to the induction of myofibroblast apoptosis [338]. Similarly, stiff matrices induce fibroblast to myofibroblast transition [339, 340], which is accompanied by the augmentation of matrix protein expression [334]. The development of matrix tension and stiffness also modifies cellular responses to TGF-β. TGF-β bioavailability is directed and modified by the transmission of tension to its associated LTBP via α_v-containing integrins [341, 342]. Therefore, myofibroblast differentiation induced by soluble TGF-β requires the development of matrix-derived tension across the cell [343, 344].

Functionally, increases in matrix stiffness that mimic fibrotic lung result in augmentation of traction forces by lung fibroblasts in response to TGF-β, whereas normal matrix stiffness does not [345]. Epithelial cells toggle their response to TGF-β stimulation that is dependent on the matrix stiffness of their environment, undergoing apoptosis on low-stiffness substrates but EMT on high-stiffness substrates [346]. Some matrix stiffness-dependent effects on cells may be durable, as fibroblasts retain the "programmed" behavior imparted by culture on a stiff matrix, even after subsequent prolonged culture on matrix with "normal" stiffness [347]. Similarly, adoptive transfer of lung fibroblasts from patients with pulmonary fibrosis induces the development of fibrotic lung lesions in mice, while those from normal lungs do not [297, 348]. The acquisition of these durable aberrant behaviors from the matrix environment may be due to epigenetic "programming," although this has not been formally demonstrated as of yet.

These observations strongly suggest that the ECM and its cellular constituents participate in a reciprocal signaling fashion during fibrosis that provides a "feed-forward" mechanism that promotes progression of fibrosis. How matrix-derived signaling varies between fibrosis and normal wound healing remains an open area of inquiry.

New Directions and Targets for Therapy

Current strategies to stop IPF progression rely heavily on the mechanistic understanding of fibrosis that has been reviewed thus far (Table 7.1). However, only two therapies (nintedanib and pirfenidone) have had successful Phase III trials that have led to FDA approval for therapy (see Chap. 13). Many additional drugs have been investigated that target known mechanisms of fibrosis, but to date these therapies have either failed to attain the desired endpoint or are still in the developmental pipeline (Table 7.1). New advances in the understanding of IPF pathogenesis will be essential for the next generation of therapies to be developed. The past decade has seen the advent of the use of unbiased GWAS investigations as well as studies of RNA profiles (mRNA, splice isoforms, microRNA, long noncoding RNA), protein expression (proteomics), epigenetic alterations (epigenomics), and metabolic alterations (metabolomics) in human samples from patients with IPF. These analyses have provided investigators with powerful new tools that facilitate pathway discovery for complex disorders such as IPF. Single-cell sequencing has more recently

Table 7.1 Mechanistic targets of therapy for IPF

	Drug		
Mechanistic target	Ineffective	Under evaluation (phase II/III)	Effective
Unknown			Pirfenidone
Receptor tyrosine kinases (VEGFR, PDGFR, FGF)			Nintedanib
Receptor Tyrosine Kinases (PDGFR, DDRs, c-Kit, c-Abl)	Imatinib		
Immune response	Prednisone Azathioprine		
Antioxidant	N-Acetylcysteine		
Coagulation	Warfarin		
TNF-α	Etanercept		
Interferon γ-1b	Interferon γ-1b		
Endothelin-1 receptor	Bosentan Ambrisentan		
LPA1 receptor		BMS-986020	
Autotaxin		GLPG1690	
Interleukin-13	Tralokinumab	Lebrikizumab	
CTGF		Pamrevlumab	
Avβ6 (via TGF-β release)		STX-100	
LoxL2 (matrix cross-linking)	AB0023		
Serum amyloid P (macrophage function)		PRM-151	
Fatty acid receptors (GPR84, GPR40)		PBI-4050	
Microbiome		Co-trimoxazole	

emerged and holds the promise of dramatically improving the resolution of the changes that occur in the underlying cell populations during the induction and progression of pulmonary fibrosis, and this novel technique may inform the discrete function of gene expression changes that are associated with IPF pathogenesis. When coupled with the various mechanistic investigations, these methodologies have opened the door for biomarker development and novel approaches to therapy.

RNA expression profiling Initial investigations of RNA expression focused on mRNA to determine how global expression profiles differed between IPF and normal lung as well as between IPF and other forms of interstitial lung disease (ILD). These studies found increased gene expression of matrix metalloproteinases, developmental signals, adhesion proteins, extracellular matrix proteins, and muscle-related proteins present in IPF lungs, when compared to normal lungs or other ILDs [106, 153, 154, 349]. Subsequent global analysis of the cumulative datasets demonstrated that WNT and TGF-β signaling pathways are highly enriched in IPF lungs [28]. Moreover, recent work has uncovered differences in mRNA expression profiles between sub-phenotypes of IPF and demonstrated distinct patterns of gene expression in IPF patients with secondary pulmonary hypertension [350], those with more progressive IPF [351–353], and patients with acute exacerbations of IPF [354].

In addition to providing insight into disease pathogenesis, a major potential use of gene expression profiling is the development of diagnostic, prognostic, and disease activity biomarkers. Several candidate biomarkers have been identified [355], but validation of these approaches and translation to clinical practice remains a future goal.

Proteomics Several studies have now been completed that have used comparative proteomics to examine lungs from patients with IPF versus lung tissues from untransplanted human donor lungs and found evidence of DNA damage stress responses, UPR, and upregulation of heat-shock proteins in the IPF lung [356, 357] Additionally, deep proteome profiling identified a unique B-cell type that was only present in IPF lungs [302]. Proteomic analysis has also been performed on peripheral blood plasma and identified alterations in host defense, wound healing, and protein phosphorylation in IPF samples, and these investigators were able to identify a minimal gene signature that was highly accurate in differentiating IPF patients from normal controls [358].

Epigenetic Regulation From a mechanistic perspective, the evolving understanding of epigenetic regulation of gene expression has opened a new area of investigation into the pathogenesis of pulmonary fibrosis (see Chap. 9). Epigenetic gene regulation refers to regulation of gene expression that occurs outside of changes in DNA germline coding and occurs via three main mechanisms: histone modifications, DNA methylation, and the effects of noncoding RNAs (microRNAs).

Unbiased oligonucleotide microarray screens to determine microRNA expression profiling have demonstrated that approximately 10% of microRNAs are

differentially regulated in IPF [27]. RNA expression profiles of noncoding RNA have been more recently characterized for IPF with identification of many developmentally associated microRNAs and regulators of TGF-β signaling [359, 360]. The first reports of differentially regulated microRNAs in IPF focused on let-7d [269] and mir-21 [270]. Let-7d is a microRNA that is downregulated by TGF-β and is decreased in the lungs of patients with IPF [269]. Let-7d is localized to the alveolar epithelium in normal lungs, and it is involved in the regulation of EMT [269]. Mir-21 expression is induced by TGF-β, and elevated levels of mir-21 are found in the lungs of IPF patients as compared to controls [270]. In contrast to let-7d, mir-21 localizes to myofibroblasts and is known to mediate many of the effects of TGF-β including the regulation of PTEN expression [361]. Mir-29 is downregulated in pulmonary fibrosis [362], and it is also responsive to TGF-β and matrix-derived mechanical cues [363].

Investigators are now beginning to use transcriptional profiling of individual cell populations using single-cell RNA sequencing, which has now been used to begin to characterize the cell population differences between normal and IPF lung. This technique has identified distinct shifts in the gene expression profiles of epithelial cell populations in fibrotic lung and provided evidence of activation of TGF-β, HIPPO/YAP, WNT, and AKT signaling localized to this compartment [96]. Forthcoming studies looking at other cell populations (immune, mesenchymal, etc.) should provide new insights into cell lineage-specific gene expression changes that are associated with pulmonary fibrosis.

An alternative mode of epigenetic regulation occurs via acquired DNA modifications in somatic cells that can "program" gene expression and pass on this information to daughter cells. One of the most common modifications that can alter gene expression is gene silencing by methylation at CpG islands [364]. Hypo- and hypermethylation of critical genes have been implicated in the development of cancers [365], but only limited investigations have been published in tissue fibrosis until recently. In the context of pulmonary fibrosis, widespread alterations in epigenetic patterning are present in IPF [366, 367], and there is upregulation of DNA methyltransferase 3a in the hyperplastic epithelium of IPF lung [367]. Several fibroblast-related genes exhibit hypermethylation and silencing in fibrosis, including the prostaglandin E2 receptor (PTGER2) [236], IP-10 [368], and Thy-1 [369], while the α-SMA promoter is hypermethylated at several CpG islands in epithelial cells, but decreased methylation was found in fibroblasts [370].

These results suggest that IPF is characterized by severe derangements in the regulatory control of gene expression, and much work remains to be done to achieve an understanding of the origins and implications of many of these observations on the mechanism of disease pathogenesis in IPF. However, several of these technologies have exciting therapeutic and diagnostic potential. The identification of key gene expression profiles may lead to the development of individual biomarkers or gene sets that may obviate the need for a surgical lung biopsy, allow for more precise identification of IPF sub-phenotypes, and identify patients at high risk for disease progression [299, 353, 371]. The identification of key microRNAs involved in IPF may allow for a novel mode of targeting deranged signaling in IPF, as a single

microRNA can target many different genes from divergent signaling pathways. Implementation of this strategy will require a more detailed understanding of the relationship of the downstream signaling pathways along with the development of drug delivery technology that utilizes this novel mode of targeting.

Summary

IPF is a disorder characterized by the presence of extensive alveolar epithelial cell injury accompanied by a robust, non-resolving wound-healing response. Robust investigations in familial cohorts of patients with FPF and sporadic IPF patients strongly suggest that genetic AEC susceptibility to injury (telomeres, MUC5B, etc.) compounded by age-related changes set the stage for exogenous stimuli to chronically injure the AEC, thereby initiating the fibrotic response. AEC injury and failure to reepithelialize the airspace perpetuate the fibrotic response in concert with TGF-β activation and WNT signaling. The reparative response in IPF is characterized by activation of the coagulation cascade, formation of a provisional matrix, and expansion of local progenitor populations for fibroblasts, all of which lead to myofibroblast populations that comprise the fibroblastic focus. Deposition and remodeling of ECM yield a stiffened, fibrotic matrix that feeds forward to perpetuate the fibrotic response. Myofibroblasts of the fibroblastic focus show evidence of resistance to apoptosis, senescence, and metabolic derangements that likely impair the normal resolution of the fibrotic response. Alterations in immune cell phenotype and function also contribute to abnormal repair.

While an effective therapy for IPF remains elusive, approaches to therapy have begun to evolve toward targeted therapies directed at the putative growth factors, receptors, and enzymes for which robust evidence for mechanistic involvement in matrix remodeling has evolved. New approaches that encompass high-throughput but also allow high-resolution assessments of the cell heterogeneity and gene expression in IPF should help identify additional targets for therapies that can halt fibrotic progression and promote reparative responses that restore tissue integrity and function.

References

1. Martin P. Wound healing – aiming for perfect skin regeneration. Science (New York, NY [Research Support, Non-U.S. Gov't Review]). 1997;276(5309):75–81.
2. Beers MF, Morrisey EE. The three R's of lung health and disease: repair, remodeling, and regeneration. J Clin Invest [Research Support, N.I.H., Extramural Review]. 2011;121(6):2065–73.
3. Shaw TJ, Martin P. Wound repair at a glance. J Cell Sci [Research Support, Non-US Gov't Review]. 2009;122(Pt 18):3209–13.
4. West JB, Mathieu-Costello O. Structure, strength, failure, and remodeling of the pulmonary blood-gas barrier. Annu Rev Physiol [Review]. 1999;61:543–72.

5. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. J Cell Sci [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S. Review]. 2010;123(Pt 24):4195–200.
6. Kuhn C 3rd, Boldt J, King TE Jr, Crouch E, Vartio T, McDonald JA. An immunohistochemical study of architectural remodeling and connective tissue synthesis in pulmonary fibrosis. Am Rev Respir Dis [Research Support, U.S. Gov't, P.H.S.]. 1989;140(6):1693–703.
7. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. Am J Respir Crit Care Med [Review]. 1998;157(4 Pt 1):1301–15.
8. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med. 2000;161(2 Pt 1):646–64.
9. 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.
10. Cool CD, Groshong SD, Rai PR, Henson PM, Stewart JS, Brown KK. Fibroblast foci are not discrete sites of lung injury or repair: the fibroblast reticulum. Am J Respir Crit Care Med [Comparative Study In Vitro Research Support, N.I.H., Extramural]. 2006;174(6):654–8.
11. King TE Jr, Schwarz MI, Brown K, Tooze JA, Colby TV, Waldron JA Jr, et al. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med [Comparative Study Research Support, U.S. Gov't, P.H.S.]. 2001;164(6):1025–32.
12. Nicholson AG, Fulford LG, Colby TV, du Bois RM, Hansell DM, Wells AU. The relationship between individual histologic features and disease progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2002;166(2):173–7.
13. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest [Review]. 2007;117(3):524–9.
14. Keogh BA, Crystal RG. Alveolitis: the key to the interstitial lung disorders. Thorax [Review]. 1982;37(1):1–10.
15. Crystal RG, Bitterman PB, Rennard SI, Hance AJ, Keogh BA. Interstitial lung diseases of unknown cause. Disorders characterized by chronic inflammation of the lower respiratory tract (first of two parts). N Engl J Med [Review]. 1984;310(3):154–66.
16. Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med [Comparative Study Multicenter Study Randomized Controlled Trial Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2012;366(21):1968–77.
17. Davies HR, Richeldi L, Walters EH. Immunomodulatory agents for idiopathic pulmonary fibrosis. Cochrane Database Syst Rev [Meta-Analysis Review]. 2003;3:CD003134.
18. Richeldi L, Davies HR, Ferrara G, Franco F. Corticosteroids for idiopathic pulmonary fibrosis. Cochrane Database Syst Rev [Review]. 2003;3:CD002880.
19. Thannickal VJ, Toews GB, White ES, Lynch JP 3rd, Martinez FJ. Mechanisms of pulmonary fibrosis. Annu Rev Med. 2004;55:395–417.
20. Selman M, King TE, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med [Consensus Development Conference Review]. 2001;134(2):136–51.
21. Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci [Review]. 2008;121(Pt 3):255–64.
22. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 2011;3(12):a005058.
23. Clark RA. Fibrin and wound healing. Ann N Y Acad Sci [Research Support, U.S. Gov't, P.H.S. Review]. 2001;936:355–67.
24. Zoz DF, Lawson WE, Blackwell TS. Idiopathic pulmonary fibrosis: a disorder of epithelial cell dysfunction. Am J Med Sci [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. 2011;341(6):435–8.

25. Selman M, Pardo A. Role of epithelial cells in idiopathic pulmonary fibrosis: from innocent targets to serial killers. Proc Am Thorac Soc [Review]. 2006;3(4):364–72.
26. Macneal K, Schwartz DA. The genetic and environmental causes of pulmonary fibrosis. Proc Am Thorac Soc. 2012;9(3):120–5.
27. Pandit KV, Milosevic J, Kaminski N. MicroRNAs in idiopathic pulmonary fibrosis. Transl Res [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 2011;157(4):191–9.
28. Selman M, Pardo A, Kaminski N. Idiopathic pulmonary fibrosis: aberrant recapitulation of developmental programs? PLoS Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2008;5(3):e62.
29. Mason RJ. Biology of alveolar type II cells. Respirology [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S. Review]. 2006;11(Suppl):S12–5.
30. Adamson IY, Bowden DH. The pathogenesis of bleomycin-induced pulmonary fibrosis in mice. Am J Pathol. 1974;77(2):185–97.
31. Bhaskaran M, Kolliputi N, Wang Y, Gou D, Chintagari NR, Liu L. Trans-differentiation of alveolar epithelial type II cells to type I cells involves autocrine signaling by transforming growth factor beta 1 through the Smad pathway. J Biol Chem [Research Support, N.I.H., Extramural]. 2007;282(6):3968–76.
32. Myers JL, Katzenstein AL. Epithelial necrosis and alveolar collapse in the pathogenesis of usual interstitial pneumonia. Chest [Case Reports]. 1988;94(6):1309–11.
33. Barbas-Filho JV, Ferreira MA, Sesso A, Kairalla RA, Carvalho CR, Capelozzi VL. Evidence of type II pneumocyte apoptosis in the pathogenesis of idiopathic pulmonary fibrosis (IFP)/usual interstitial pneumonia (UIP). J Clin Pathol [Research Support, Non-U.S. Gov't]. 2001;54(2):132–8.
34. Kawanami O, Ferrans VJ, Crystal RG. Structure of alveolar epithelial cells in patients with fibrotic lung disorders. Lab Investig. 1982;46(1):39–53.
35. Brody AR, Soler P, Basset F, Haschek WM, Witschi H. Epithelial-mesenchymal associations of cells in human pulmonary fibrosis and in BHT-oxygen-induced fibrosis in mice. Exp Lung Res [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1981;2(3):207–20.
36. Kasper M, Haroske G. Alterations in the alveolar epithelium after injury leading to pulmonary fibrosis. Histol Histopathol [Research Support, Non-U.S. Gov't Review]. 1996;11(2):463–83.
37. Nogee LM, Dunbar AE 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. N Engl J Med [Case Reports Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2001;344(8):573–9.
38. Thomas AQ, Lane K, Phillips J 3rd, Prince M, Markin C, Speer M, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2002;165(9):1322–8.
39. Mulugeta S, Maguire JA, Newitt JL, Russo SJ, Kotorashvili A, Beers MF. Misfolded BRICHOS SP-C mutant proteins induce apoptosis via caspase-4- and cytochrome c-related mechanisms. Am J Physiol Lung Cell Mol Physiol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007;293(3):L720–9.
40. Mulugeta S, Nguyen V, Russo SJ, Muniswamy M, Beers MF. A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and caspase 3 activation. Am J Respir Cell Mol Biol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2005;32(6):521–30.
41. Glasser SW, Detmer EA, Ikegami M, Na CL, Stahlman MT, Whitsett JA. Pneumonitis and emphysema in sp-C gene targeted mice. J Biol Chem [Research Support, U.S. Gov't, P.H.S.]. 2003;278(16):14291–8.
42. Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, et al. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. Am J Hum Genet [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2009;84(1):52–9.

43. van Moorsel CH, Ten Klooster L, van Oosterhout MF, de Jong PA, Adams H, van Wouter ESH, et al. SFTPA2 mutations in familial and sporadic idiopathic interstitial pneumonia. Am J Respir Crit Care Med [Case Reports Letter Research Support, Non-U.S. Gov't]. 2015;192(10):1249–52.
44. 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 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2010;285(29):22103–13.
45. Hornsby PJ. Telomerase and the aging process. Exp Gerontol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 2007;42(7):575–81.
46. Moodley YP, Scaffidi AK, Misso NL, Keerthisingam C, McAnulty RJ, Laurent GJ, et al. Fibroblasts isolated from normal lungs and those with idiopathic pulmonary fibrosis differ in interleukin-6/gp130-mediated cell signaling and proliferation. Am J Pathol [Research Support, Non-U.S. Gov't]. 2003;163(1):345–54.
47. Utz JP, Ryu JH, Myers JL, Michels VV. Usual interstitial pneumonia complicating dyskeratosis congenita. Mayo Clin Proc [Case Reports]. 2005;80(6):817–21.
48. Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. Proc Natl Acad Sci U S A [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. 2007;104(18):7552–7.
49. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007;356(13):1317–26.
50. Diaz de Leon A, Cronkhite JT, Katzenstein AL, Godwin JD, Raghu G, Glazer CS, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. PLoS One [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2010;5(5):e10680.
51. 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 [Research Support, N.I.H., Extramural]. 2008;178(7):729–37.
52. Kropski JA, Mitchell DB, Markin C, Polosukhin VV, Choi L, Johnson JE, et al. A novel dyskerin (DKC1) mutation is associated with familial interstitial pneumonia. Chest [Case Reports Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S.]. 2014;146(1):e1–7.
53. Alder JK, Stanley SE, Wagner CL, Hamilton M, Hanumanthu VS, Armanios M. Exome sequencing identifies mutant TINF2 in a family with pulmonary fibrosis. Chest [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2015;147(5):1361–8.
54. Kannengiesser C, Borie R, Menard C, Reocreux M, Nitschke P, Gazal S, et al. Heterozygous RTEL1 mutations are associated with familial pulmonary fibrosis. Eur Respir J [Research Support, Non-U.S. Gov't]. 2015;46(2):474–85.
55. Stuart BD, Choi J, Zaidi S, Xing C, Holohan B, Chen R, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. Nat Genet [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2015;47(5):512–7.
56. Petrovski S, Todd JL, Durheim MT, Wang Q, Chien JW, Kelly FL, et al. An exome sequencing study to assess the role of rare genetic variation in pulmonary fibrosis. Am J Respir Crit Care Med. 2017;196(1):82–93.
57. Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. Proc Natl Acad Sci U S A [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2008;105(35):13051–6.
58. Mushiroda T, Wattanapokayakit S, Takahashi A, Nukiwa T, Kudoh S, Ogura T, et al. A genome-wide association study identifies an association of a common variant in TERT with susceptibility to idiopathic pulmonary fibrosis. J Med Genet. 2008;45(10):654–6.

59. Pulkkinen V, Bruce S, Rintahaka J, Hodgson U, Laitinen T, Alenius H, et al. ELMOD2, a candidate gene for idiopathic pulmonary fibrosis, regulates antiviral responses. Faseb J [Research Support, Non-U.S. Gov't]. 2010;24(4):1167–77.
60. Hodgson U, Pulkkinen V, Dixon M, Peyrard-Janvid M, Rehn M, Lahermo P, et al. ELMOD2 is a candidate gene for familial idiopathic pulmonary fibrosis. Am J Hum Genet [Research Support, Non-US Gov't]. 2006;79(1):149–54.
61. 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 [Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't]. 2011;364(16):1503–12.
62. Zhang Y, Noth I, Garcia JG, Kaminski N. A variant in the promoter of MUC5B and idiopathic pulmonary fibrosis. N Engl J Med [Letter Research Support, N.I.H., Extramural]. 2011;364(16):1576–7.
63. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, Steele MP, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. Nat Genet [Meta-Analysis Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. 2013;45(6):613–20.
64. 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 [Comparative Study Multicenter Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2013;1(4):309–17.
65. Raghu G, Freudenberger TD, Yang S, Curtis JR, Spada C, Hayes J, et al. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. Eur Respir J. 2006;27(1):136–42.
66. Tobin RW, Pope CE 2nd, Pellegrini CA, Emond MJ, Sillery J, Raghu G. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Comparative Study]. 1998;158(6):1804–8.
67. Raghu G, Meyer KC. Silent gastro-oesophageal reflux and microaspiration in IPF: mounting evidence for anti-reflux therapy? Eur Respir J [Comment Editorial]. 2012;39(2):242–5.
68. Lee JS, Ryu JH, Elicker BM, Lydell CP, Jones KD, Wolters PJ, et al. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Research Support, N.I.H., Extramural]. 2011;184(12):1390–4.
69. Baumgartner KB, Samet JM, Stidley CA, Colby TV, Waldron JA. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Multicenter Study Research Support, U.S. Gov't, P.H.S.]. 1997;155(1):242–8.
70. Steele MP, Speer MC, Loyd JE, Brown KK, Herron A, Slifer SH, et al. Clinical and pathologic features of familial interstitial pneumonia. Am J Respir Crit Care Med [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S.]. 2005;172(9):1146–52.
71. Oh CK, Murray LA, Molfino NA. Smoking and idiopathic pulmonary fibrosis. Pulm Med. 2012;2012:808260.
72. Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease? Proc Am Thorac Soc [Review]. 2006;3(4):293–8.
73. Vassilakis DA, Sourvinos G, Spandidos DA, Siafakas NM, Bouros D. Frequent genetic alterations at the microsatellite level in cytologic sputum samples of patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Research Support, Non-U.S. Gov't]. 2000;162(3 Pt 1):1115–9.
74. Naik PK, Moore BB. Viral infection and aging as cofactors for the development of pulmonary fibrosis. Expert Rev Respir Med [Research Support, N.I.H., Extramural Review]. 2010;4(6):759–71.
75. Lasithiotaki I, Antoniou KM, Vlahava VM, Karagiannis K, Spandidos DA, Siafakas NM, et al. Detection of herpes simplex virus type-1 in patients with fibrotic lung diseases. PLoS One [Research Support, Non-U.S. Gov't]. 2011;6(12):e27800.

76. Pulkkinen V, Salmenkivi K, Kinnula VL, Sutinen E, Halme M, Hodgson U, et al. A novel screening method detects herpesviral DNA in the idiopathic pulmonary fibrosis lung. Ann Med [Research Support, Non-U.S. Gov't]. 2012;44(2):178–86.
77. Tang YW, Johnson JE, Browning PJ, Cruz-Gervis RA, Davis A, Graham BS, et al. Herpesvirus DNA is consistently detected in lungs of patients with idiopathic pulmonary fibrosis. J Clin Microbiol. 2003;41(6):2633–40.
78. Lawson WE, Crossno PF, Polosukhin VV, Roldan J, Cheng DS, Lane KB, et al. Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. Am J Physiol Lung Cell Mol Physiol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2008;294(6):L1119–26.
79. Torres-Gonzalez E, Bueno M, Tanaka A, Krug LT, Cheng DS, Polosukhin VV, et al. Role of endoplasmic reticulum stress in age-related susceptibility to lung fibrosis. Am J Respir Cell Mol Biol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2012;46(6):748–56.
80. Han MK, Zhou Y, Murray S, Tayob N, Noth I, Lama VN, et al. Lung microbiome and disease progression in idiopathic pulmonary fibrosis: an analysis of the COMET study. Lancet Respir Med [Clinical Trial Research Support, N.I.H., Extramural]. 2014;2(7):548–56.
81. Molyneaux PL, Cox MJ, Willis-Owen SA, Mallia P, Russell KE, Russell AM, et al. The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2014;190(8):906–13.
82. Sisson TH, Mendez M, Choi K, Subbotina N, Courey A, Cunningham A, et al. Targeted injury of type II alveolar epithelial cells induces pulmonary fibrosis. Am J Respir Crit Care Med [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. 2010;181(3):254–63.
83. Adamson IY, Young L, Bowden DH. Relationship of alveolar epithelial injury and repair to the induction of pulmonary fibrosis. Am J Pathol [Research Support, Non-U.S. Gov't]. 1988;130(2):377–83.
84. Evans MJ, Cabral LJ, Stephens RJ, Freeman G. Renewal of alveolar epithelium in the rat following exposure to NO2. Am J Pathol. 1973;70(2):175–98.
85. Rock JR, Barkauskas CE, Cronce MJ, Xue Y, Harris JR, Liang J, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. Proc Natl Acad Sci U S A [Comparative Study Research Support, N.I.H., Extramural]. 2011;108(52):E1475–83.
86. Barkauskas CE, Cronce MJ, Rackley CR, Bowie EJ, Keene DR, Stripp BR, et al. Type 2 alveolar cells are stem cells in adult lung. J Clin Invest [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2013;123(7):3025–36.
87. Vaughan AE, Brumwell AN, Xi Y, Gotts JE, Brownfield DG, Treutlein B, et al. Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. Nature [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2015;517(7536):621–5.
88. Chilosi M, Poletti V, Murer B, Lestani M, Cancellieri A, Montagna L, et al. Abnormal re-epithelialization and lung remodeling in idiopathic pulmonary fibrosis: the role of deltaN-p63. Lab Invest [Research Support, Non-U.S. Gov't]. 2002;82(10):1335–45.
89. Perl AK, Riethmacher D, Whitsett JA. Conditional depletion of airway progenitor cells induces peribronchiolar fibrosis. Am J Respir Crit Care Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2011;183(4):511–21.
90. Fehrenbach H, Kasper M, Tschernig T, Shearman MS, Schuh D, Muller M. Receptor for advanced glycation endproducts (RAGE) exhibits highly differential cellular and subcellular localisation in rat and human lung. Cell Mol Biol (Noisy-le-grand) [Research Support, Non-U.S. Gov't]. 1998;44(7):1147–57.
91. Demling N, Ehrhardt C, Kasper M, Laue M, Knels L, Rieber EP. Promotion of cell adherence and spreading: a novel function of RAGE, the highly selective differentiation marker of human alveolar epithelial type I cells. Cell Tissue Res. 2006;323(3):475–88.

92. Queisser MA, Kouri FM, Konigshoff M, Wygrecka M, Schubert U, Eickelberg O, et al. Loss of RAGE in pulmonary fibrosis: molecular relations to functional changes in pulmonary cell types. Am J Respir Cell Mol Biol [Research Support, Non-U.S. Gov't]. 2008;39(3):337–45.
93. Englert JM, Hanford LE, Kaminski N, Tobolewski JM, Tan RJ, Fattman CL, et al. A role for the receptor for advanced glycation end products in idiopathic pulmonary fibrosis. Am J Pathol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2008;172(3):583–91.
94. Blasco MA. Telomere length, stem cells and aging. Nat Chem Biol [Research Support, Non-U.S. Gov't Review]. 2007;3(10):640–9.
95. Waisberg DR, Barbas-Filho JV, Parra ER, Fernezlian S, de Carvalho CR, Kairalla RA, et al. Abnormal expression of telomerase/apoptosis limits type II alveolar epithelial cell replication in the early remodeling of usual interstitial pneumonia/idiopathic pulmonary fibrosis. Hum Pathol [Research Support, Non-U.S. Gov't]. 2010;41(3):385–91.
96. Xu Y, Mizuno T, Sridharan A, Du Y, Guo M, Tang J, et al. Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis. JCI Insight. 2016;1(20):e90558.
97. Zavadil J, Bottinger EP. TGF-beta and epithelial-to-mesenchymal transitions. Oncogene [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S. Review]. 2005;24(37):5764–74.
98. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol [Research Support, Non-U.S. Gov't Review]. 2006;7(2):131–42.
99. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer [Review]. 2002;2(6):442–54.
100. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest [Research Support, U.S. Gov't, P.H.S.]. 2002;110(3):341–50.
101. Rowe RG, Lin Y, Shimizu-Hirota R, Hanada S, Neilson EG, Greenson JK, et al. Hepatocyte-derived Snail1 propagates liver fibrosis progression. Mol Cell Biol [Research Support, N.I.H., Extramural]. 2011;31(12):2392–403.
102. Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol [Review]. 2008;294(2):L152–60.
103. Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, et al. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. Proc Natl Acad Sci U S A [Research Support, N.I.H., Extramural]. 2006;103(35):13180–5.
104. Tanjore H, Xu XC, Polosukhin VV, Degryse AL, Li B, Han W, et al. Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. Am J Respir Crit Care Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2009;180(7):657–65.
105. Marmai C, Sutherland RE, Kim KK, Dolganov GM, Fang X, Kim SS, et al. Alveolar epithelial cells express mesenchymal proteins in patients with idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2011;301(1):L71–8.
106. Bridges RS, Kass D, Loh K, Glackin C, Borczuk AC, Greenberg S. Gene expression profiling of pulmonary fibrosis identifies Twist1 as an antiapoptotic molecular "rectifier" of growth factor signaling. Am J Pathol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2009;175(6):2351–61.
107. Jayachandran A, Konigshoff M, Yu H, Rupniewska E, Hecker M, Klepetko W, et al. SNAI transcription factors mediate epithelial-mesenchymal transition in lung fibrosis. Thorax [Research Support, Non-U.S. Gov't]. 2009;64(12):1053–61.
108. 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 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2005;166(5):1321–32.

109. Shannon JM, Hyatt BA. Epithelial-mesenchymal interactions in the developing lung. Annu Rev Physiol [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. 2004;66:625–45.
110. Cardoso WV. Molecular regulation of lung development. Annu Rev Physiol [Research Support, U.S. Gov't, P.H.S. Review]. 2001;63:471–94.
111. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell [Research Support, Non-U.S. Gov't Review]. 2009;139(5):871–90.
112. Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. Cell Res [Review]. 2009;19(2):156–72.
113. Kim KK, Wei Y, Szekeres C, Kugler MC, Wolters PJ, Hill ML, et al. Epithelial cell alpha3beta1 integrin links beta-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. J Clin Invest [Research Support, N.I.H., Extramural]. 2009;119(1):213–24.
114. Masszi A, Fan L, Rosivall L, McCulloch CA, Rotstein OD, Mucsi I, et al. Integrity of cell-cell contacts is a critical regulator of TGF-beta 1-induced epithelial-to-myofibroblast transition: role for beta-catenin. Am J Pathol [Research Support, Non-U.S. Gov't]. 2004;165(6):1955–67.
115. Koli K, Myllarniemi M, Vuorinen K, Salmenkivi K, Ryynanen MJ, Kinnula VL, et al. Bone morphogenetic protein-4 inhibitor gremlin is overexpressed in idiopathic pulmonary fibrosis. Am J Pathol [Research Support, Non-U.S. Gov't]. 2006;169(1):61–71.
116. Mosteiro L, Pantoja C, Alcazar N, Marion RM, Chondronasiou D, Rovira M, et al. Tissue damage and senescence provide critical signals for cellular reprogramming in vivo. Science. 2016;354(6315):aaf4445.
117. Hashimoto M, Asai A, Kawagishi H, Mikawa R, Iwashita Y, Kanayama K, et al. Elimination of p19(ARF)-expressing cells enhances pulmonary function in mice. JCI Insight. 2016;1(12):e87732.
118. Minagawa S, Araya J, Numata T, Nojiri S, Hara H, Yumino Y, et al. Accelerated epithelial cell senescence in IPF and the inhibitory role of SIRT6 in TGF-beta-induced senescence of human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2011;300(3):L391–401.
119. Disayabutr S, Kim EK, Cha SI, Green G, Naikawadi RP, Jones KD, et al. miR-34 miRNAs regulate cellular senescence in type II alveolar epithelial cells of patients with idiopathic pulmonary fibrosis. PLoS One. 2016;11(6):e0158367.
120. Orjalo AV, Bhaumik D, Gengler BK, Scott GK, Campisi J. Cell surface-bound IL-1alpha is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. Proc Natl Acad Sci U S A. 2009;106(40):17031–6.
121. 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.
122. Aquino-Galvez A, Gonzalez-Avila G, Perez-Rodriguez M, Partida-Rodriguez O, Nieves-Ramirez M, Pina-Ramirez I, et al. Analysis of heat shock protein 70 gene polymorphisms Mexican patients with idiopathic pulmonary fibrosis. BMC Pulm Med [Research Support, Non-U.S. Gov't]. 2015;15:129.
123. Korfei M, Ruppert C, Mahavadi P, Henneke I, Markart P, Koch M, et al. Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2008;178(8):838–46.
124. Tanjore H, Cheng DS, Degryse AL, Zoz DF, Abdolrasulnia R, Lawson WE, et al. Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress. J Biol Chem [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. 2011;286(35):30972–80.
125. Naidoo N. The endoplasmic reticulum stress response and aging. Rev Neurosci [Review]. 2009;20(1):23–37.
126. Fink AL. Chaperone-mediated protein folding. Physiol Rev. 1999;79(2):425–49.
127. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194–217.

128. Patel AS, Lin L, Geyer A, Haspel JA, An CH, Cao J, et al. Autophagy in idiopathic pulmonary fibrosis. PLoS One [Research Support, N.I.H., Extramural]. 2012;7(7):e41394.
129. Romero Y, Bueno M, Ramirez R, Alvarez D, Sembrat JC, Goncharova EA, et al. mTORC1 activation decreases autophagy in aging and idiopathic pulmonary fibrosis and contributes to apoptosis resistance in IPF fibroblasts. Aging Cell. 2016;113:7291–3.
130. Sosulski ML, Gongora R, Danchuk S, Dong C, Luo F, Sanchez CG. Deregulation of selective autophagy during aging and pulmonary fibrosis: the role of TGFbeta1. Aging Cell. 2015;14(5):774–83.
131. 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.
132. Huang LS, Berdyshev EV, Tran JT, Xie L, Chen J, Ebenezer DL, et al. Sphingosine-1-phosphate lyase is an endogenous suppressor of pulmonary fibrosis: role of S1P signalling and autophagy. Thorax. 2015;70(12):1138–48.
133. Cabrera S, Maciel M, Herrera I, Nava T, Vergara F, Gaxiola M, et al. Essential role for the ATG4B protease and autophagy in bleomycin-induced pulmonary fibrosis. Autophagy. 2015;11(4):670–84.
134. Bueno M, Lai YC, Romero Y, Brands J, St Croix CM, Kamga C, et al. PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. J Clin Invest [Research Support, N.I.H., Extramural]. 2015;125(2):521–38.
135. Braidy N, Guillemin GJ, Mansour H, Chan-Ling T, Poljak A, Grant R. Age related changes in NAD+ metabolism oxidative stress and Sirt1 activity in wistar rats. PLoS One [Research Support, Non-U.S. Gov't]. 2011;6(4):e19194.
136. Panduri V, Liu G, Surapureddi S, Kondapalli J, Soberanes S, de Souza-Pinto NC, et al. Role of mitochondrial hOGG1 and aconitase in oxidant-induced lung epithelial cell apoptosis. Free Radic Biol Med. 2009;47(6):750–9.
137. Jablonski RP, Kim SJ, Cheresh P, Williams DB, Morales-Nebreda L, Cheng Y, et al. SIRT3 deficiency promotes lung fibrosis by augmenting alveolar epithelial cell mitochondrial DNA damage and apoptosis. FASEB J. 2017;31(6):2520–32.
138. Patel AS, Song JW, Chu SG, Mizumura K, Osorio JC, Shi Y, et al. Epithelial cell mitochondrial dysfunction and PINK1 are induced by transforming growth factor-beta1 in pulmonary fibrosis. PLoS One [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2015;10(3):e0121246.
139. Fehrenbach H. Alveolar epithelial type II cell: defender of the alveolus revisited. Respir Res [Research Support, Non-U.S. Gov't Review]. 2001;2(1):33–46.
140. Sirianni FE, Chu FS, Walker DC. Human alveolar wall fibroblasts directly link epithelial type 2 cells to capillary endothelium. Am J Respir Crit Care Med [Research Support, U.S. Gov't, P.H.S.]. 2003;168(12):1532–7.
141. Portnoy J, Pan T, Dinarello CA, Shannon JM, Westcott JY, Zhang L, et al. Alveolar type II cells inhibit fibroblast proliferation: role of IL-1alpha. Am J Physiol Lung Cell Mol Physiol [Research Support, N.I.H., Extramural]. 2006;290(2):L307–16.
142. Chauncey JB, Peters-Golden M, Simon RH. Arachidonic acid metabolism by rat alveolar epithelial cells. Lab Invest [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. 1988;58(2):133–40.
143. Lipchik RJ, Chauncey JB, Paine R, Simon RH, Peters-Golden M. Arachidonate metabolism increases as rat alveolar type II cells differentiate in vitro. Am J Phys. 1990;259(2 Pt 1):L73–80.
144. Lama V, Moore BB, Christensen P, Toews GB, Peters-Golden M. Prostaglandin E2 synthesis and suppression of fibroblast proliferation by alveolar epithelial cells is cyclooxygenase-2-dependent. Am J Respir Cell Mol Biol [Research Support, U.S. Gov't, P.H.S.]. 2002;27(6):752–8.
145. Bitterman PB, Wewers MD, Rennard SI, Adelberg S, Crystal RG. Modulation of alveolar macrophage-driven fibroblast proliferation by alternative macrophage mediators. J Clin Invest. 1986;77(3):700–8.

146. Kohyama T, Ertl RF, Valenti V, Spurzem J, Kawamoto M, Nakamura Y, et al. Prostaglandin E(2) inhibits fibroblast chemotaxis. Am J Physiol Lung Cell Mol Physiol [Research Support, Non-U.S. Gov't]. 2001;281(5):L1257–63.
147. Korn JH, Halushka PV, LeRoy EC. Mononuclear cell modulation of connective tissue function: suppression of fibroblast growth by stimulation of endogenous prostaglandin production. J Clin Invest [Research Support, U.S. Gov't, P.H.S.]. 1980;65(2):543–54.
148. Goldstein RH, Polgar P. The effect and interaction of bradykinin and prostaglandins on protein and collagen production by lung fibroblasts. J Biol Chem [Research Support, U.S. Gov't, P.H.S.]. 1982;257(15):8630–3.
149. Borok Z, Gillissen A, Buhl R, Hoyt RF, Hubbard RC, Ozaki T, et al. Augmentation of functional prostaglandin E levels on the respiratory epithelial surface by aerosol administration of prostaglandin E. Am Rev Respir Dis [Comparative Study Research Support, Non-U.S. Gov't]. 1991;144(5):1080–4.
150. Huang SK, Wettlaufer SH, Hogaboam CM, Flaherty KR, Martinez FJ, Myers JL, et al. Variable prostaglandin E2 resistance in fibroblasts from patients with usual interstitial pneumonia. Am J Respir Crit Care Med [Research Support, N.I.H., Extramural]. 2008;177(1):66–74.
151. Konigshoff M, Eickelberg O. WNT signaling in lung disease: a failure or a regeneration signal? Am J Respir Cell Mol Biol [Research Support, Non-U.S. Gov't Review]. 2010;42(1):21–31.
152. Mucenski ML, Nation JM, Thitoff AR, Besnard V, Xu Y, Wert SE, et al. Beta-catenin regulates differentiation of respiratory epithelial cells in vivo. Am J Physiol Lung Cell Mol Physiol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2005;289(6):L971–9.
153. Selman M, Pardo A, Barrera L, Estrada A, Watson SR, Wilson K, et al. Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis. Am J Respir Crit Care Med [Clinical Trial Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2006;173(2):188–98.
154. 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 [Research Support, Non-U.S. Gov't]. 2002;99(9):6292–7.
155. 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 [Comparative Study]. 2005;2(9):e251.
156. Yang IV, Burch LH, Steele MP, Savov JD, Hollingsworth JW, McElvania-Tekippe E, et al. Gene expression profiling of familial and sporadic interstitial pneumonia. Am J Respir Crit Care Med [Comparative Study Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural]. 2007;175(1):45–54.
157. Chilosi M, Poletti V, Zamo A, Lestani M, Montagna L, Piccoli P, et al. Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. Am J Pathol [Research Support, Non-U.S. Gov't]. 2003;162(5):1495–502.
158. Konigshoff M, Balsara N, Pfaff EM, Kramer M, Chrobak I, Seeger W, et al. Functional Wnt signaling is increased in idiopathic pulmonary fibrosis. PLoS One [Research Support, Non-U.S. Gov't]. 2008;3(5):e2142.
159. Konigshoff M, Kramer M, Balsara N, Wilhelm J, Amarie OV, Jahn A, et al. WNT1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. J Clin Invest [Research Support, Non-U.S. Gov't]. 2009;119(4):772–87.
160. Kapanci Y, Desmouliere A, Pache JC, Redard M, Gabbiani G. Cytoskeletal protein modulation in pulmonary alveolar myofibroblasts during idiopathic pulmonary fibrosis. Possible role of transforming growth factor beta and tumor necrosis factor alpha. Am J Respir Crit Care Med [Research Support, Non-U.S. Gov't]. 1995;152(6 Pt 1):2163–9.
161. Khalil N, O'Connor RN, Flanders KC, Unruh H. TGF-beta 1, but not TGF-beta 2 or TGF-beta 3, is differentially present in epithelial cells of advanced pulmonary fibrosis: an immunohistochemical study. Am J Respir Cell Mol Biol [Research Support, Non-U.S. Gov't]. 1996;14(2):131–8.

162. Antoniades HN, Bravo MA, Avila RE, Galanopoulos T, Neville-Golden J, Maxwell M, et al. Platelet-derived growth factor in idiopathic pulmonary fibrosis. J Clin Invest [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1990;86(4):1055–64.
163. Antoniades HN, Neville-Golden J, Galanopoulos T, Kradin RL, Valente AJ, Graves DT. Expression of monocyte chemoattractant protein 1 mRNA in human idiopathic pulmonary fibrosis. Proc Natl Acad Sci U S A [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1992;89(12):5371–5.
164. Pan LH, Yamauchi K, Uzuki M, Nakanishi T, Takigawa M, Inoue H, et al. Type II alveolar epithelial cells and interstitial fibroblasts express connective tissue growth factor in IPF. Eur Respir J [Research Support, Non-U.S. Gov't]. 2001;17(6):1220–7.
165. Giaid A, Michel RP, Stewart DJ, Sheppard M, Corrin B, Hamid Q. Expression of endothelin-1 in lungs of patients with cryptogenic fibrosing alveolitis. Lancet [Research Support, Non-U.S. Gov't]. 1993;341(8860):1550–4.
166. Nash JR, McLaughlin PJ, Butcher D, Corrin B. Expression of tumour necrosis factor-alpha in cryptogenic fibrosing alveolitis. Histopathology. 1993;22(4):343–7.
167. Piguet PF, Ribaux C, Karpuz V, Grau GE, Kapanci Y. Expression and localization of tumor necrosis factor-alpha and its mRNA in idiopathic pulmonary fibrosis. Am J Pathol [Research Support, Non-U.S. Gov't]. 1993;143(3):651–5.
168. Dancer RC, Wood AM, Thickett DR. Metalloproteinases in idiopathic pulmonary fibrosis. Eur Respir J [Review]. 2011;38(6):1461–7.
169. Maitra M, Cano CA, Garcia CK. Mutant surfactant A2 proteins associated with familial pulmonary fibrosis and lung cancer induce TGF-beta1 secretion. Proc Natl Acad Sci U S A [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Retracted Publication]. 2012;109(51):21064–9.
170. Corrin B, Dewar A, Rodriguez-Roisin R, Turner-Warwick M. Fine structural changes in cryptogenic fibrosing alveolitis and asbestosis. J Pathol. 1985;147(2):107–19.
171. Mogulkoc N, Brutsche MH, Bishop PW, Murby B, Greaves MS, Horrocks AW, et al. Pulmonary (99m)Tc-DTPA aerosol clearance and survival in usual interstitial pneumonia (UIP). Thorax. 2001;56(12):916–23.
172. McKeown S, Richter AG, O'Kane C, McAuley DF, Thickett DR. MMP expression and abnormal lung permeability are important determinants of outcome in IPF. Eur Respir J [Research Support, Non-U.S. Gov't]. 2009;33(1):77–84.
173. Tager AM, LaCamera P, Shea BS, Campanella GS, Selman M, Zhao Z, et al. The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. Nat Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2008;14(1):45–54.
174. Basset F, Ferrans VJ, Soler P, Takemura T, Fukuda Y, Crystal RG. Intraluminal fibrosis in interstitial lung disorders. Am J Pathol. 1986;122(3):443–61.
175. Gross TJ, Simon RH, Sitrin RG. Tissue factor procoagulant expression by rat alveolar epithelial cells. Am J Respir Cell Mol Biol [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1992;6(4):397–403.
176. Kotani I, Sato A, Hayakawa H, Urano T, Takada Y, Takada A. Increased procoagulant and antifibrinolytic activities in the lungs with idiopathic pulmonary fibrosis. Thromb Res. 1995;77(6):493–504.
177. Chambers RC, Scotton CJ. Coagulation cascade proteinases in lung injury and fibrosis. Proc Am Thorac Soc. 2012;9(3):96–101.
178. Chapman HA. Disorders of lung matrix remodeling. J Clin Invest [Research Support, U.S. Gov't, P.H.S. Review]. 2004;113(2):148–57.
179. Marshall BC, Brown BR, Rothstein MA, Rao NV, Hoidal JR, Rodgers GM. Alveolar epithelial cells express both plasminogen activator and tissue factor. Potential role in repair of lung injury. Chest [Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. 1991;99(3 Suppl):25S–7S.
180. Imokawa S, Sato A, Hayakawa H, Kotani M, Urano T, Takada A. Tissue factor expression and fibrin deposition in the lungs of patients with idiopathic pulmonary fibrosis and systemic sclerosis. Am J Respir Crit Care Med [Comparative Study]. 1997;156(2 Pt 1):631–6.

181. Gunther A, Mosavi P, Ruppert C, Heinemann S, Temmesfeld B, Velcovsky HG, et al. Enhanced tissue factor pathway activity and fibrin turnover in the alveolar compartment of patients with interstitial lung disease. Thromb Haemost [Comparative Study Research Support, Non-U.S. Gov't]. 2000;83(6):853–60.
182. Fujii M, Hayakawa H, Urano T, Sato A, Chida K, Nakamura H, et al. Relevance of tissue factor and tissue factor pathway inhibitor for hypercoagulable state in the lungs of patients with idiopathic pulmonary fibrosis. Thromb Res [Comparative Study]. 2000;99(2):111–7.
183. Grainger DJ, Wakefield L, Bethell HW, Farndale RW, Metcalfe JC. Release and activation of platelet latent TGF-beta in blood clots during dissolution with plasmin. Nat Med [Research Support, Non-U.S. Gov't]. 1995;1(9):932–7.
184. Eitzman DT, McCoy RD, Zheng X, Fay WP, Shen T, Ginsburg D, et al. Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. J Clin Invest [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1996;97(1):232–7.
185. Howell DC, Johns RH, Lasky JA, Shan B, Scotton CJ, Laurent GJ, et al. Absence of proteinase-activated receptor-1 signaling affords protection from bleomycin-induced lung inflammation and fibrosis. Am J Pathol [Research Support, Non-U.S. Gov't]. 2005;166(5):1353–65.
186. Scotton CJ, Krupiczojc MA, Konigshoff M, Mercer PF, Lee YC, Kaminski N, et al. Increased local expression of coagulation factor X contributes to the fibrotic response in human and murine lung injury. J Clin Invest [Research Support, Non-U.S. Gov't]. 2009;119(9):2550–63.
187. Wygrecka M, Kwapiszewska G, Jablonska E, von Gerlach S, Henneke I, Zakrzewicz D, et al. Role of protease-activated receptor-2 in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [In Vitro Research Support, Non-U.S. Gov't]. 2011;183(12):1703–14.
188. Noth I, Anstrom KJ, Calvert SB, de Andrade J, Flaherty KR, Glazer C, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186(1):88–95.
189. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast. One function, multiple origins. Am J Pathol. 2007;170:1807–16.
190. Serini G, Bochaton-Piallat ML, Ropraz P, Geinoz A, Borsi L, Zardi L, et al. The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. J Cell Biol. 1998;142(3):873–81.
191. Kapanci Y, Ribaux C, Chaponnier C, Gabbiani G. Cytoskeletal features of alveolar myofibroblasts and pericytes in normal human and rat lung. J Histochem Cytochem [Research Support, Non-U.S. Gov't]. 1992;40(12):1955–63.
192. Darby I, Skalli O, Gabbiani G. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Investig. 1990;63(1):21–9.
193. Ehrlich HP, Desmouliere A, Diegelmann RF, Cohen IK, Compton CC, Garner WL, et al. Morphological and immunochemical differences between keloid and hypertrophic scar. Am J Pathol. 1994;145(1):105–13.
194. Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. J Pathol. 2003;200(4):500–3.
195. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Phys. 1999;277(1 Pt 1):C1–9.
196. Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmouliere A, Varga J, et al. Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. Am J Pathol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 2012;180(4):1340–55.
197. Kuhn C, McDonald JA. The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. Am J Pathol. 1991;138(5):1257–65.
198. Xia H, Bodempudi V, Benyumov A, Hergert P, Tank D, Herrera J, et al. Identification of a cell-of-origin for fibroblasts comprising the fibrotic reticulum in idiopathic pulmonary fibrosis. Am J Pathol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2014;184(5):1369–83.

199. Phan SH. Genesis of the myofibroblast in lung injury and fibrosis. Proc Am Thorac Soc. 2012;9(3):148–52.
200. Strieter RM, Keeley EC, Hughes MA, Burdick MD, Mehrad B. The role of circulating mesenchymal progenitor cells (fibrocytes) in the pathogenesis of pulmonary fibrosis. J Leukoc Biol [Research Support, N.I.H., Extramural Review]. 2009;86(5):1111–8.
201. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1994;1(1):71–81.
202. Ebihara Y, Masuya M, Larue AC, Fleming PA, Visconti RP, Minamiguchi H, et al. Hematopoietic origins of fibroblasts: II. In vitro studies of fibroblasts, CFU-F, and fibrocytes. Exp Hematol [In Vitro Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S.]. 2006;34(2):219–29.
203. Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. Bone marrow-derived progenitor cells in pulmonary fibrosis. J Clin Invest [Research Support, U.S. Gov't, P.H.S.]. 2004;113(2):243–52.
204. 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 [Research Support, U.S. Gov't, P.H.S.]. 2004;114(3):438–46.
205. Epperly MW, Guo H, Gretton JE, Greenberger JS. Bone marrow origin of myofibroblasts in irradiation pulmonary fibrosis. Am J Respir Cell Mol Biol [Research Support, U.S. Gov't, P.H.S.]. 2003;29(2):213–24.
206. Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am J Pathol [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S.]. 2010;176(1):85–97.
207. Yokota T, Kawakami Y, Nagai Y, Ma JX, Tsai JY, Kincade PW, et al. Bone marrow lacks a transplantable progenitor for smooth muscle type alpha-actin-expressing cells. Stem Cells [Research Support, N.I.H., Extramural]. 2006;24(1):13–22.
208. Strieter RM. Pathogenesis and natural history of usual interstitial pneumonia: the whole story or the last chapter of a long novel. Chest. 2005;128(5 Suppl 1):526S–32S.
209. Mehrad B, Burdick MD, Zisman DA, Keane MP, Belperio JA, Strieter RM. Circulating peripheral blood fibrocytes in human fibrotic interstitial lung disease. Biochem Biophys Res Commun [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007;353(1):104–8.
210. Andersson-Sjoland A, de Alba CG, Nihlberg K, Becerril C, Ramirez R, Pardo A, et al. Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. Int J Biochem Cell Biol [Research Support, Non-U.S. Gov't]. 2008;40(10):2129–40.
211. 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 [Research Support, Non-U.S. Gov't]. 2009;179(7):588–94.
212. Willis BC, Borok Z. TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease. Am J Physiol Lung Cell Mol Physiol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 2007;293(3):L525–34.
213. Gabasa M, Duch P, Jorba I, Gimenez A, Lugo R, Pavelescu I, et al. Epithelial contribution to the profibrotic stiff microenvironment and myofibroblast population in lung fibrosis. Mol Biol Cell. 2017;28(26):3741–55.
214. Chapman HA. Epithelial responses to lung injury: role of the extracellular matrix. Proc Am Thorac Soc. 2012;9(3):89–95.
215. Chapman HA, Li X, Alexander JP, Brumwell A, Lorizio W, Tan K, et al. Integrin alpha-6beta4 identifies an adult distal lung epithelial population with regenerative potential in mice. J Clin Invest [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2011;121(7):2855–62.
216. Hecker L, Logsdon NJ, Kurundkar D, Kurundkar A, Bernard K, Hock T, et al. Reversal of persistent fibrosis in aging by targeting Nox4-Nrf2 redox imbalance. Sci Transl Med. 2014;6(231):231ra47.

217. Zhang K, Rekhter MD, Gordon D, Phan SH. Myofibroblasts and their role in lung collagen gene expression during pulmonary fibrosis. A combined immunohistochemical and in situ hybridization study. Am J Pathol [Research Support, U.S. Gov't, P.H.S.]. 1994;145(1):114–25.
218. Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol. 1993;122(1):103–11.
219. Phan SH. Fibroblast phenotypes in pulmonary fibrosis. Am J Respir Cell Mol Biol [Review]. 2003;29(3 Suppl):S87–92.
220. Hagood JS, Lasky JA, Nesbitt JE, Segarini P. Differential expression, surface binding, and response to connective tissue growth factor in lung fibroblast subpopulations. Chest [Research Support, U.S. Gov't, P.H.S.]. 2001;120(1 Suppl):64S–6S.
221. Hagood JS, Prabhakaran P, Kumbla P, Salazar L, MacEwen MW, Barker TH, et al. Loss of fibroblast Thy-1 expression correlates with lung fibrogenesis. Am J Pathol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2005;167(2):365–79.
222. Sanders YY, Kumbla P, Hagood JS. Enhanced myofibroblastic differentiation and survival in Thy-1(-) lung fibroblasts. Am J Respir Cell Mol Biol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007;36(2):226–35.
223. Xia H, Gilbertsen A, Herrera J, Racila E, Smith K, Peterson M, et al. Calcium-binding protein S100A4 confers mesenchymal progenitor cell fibrogenicity in idiopathic pulmonary fibrosis. J Clin Invest. 2017;127(7):2586–97.
224. Zepp JA, Zacharias WJ, Frank DB, Cavanaugh CA, Zhou S, Morley MP, et al. Distinct mesenchymal lineages and niches promote epithelial self-renewal and myofibrogenesis in the lung. Cell. 2017;170(6):1134–48 e10.
225. Xie T, Wang Y, Deng N, Huang G, Taghavifar F, Geng Y, et al. Single-cell deconvolution of fibroblast heterogeneity in mouse pulmonary fibrosis. Cell Rep. 2018;22(13):3625–40.
226. Emblom-Callahan MC, Chhina MK, Shlobin OA, Ahmad S, Reese ES, Iyer EP, et al. Genomic phenotype of non-cultured pulmonary fibroblasts in idiopathic pulmonary fibrosis. Genomics. 2010;96(3):134–45.
227. Xia H, Diebold D, Nho R, Perlman D, Kleidon J, Kahm J, et al. Pathological integrin signaling enhances proliferation of primary lung fibroblasts from patients with idiopathic pulmonary fibrosis. J Exp Med [Research Support, N.I.H., Extramural]. 2008;205(7):1659–72.
228. Ramos C, Montano M, Garcia-Alvarez J, Ruiz V, Uhal BD, Selman M, et al. Fibroblasts from idiopathic pulmonary fibrosis and normal lungs differ in growth rate, apoptosis, and tissue inhibitor of metalloproteinases expression. Am J Respir Cell Mol Biol. 2001;24(5):591–8.
229. Torry DJ, Richards CD, Podor TJ, Gauldie J. Anchorage-independent colony growth of pulmonary fibroblasts derived from fibrotic human lung tissue. J Clin Invest [Research Support, Non-U.S. Gov't]. 1994;93(4):1525–32.
230. Larsson O, Diebold D, Fan D, Peterson M, Nho RS, Bitterman PB, et al. Fibrotic myofibroblasts manifest genome-wide derangements of translational control. PLoS One [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2008;3(9):e3220.
231. White ES, Atrasz RG, Hu B, Phan SH, Stambolic V, Mak TW, et al. Negative regulation of myofibroblast differentiation by PTEN (phosphatase and tensin homolog deleted on chromosome 10). Am J Respir Crit Care Med [Research Support, N.I.H., Extramural]. 2006;173(1):112–21.
232. Okamoto T, Schlegel A, Scherer PE, Lisanti MP. Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. J Biol Chem [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. 1998;273(10):5419–22.
233. Liu P, Ying Y, Ko YG, Anderson RG. Localization of platelet-derived growth factor-stimulated phosphorylation cascade to caveolae. J Biol Chem [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1996;271(17):10299–303.

234. Wang XM, Zhang Y, Kim HP, Zhou Z, Feghali-Bostwick CA, Liu F, et al. Caveolin-1: a critical regulator of lung fibrosis in idiopathic pulmonary fibrosis. J Exp Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2006;203(13):2895–906.
235. Sagana RL, Yan M, Cornett AM, Tsui JL, Stephenson DA, Huang SK, et al. Phosphatase and tensin homologue on chromosome 10 (PTEN) directs prostaglandin E2-mediated fibroblast responses via regulation of E prostanoid 2 receptor expression. J Biol Chem [Research Support, N.I.H., Extramural]. 2009;284(47):32264–71.
236. Huang SK, Fisher AS, Scruggs AM, White ES, Hogaboam CM, Richardson BC, et al. Hypermethylation of PTGER2 confers prostaglandin E2 resistance in fibrotic fibroblasts from humans and mice. Am J Pathol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2010;177(5):2245–55.
237. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, et al. Cellular senescence mediates fibrotic pulmonary disease. Nat Commun. 2017;8:14532.
238. Alvarez D, Cardenes N, Sellares J, Bueno M, Corey C, Hanumanthu VS, et al. IPF lung fibroblasts have a senescent phenotype. Am J Physiol Lung Cell Mol Physiol. 2017;313(6):L1164–L73.
239. Kobayashi K, Araya J, Minagawa S, Hara H, Saito N, Kadota T, et al. Involvement of PARK2-mediated mitophagy in idiopathic pulmonary fibrosis pathogenesis. J Immunol. 2016;197(2):504–16.
240. Kane CJ, Hebda PA, Mansbridge JN, Hanawalt PC. Direct evidence for spatial and temporal regulation of transforming growth factor beta 1 expression during cutaneous wound healing. J Cell Physiol [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1991;148(1):157–73.
241. Gauldie J, Bonniaud P, Sime P, Ask K, Kolb M. TGF-beta, Smad3 and the process of progressive fibrosis. Biochem Soc Trans [Review]. 2007;35(Pt 4):661–4.
242. Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. Faseb J [Research Support, Non-U.S. Gov't Review]. 2004;18(7):816–27.
243. Sheppard D. Transforming growth factor beta: a central modulator of pulmonary and airway inflammation and fibrosis. Proc Am Thorac Soc [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 2006;3(5):413–7.
244. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci U S A [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1991;88(15):6642–6.
245. Khalil N, O'Connor RN, Unruh HW, Warren PW, Flanders KC, Kemp A, et al. Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol [Research Support, Non-U.S. Gov't]. 1991;5(2):155–62.
246. Giri SN, Hyde DM, Hollinger MA. Effect of antibody to transforming growth factor beta on bleomycin induced accumulation of lung collagen in mice. Thorax [Research Support, U.S. Gov't, P.H.S.]. 1993;48(10):959–66.
247. Wang Q, Wang Y, Hyde DM, Gotwals PJ, Koteliansky VE, Ryan ST, et al. Reduction of bleomycin induced lung fibrosis by transforming growth factor beta soluble receptor in hamsters. Thorax. 1999;54(9):805–12.
248. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. J Cell Sci [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. 2003;116(Pt 2):217–24.
249. Hynes RO. The extracellular matrix: not just pretty fibrils. Science (New York, NY) [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 2009;326(5957):1216–9.
250. Rifkin DB. Latent transforming growth factor-beta (TGF-beta) binding proteins: orchestrators of TGF-beta availability. J Biol Chem [Review]. 2005;280(9):7409–12.

251. Jenkins G. The role of proteases in transforming growth factor-beta activation. Int J Biochem Cell Biol [Review]. 2008;40(6–7):1068–78.
252. Jenkins RG, Su X, Su G, Scotton CJ, Camerer E, Laurent GJ, et al. Ligation of protease-activated receptor 1 enhances alpha(v)beta6 integrin-dependent TGF-beta activation and promotes acute lung injury. J Clin Invest [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2006;116(6):1606–14.
253. Xu MY, Porte J, Knox AJ, Weinreb PH, Maher TM, Violette SM, et al. Lysophosphatidic acid induces alphavbeta6 integrin-mediated TGF-beta activation via the LPA2 receptor and the small G protein G alpha(q). Am J Pathol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2009;174(4):1264–79.
254. Hinz B. Mechanical aspects of lung fibrosis: a spotlight on the myofibroblast. Proc Am Thorac Soc. 2012;9(3):137–47.
255. Hoyles RK, Derrett-Smith EC, Khan K, Shiwen X, Howat SL, Wells AU, et al. An essential role for resident fibroblasts in experimental lung fibrosis is defined by lineage-specific deletion of high-affinity type II transforming growth factor beta receptor. Am J Respir Crit Care Med [Research Support, Non-U.S. Gov't]. 2011;183(2):249–61.
256. Hashimoto S, Gon Y, Takeshita I, Matsumoto K, Maruoka S, Horie T. Transforming growth factor-beta1 induces phenotypic modulation of human lung fibroblasts to myofibroblast through a c-Jun-NH2-terminal kinase-dependent pathway. Am J Respir Crit Care Med. 2001;163(1):152–7.
257. Shi-wen X, Parapuram SK, Pala D, Chen Y, Carter DE, Eastwood M, et al. Requirement of transforming growth factor beta-activated kinase 1 for transforming growth factor beta-induced alpha-smooth muscle actin expression and extracellular matrix contraction in fibroblasts. Arthritis Rheum. 2009;60(1):234–41.
258. Wilkes MC, Mitchell H, Penheiter SG, Dore JJ, Suzuki K, Edens M, et al. Transforming growth factor-beta activation of phosphatidylinositol 3-kinase is independent of Smad2 and Smad3 and regulates fibroblast responses via p21-activated kinase-2. Cancer Res [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2005;65(22):10431–40.
259. Horowitz JC, Rogers DS, Sharma V, Vittal R, White ES, Cui Z, et al. Combinatorial activation of FAK and AKT by transforming growth factor-beta1 confers an anoikis-resistant phenotype to myofibroblasts. Cell Signal [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007;19(4):761–71.
260. Thannickal VJ, Lee DY, White ES, Cui Z, Larios JM, Chacon R, et al. Myofibroblast differentiation by transforming growth factor-beta1 is dependent on cell adhesion and integrin signaling via focal adhesion kinase. J Biol Chem. 2003;278(14):12384–9.
261. Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH, et al. Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis. J Clin Invest [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2004;114(9):1308–16.
262. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature [Review]. 2003;425(6958):577–84.
263. Sandbo N, Lau A, Kach J, Ngam C, Yau D, Dulin NO. Delayed stress fiber formation mediates pulmonary myofibroblast differentiation in response to TGF-beta. Am J Physiol Lung Cell Mol Physiol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2011;301(5):L656–66.
264. Hecker L, Vittal R, Jones T, Jagirdar R, Luckhardt TR, Horowitz JC, et al. NADPH oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. Nat Med [Research Support, N.I.H., Extramural]. 2009;15(9):1077–81.
265. Sandbo N, Dulin N. Actin cytoskeleton in myofibroblast differentiation: ultrastructure defining form and driving function. Transl Res [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 2011;158(4):181–96.
266. Horowitz JC, Lee DY, Waghray M, Keshamouni VG, Thomas PE, Zhang H, et al. Activation of the pro-survival phosphatidylinositol 3-kinase/AKT pathway by transforming growth

factor-beta1 in mesenchymal cells is mediated by p38 MAPK-dependent induction of an autocrine growth factor. J Biol Chem. 2004;279(2):1359–67.
267. Varga J, Jimenez SA. Stimulation of normal human fibroblast collagen production and processing by transforming growth factor-beta. Biochem Biophys Res Commun [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1986;138(2):974–80.
268. Ignotz RA, Massague J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem. 1986;261(9):4337–45.
269. Pandit KV, Corcoran D, Yousef H, Yarlagadda M, Tzouvelekis A, Gibson KF, et al. Inhibition and role of let-7d in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Clinical Trial Research Support, N.I.H., Extramural]. 2010;182(2):220–9.
270. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. J Exp Med [Research Support, N.I.H., Extramural]. 2010;207(8):1589–97.
271. Aoki J. Mechanisms of lysophosphatidic acid production. Semin Cell Dev Biol [Review]. 2004;15(5):477–89.
272. Funke M, Zhao Z, Xu Y, Chun J, Tager AM. The lysophosphatidic acid receptor LPA1 promotes epithelial cell apoptosis after lung injury. Am J Respir Cell Mol Biol [Research Support, N.I.H., Extramural]. 2012;46(3):355–64.
273. Sakai T, Peyruchaud O, Fassler R, Mosher DF. Restoration of beta1A integrins is required for lysophosphatidic acid-induced migration of beta1-null mouse fibroblastic cells. J Biol Chem [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1998;273(31):19378–82.
274. Fonseca C, Abraham D, Renzoni EA. Endothelin in pulmonary fibrosis. Am J Respir Cell Mol Biol [Review]. 2011;44(1):1–10.
275. Uhal BD, Li X, Piasecki CC, Molina-Molina M. Angiotensin signalling in pulmonary fibrosis. Int J Biochem Cell Biol [Review]. 2012;44(3):465–8.
276. Trojanowska M. Role of PDGF in fibrotic diseases and systemic sclerosis. Rheumatology (Oxford) [Review]. 2008;47(Suppl 5):v2–4.
277. Hardie WD, Hagood JS, Dave V, Perl AK, Whitsett JA, Korfhagen TR, et al. Signaling pathways in the epithelial origins of pulmonary fibrosis. Cell Cycle [Research Support, N.I.H., Extramural Review]. 2010;9(14):2769–76.
278. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE Jr. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. Chest [Research Support, N.I.H., Extramural]. 2008;133(1):226–32.
279. Desai B, Mattson J, Paintal H, Nathan M, Shen F, Beaumont M, et al. Differential expression of monocyte/macrophage- selective markers in human idiopathic pulmonary fibrosis. Exp Lung Res. 2011;37(4):227–38.
280. Pan LH, Ohtani H, Yamauchi K, Nagura H. Co-expression of TNF alpha and IL-1 beta in human acute pulmonary fibrotic diseases: an immunohistochemical analysis. Pathol Int. 1996;46(2):91–9.
281. Zhang Y, Lee TC, Guillemin B, Yu MC, Rom WN. Enhanced IL-1 beta and tumor necrosis factor-alpha release and messenger RNA expression in macrophages from idiopathic pulmonary fibrosis or after asbestos exposure. J Immunol [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1993;150(9):4188–96.
282. Wilson MS, Madala SK, Ramalingam TR, Gochuico BR, Rosas IO, Cheever AW, et al. Bleomycin and IL-1beta-mediated pulmonary fibrosis is IL-17A dependent. J Exp Med [Research Support, N.I.H., Intramural]. 2010;207(3):535–52.
283. Wallace WA, Ramage EA, Lamb D, Howie SE. A type 2 (Th2-like) pattern of immune response predominates in the pulmonary interstitium of patients with cryptogenic fibrosing alveolitis (CFA). Clin Exp Immunol [Research Support, Non-U.S. Gov't]. 1995;101(3):436–41.
284. Wynn TA. Integrating mechanisms of pulmonary fibrosis. J Exp Med [Research Support, N.I.H., Intramural Review]. 2011;208(7):1339–50.

285. Park SW, Ahn MH, Jang HK, Jang AS, Kim DJ, Koh ES, et al. Interleukin-13 and its receptors in idiopathic interstitial pneumonia: clinical implications for lung function. J Korean Med Sci [Research Support, Non-U.S. Gov't]. 2009;24(4):614–20.
286. Moore BB, Kolodsick JE, Thannickal VJ, Cooke K, Moore TA, Hogaboam C, et al. CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. Am J Pathol [Research Support, U.S. Gov't, P.H.S.]. 2005;166(3):675–84.
287. Agostini C, Gurrieri C. Chemokine/cytokine cocktail in idiopathic pulmonary fibrosis. Proc Am Thorac Soc [Research Support, Non-U.S. Gov't Review]. 2006;3(4):357–63.
288. 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 [Research Support, N.I.H., Extramural]. 2007;176(1):78–89.
289. Car BD, Meloni F, Luisetti M, Semenzato G, Gialdroni-Grassi G, Walz A. Elevated IL-8 and MCP-1 in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. Am J Respir Crit Care Med [Comparative Study Research Support, Non-U.S. Gov't]. 1994;149(3 Pt 1):655–9.
290. Standiford TJ, Kunkel SL, Liebler JM, Burdick MD, Gilbert AR, Strieter RM. Gene expression of macrophage inflammatory protein-1 alpha from human blood monocytes and alveolar macrophages is inhibited by interleukin-4. Am J Respir Cell Mol Biol [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1993;9(2):192–8.
291. Standiford TJ, Rolfe MR, Kunkel SL, Lynch JP 3rd, Becker FS, Orringer MB, et al. Altered production and regulation of monocyte chemoattractant protein-1 from pulmonary fibroblasts isolated from patients with idiopathic pulmonary fibrosis. Chest [Comparative Study]. 1993;103(2 Suppl):121S.
292. Standiford TJ, Rolfe MW, Kunkel SL, Lynch JP 3rd, Burdick MD, Gilbert AR, et al. Macrophage inflammatory protein-1 alpha expression in interstitial lung disease. J Immunol [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1993;151(5):2852–63.
293. Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. Semin Liver Dis [Review]. 2010;30(3):245–57.
294. 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.
295. Gibbons MA, MacKinnon AC, Ramachandran P, Dhaliwal K, Duffin R, Phythian-Adams AT, et al. Ly6Chi monocytes direct alternatively activated profibrotic macrophage regulation of lung fibrosis. Am J Respir Crit Care Med [Comparative Study Research Support, Non-U.S. Gov't]. 2011;184(5):569–81.
296. 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.
297. Trujillo G, Meneghin A, Flaherty KR, Sholl LM, Myers JL, Kazerooni EA, et al. TLR9 differentiates rapidly from slowly progressing forms of idiopathic pulmonary fibrosis. Sci Transl Med [Research Support, N.I.H., Extramural]. 2010;2(57):57ra82.
298. Huang Y, Ma SF, Espindola MS, Vij R, Oldham JM, Huffnagle GB, et al. Microbes are associated with host innate immune response in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2017;196(2):208–19.
299. Herazo-Maya JD, Noth I, Duncan SR, Kim S, Ma SF, Tseng GC, et al. Peripheral blood mononuclear cell gene expression profiles predict poor outcome in idiopathic pulmonary fibrosis. Sci Transl Med. 2013;5(205):205ra136.
300. Gilani SR, Vuga LJ, Lindell KO, Gibson KF, Xue J, Kaminski N, et al. CD28 down-regulation on circulating CD4 T-cells is associated with poor prognoses of patients with idiopathic pulmonary fibrosis. PLoS One. 2010;5(1):e8959.
301. Fingerlin TE, Zhang W, Yang IV, Ainsworth HC, Russell PH, Blumhagen RZ, et al. Genome-wide imputation study identifies novel HLA locus for pulmonary fibrosis and potential role for auto-immunity in fibrotic idiopathic interstitial pneumonia. BMC Genet. 2016;17(1):74.

302. Schiller HB, Mayr CH, Leuschner G, Strunz M, Staab-Weijnitz C, Preisendorfer S, et al. Deep proteome profiling reveals common prevalence of MZB1-positive plasma B cells in human lung and skin fibrosis. Am J Respir Crit Care Med. 2017;196(10):1298–310.
303. Turner-Warwick M. Precapillary systemic-pulmonary anastomoses. Thorax. 1963;18:225–37.
304. Simler NR, Brenchley PE, Horrocks AW, Greaves SM, Hasleton PS, Egan JJ. Angiogenic cytokines in patients with idiopathic interstitial pneumonia. Thorax [Clinical Trial Comparative Study Controlled Clinical Trial]. 2004;59(7):581–5.
305. Parra ER, David YR, da Costa LR, Ab'Saber A, Sousa R, Kairalla RA, et al. Heterogeneous remodeling of lung vessels in idiopathic pulmonary fibrosis. Lung [Research Support, Non-US Gov't]. 2005;183(4):291–300.
306. 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 [Comparative Study Evaluation Studies Research Support, Non-U.S. Gov't]. 2004;169(11):1203–8.
307. Renzoni EA, Walsh DA, Salmon M, Wells AU, Sestini P, Nicholson AG, et al. Interstitial vascularity in fibrosing alveolitis. Am J Respir Crit Care Med. 2003;167(3):438–43.
308. Sumi M, Satoh H, Kagohashi K, Ishikawa H, Sekizawa K. Increased serum levels of endostatin in patients with idiopathic pulmonary fibrosis. J Clin Lab Anal. 2005;19(4):146–9.
309. Cosgrove GP, Brown KK, Schiemann WP, Serls AE, Parr JE, Geraci MW, et al. Pigment epithelium-derived factor in idiopathic pulmonary fibrosis: a role in aberrant angiogenesis. Am J Respir Crit Care Med [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2004;170(3):242–51.
310. Raghu G, Striker LJ, Hudson LD, Striker GE. Extracellular matrix in normal and fibrotic human lungs. Am Rev Respir Dis [Research Support, U.S. Gov't, P.H.S.]. 1985;131(2):281–9.
311. Kaarteenaho-Wiik R, Lammi L, Lakari E, Kinnula VL, Risteli J, Ryhanen L, et al. Localization of precursor proteins and mRNA of type I and III collagens in usual interstitial pneumonia and sarcoidosis. J Mol Histol [Research Support, Non-U.S. Gov't]. 2005;36(6–7):437–46.
312. Selman M, Ruiz V, Cabrera S, Segura L, Ramirez R, Barrios R, et al. TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? Am J Physiol Lung Cell Mol Physiol [Research Support, Non-U.S. Gov't]. 2000;279(3):L562–74.
313. Montano M, Ramos C, Gonzalez G, Vadillo F, Pardo A, Selman M. Lung collagenase inhibitors and spontaneous and latent collagenase activity in idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. Chest. 1989;96(5):1115–9.
314. Gadek JE, Kelman JA, Fells G, Weinberger SE, Horwitz AL, Reynolds HY, et al. Collagenase in the lower respiratory tract of patients with idiopathic pulmonary fibrosis. N Engl J Med. 1979;301(14):737–42.
315. Garcia-Alvarez J, Ramirez R, Sampieri CL, Nuttall RK, Edwards DR, Selman M, et al. Membrane type-matrix metalloproteinases in idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis [Comparative Study Research Support, Non-U.S. Gov't]. 2006;23(1):13–21.
316. Pardo A, Selman M, Kaminski N. Approaching the degradome in idiopathic pulmonary fibrosis. Int J Biochem Cell Biol [Review]. 2008;40(6–7):1141–55.
317. Agnihotri R, Crawford HC, Haro H, Matrisian LM, Havrda MC, Liaw L. Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin). J Biolog Chem [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2001;276(30):28261–7.
318. Loffek S, Schilling O, Franzke CW. Series ":matrix metalloproteinases in lung health and disease": biological role of matrix metalloproteinases: a critical balance. Eur Respir J [Research Support, Non-U.S. Gov't Review]. 2011;38(1):191–208.
319. Fukuda Y, Ishizaki M, Kudoh S, Kitaichi M, Yamanaka N. Localization of matrix metalloproteinases-1, -2, and -9 and tissue inhibitor of metalloproteinase-2 in interstitial lung diseases. Lab Invest [Research Support, Non-U.S. Gov't]. 1998;78(6):687–98.
320. Hayashi T, Stetler-Stevenson WG, Fleming MV, Fishback N, Koss MN, Liotta LA, et al. Immunohistochemical study of metalloproteinases and their tissue inhibitors in the lungs of patients with diffuse alveolar damage and idiopathic pulmonary fibrosis. Am J Pathol. 1996;149(4):1241–56.

321. Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. Am J Respir Cell Mol Biol [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. 2003;28(1):12–24.
322. Lemjabbar H, Gosset P, Lechapt-Zalcman E, Franco-Montoya ML, Wallaert B, Harf A, et al. Overexpression of alveolar macrophage gelatinase B (MMP-9) in patients with idiopathic pulmonary fibrosis: effects of steroid and immunosuppressive treatment. Am J Respir Cell Mol Biol. 1999;20(5):903–13.
323. Suga M, Iyonaga K, Okamoto T, Gushima Y, Miyakawa H, Akaike T, et al. Characteristic elevation of matrix metalloproteinase activity in idiopathic interstitial pneumonias. Am J Respir Crit Care Med [Research Support, Non-U.S. Gov't]. 2000;162(5):1949–56.
324. Egeblad M, Nakasone ES, Werb Z. Tumors as organs: complex tissues that interface with the entire organism. Dev Cell [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 2010;18(6):884–901.
325. Muro AF, Moretti FA, Moore BB, Yan M, Atrasz RG, Wilke CA, et al. An essential role for fibronectin extra type III domain A in pulmonary fibrosis. Am J Respir Crit Care Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2008;177(6):638–45.
326. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc Natl Acad Sci U S A [Randomized Controlled Trial Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007;104(40):15858–63.
327. Okamoto M, Hoshino T, Kitasato Y, Sakazaki Y, Kawayama T, Fujimoto K, et al. Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. Eur Respir J [Research Support, Non-U.S. Gov't]. 2011;37(5):1119–27.
328. Uchida M, Shiraishi H, Ohta S, Arima K, Taniguchi K, Suzuki S, et al. Periostin, a matricellular protein, plays a role in the induction of chemokines in pulmonary fibrosis. Am J Respir Cell Mol Biol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2012;46(5):677–86.
329. Li Y, Jiang D, Liang J, Meltzer EB, Gray A, Miura R, et al. Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. J Exp Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2011;208(7):1459–71.
330. Sottile J, Hocking DC. Fibronectin polymerization regulates the composition and stability of extracellular matrix fibrils and cell-matrix adhesions. Mol Biol Cell [Research Support, U.S. Gov't, P.H.S.]. 2002;13(10):3546–59.
331. Lucero HA, Kagan HM. Lysyl oxidase: an oxidative enzyme and effector of cell function. Cell Mol Life Sci [Research Support, N.I.H., Extramural Review]. 2006;63(19–20):2304–16.
332. Olsen KC, Sapinoro RE, Kottmann RM, Kulkarni AA, Iismaa SE, Johnson GV, et al. Transglutaminase 2 and its role in pulmonary fibrosis. Am J Respir Crit Care Med [Research Support, N.I.H., Extramural]. 2011;184(6):699–707.
333. Liu F, Tschumperlin DJ. Micro-mechanical characterization of lung tissue using atomic force microscopy. J Vis Exp [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S. Video-Audio Media]. 2011;54:2911.
334. Liu F, Mih JD, Shea BS, Kho AT, Sharif AS, Tager AM, et al. Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. J Cell Biol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. 2010;190(4):693–706.
335. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science (New York, NY). 2005;310(5751):1139–43.
336. Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am J Pathol. 2001;159(3):1009–20.
337. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. 2002;3(5):349–63.

338. Grinnell F, Zhu M, Carlson MA, Abrams JM. Release of mechanical tension triggers apoptosis of human fibroblasts in a model of regressing granulation tissue. Exp Cell Res [Research Support, U.S. Gov't, P.H.S.]. 1999;248(2):608–19.
339. Huang X, Yang N, Fiore VF, Barker TH, Sun Y, Morris SW, et al. Matrix stiffness-induced myofibroblast differentiation is mediated by intrinsic Mechanotransduction. Am J Respir Cell Mol Biol. 2012;47:340–8.
340. Goffin JM, Pittet P, Csucs G, Lussi JW, Meister JJ, Hinz B. Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. J Cell Biol [Research Support, Non-U.S. Gov't]. 2006;172(2):259–68.
341. Maeda T, Sakabe T, Sunaga A, Sakai K, Rivera AL, Keene DR, et al. Conversion of mechanical force into TGF-beta-mediated biochemical signals. Curr Biol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2011;21(11):933–41.
342. Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T, et al. Latent TGF-beta structure and activation. Nature [Research Support, Non-U.S. Gov't]. 2011;474(7351):343–9.
343. Arora PD, Narani N, McCulloch CA. The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. Am J Pathol. 1999;154(3):871–82.
344. Li Z, Dranoff JA, Chan EP, Uemura M, Sevigny J, Wells RG. Transforming growth factor-beta and substrate stiffness regulate portal fibroblast activation in culture. Hepatology [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007;46(4):1246–56.
345. Marinkovic A, Mih JD, Park JA, Liu F, Tschumperlin DJ. Improved throughput traction microscopy reveals pivotal role for matrix stiffness in fibroblast contractility and TGF-beta responsiveness. Am J Physiol Lung Cell Mol Physiol. 2012;303(3):L169–80.
346. Leight JL, Wozniak MA, Chen S, Lynch ML, Chen CS. Matrix rigidity regulates a switch between TGF-beta1-induced apoptosis and epithelial-mesenchymal transition. Mol Biol Cell [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. 2012;23(5):781–91.
347. Balestrini JL, Chaudhry S, Sarrazy V, Koehler A, Hinz B. The mechanical memory of lung myofibroblasts. Integr Biol (Camb) [Research Support, Non-U.S. Gov't]. 2012;4(4):410–21.
348. Pierce EM, Carpenter K, Jakubzick C, Kunkel SL, Flaherty KR, Martinez FJ, et al. Therapeutic targeting of CC ligand 21 or CC chemokine receptor 7 abrogates pulmonary fibrosis induced by the adoptive transfer of human pulmonary fibroblasts to immunodeficient mice. Am J Pathol [Research Support, N.I.H., Extramural]. 2007;170(4):1152–64.
349. Chadalavada RS, Korkola JE, Houldsworth J, Olshen AB, Bosl GJ, Studer L, et al. Constitutive gene expression predisposes morphogen-mediated cell fate responses of NT2/D1 and 27X-1 human embryonal carcinoma cells. Stem Cells [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007;25(3):771–8.
350. Rajkumar R, Konishi K, Richards TJ, Ishizawar DC, Wiechert AC, Kaminski N, et al. Genomewide RNA expression profiling in lung identifies distinct signatures in idiopathic pulmonary arterial hypertension and secondary pulmonary hypertension. Am J Physiol Heart Circ Physiol [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2010;298(4):H1235–48.
351. Selman M, Carrillo G, Estrada A, Mejia M, Becerril C, Cisneros J, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. PLoS One [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007;2(5):e482.
352. Boon K, Bailey NW, Yang J, Steel MP, Groshong S, Kervitsky D, et al. Molecular phenotypes distinguish patients with relatively stable from progressive idiopathic pulmonary fibrosis (IPF). PLoS One. 2009;4(4):e5134.
353. Huang Y, Ma SF, Vij R, Oldham JM, Herazo-Maya J, Broderick SM, et al. A functional genomic model for predicting prognosis in idiopathic pulmonary fibrosis. BMC Pulm Med [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2015;15:147.

354. 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 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2009;180(2):167–75.
355. 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 [Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't]. 2008;5(4):e93.
356. Korfei M, Schmitt S, Ruppert C, Henneke I, Markart P, Loeh B, et al. Comparative proteomic analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF) and lung transplant donor lungs. J Proteome Res [Comparative Study Research Support, Non-U.S. Gov't]. 2011;10(5):2185–205.
357. Korfei M, von der Beck D, Henneke I, Markart P, Ruppert C, Mahavadi P, et al. Comparative proteome analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP) and organ donors. J Proteome. 2013;85:109–28.
358. O'Dwyer DN, Norman KC, Xia M, Huang Y, Gurczynski SJ, Ashley SL, et al. The peripheral blood proteome signature of idiopathic pulmonary fibrosis is distinct from normal and is associated with novel immunological processes. Sci Rep. 2017;7:46560.
359. Milosevic J, Pandit K, Magister M, Rabinovich E, Ellwanger DC, Yu G, et al. Profibrotic role of miR-154 in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2012;47(6):879–87.
360. Pandit KV, Milosevic J. MicroRNA regulatory networks in idiopathic pulmonary fibrosis. Biochem Cell Biol. 2015;93(2):129–37.
361. Dey N, Ghosh-Choudhury N, Kasinath BS, Choudhury GG. TGFbeta-stimulated microRNA-21 utilizes PTEN to orchestrate AKT/mTORC1 signaling for mesangial cell hypertrophy and matrix expansion. PLoS One. 2012;7(8):e42316.
362. Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F, et al. miR-29 is a major regulator of genes associated with pulmonary fibrosis. Am J Respir Cell Mol Biol. 2011;45(2):287–94.
363. Parker MW, Rossi D, Peterson M, Smith K, Sikstrom K, White ES, et al. Fibrotic extracellular matrix activates a profibrotic positive feedback loop. J Clin Invest. 2014;124(4):1622–35.
364. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. Science (New York, NY) [Research Support, U.S. Gov't, P.H.S.]. 2001;293(5532):1068–70.
365. Rauch TA, Zhong X, Wu X, Wang M, Kernstine KH, Wang Z, et al. High-resolution mapping of DNA hypermethylation and hypomethylation in lung cancer. Proc Natl Acad Sci U S A [Research Support, N.I.H., Extramural]. 2008;105(1):252–7.
366. Rabinovich EI, Kapetanaki MG, Steinfeld I, Gibson KF, Pandit KV, Yu G, et al. Global methylation patterns in idiopathic pulmonary fibrosis. PLoS One [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2012;7(4):e33770.
367. Sanders YY, Ambalavanan N, Halloran B, Zhang X, Liu H, Crossman DK, et al. Altered DNA methylation profile in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186(6):525–35.
368. Coward WR, Watts K, Feghali-Bostwick CA, Jenkins G, Pang L. Repression of IP-10 by interactions between histone deacetylation and hypermethylation in idiopathic pulmonary fibrosis. Mol Cell Biol [Research Support, Non-U.S. Gov't]. 2010;30(12):2874–86.
369. Ramirez G, Hagood JS, Sanders Y, Ramirez R, Becerril C, Segura L, et al. Absence of Thy-1 results in TGF-beta induced MMP-9 expression and confers a profibrotic phenotype to human lung fibroblasts. Lab Invest [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2011;91(8):1206–18.
370. Hu B, Gharaee-Kermani M, Wu Z, Phan SH. Epigenetic regulation of myofibroblast differentiation by DNA methylation. Am J Pathol [Research Support, N.I.H., Extramural]. 2010;177(1):21–8.
371. 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):857–68.

Chapter 8
Genetics of Pulmonary Fibrosis

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Introduction

Interstitial lung disease (ILD) is a heterogeneous group of disorders characterized by abnormalities in the space between the alveolar epithelial cells and the capillary vascular endothelial cells. The observation that a progressive lung fibrosis can affect multiple members of the same family demonstrates a role of genetics in the underlying pathogenesis of this disease. Recent research in this field has led to a deeper understanding of the genes and genetic variants that are linked to familial pulmonary fibrosis (FPF). The same genetic mechanisms important in FPF are also relevant to sporadic forms of ILD, especially idiopathic pulmonary fibrosis (IPF). These insights have begun to reveal the molecular basis of a disease that was initially thought to be of unknown cause.

Epidemiology and Clinical Manifestations of Familial Pulmonary Fibrosis

Familial pulmonary fibrosis (FPF) is characterized by the presence of pulmonary fibrosis in two or more individuals from the same family. It encompasses

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those with familial interstitial pneumonia (FIP), in which affected individuals have a diagnosis of one of several different idiopathic interstitial pneumonias (IIPs) [1]. Affected individuals in FPF kindreds also may have a diagnosis of a fibrotic ILD of known cause, such as chronic hypersensitivity pneumonitis [2, 3]. In FPF families, IPF is often the most common diagnosis [1, 3]. Vertical transmission and an autosomal dominant pattern of inheritance are seen in most kindreds.

FPF is uncommon in the general population, with a prevalence estimated to be 1.3–5.9 per million [4]. In comparison, the prevalence of idiopathic pulmonary fibrosis (IPF), the most common IIP, has been estimated to be between 2 and 42 per 100,000 persons [5]. A positive family history, or the occurrence of another first- or second-degree family member with a fibrotic ILD, has been reported in 2–20% of cases [4, 6, 7]. This wide range is likely explained by various definitions used, as well as the various cohorts studied.

The diversity of clinical manifestations of FPF was first demonstrated by Steele and colleagues in 2005 [1]. This study evaluated 111 families with 2 or more relatives affected by an IIP and found that 45% of families included patients with different IIP subtypes. Subsequent data have confirmed this heterogeneity, finding that both IIPs and ILDs of known cause may be present within the same family [3, 8]. One series of 289 FPF patients reported an unclassifiable radiographic presentation in 50%, usual interstitial pneumonia in 22%, nonspecific interstitial pneumonia (NSIP) in 12%, and organizing pneumonia (OP) in 1% [8]. Newton and colleagues determined multidisciplinary diagnoses for 115 patients with FPF and heterozygous mutations in 4 telomere-related genes [3]. A diagnosis of IPF was found in 46% of patients, unclassifiable ILD in 20%, chronic hypersensitivity pneumonitis (HP) in 12%, pleuroparenchymal fibroelastosis (PPFE) in 10%, interstitial pneumonia with autoimmune features (IPAF) in 7%, an IIP other than IPF in 4%, and connective tissue disease-associated ILD in 3%.

Retrospective studies of patients with HP and pleuroparenchymal fibroelastosis (PPFE) have supported these findings. A retrospective Japanese study of patients with HP revealed that 17.5% of HP patients have a family history of pulmonary fibrosis [9], while a study of 12 PPFE patients revealed that 2 had a family history of pulmonary fibrosis [10].

Even asymptomatic first-degree relatives of FPF patients may exhibit a variety of manifestations of subclinical pulmonary disease, including radiographic abnormalities, reduced single-breath diffusion capacity, and reduced recruitment of diffusion capacity with exercise [11]. Another study found radiographic abnormalities in 14% of asymptomatic first-degree relatives of patients with FPF at a mean age of 50.8 years [12]. The most common findings included septal thickening, peribronchovascular thickening, subpleural reticulations, and ground-glass opacities. Transbronchial biopsies were abnormal in 35% with histopathologic findings of interstitial fibrosis, peribronchiolar fibrosis, chronic inflammation, granulomas, and respiratory bronchiolitis. These studies demonstrate that even the earliest subclinical pulmonary manifestations of lung disease in asymptomatic relatives are diverse.

Classification of Genetic Variants

Genetic variants are alterations in the DNA sequence that differ from a reference sequence. Variants are predominantly classified by allele frequency. Common variants have a minor allele frequency (MAF) >5%. Variants that are termed "rare" are much less common in the population, generally with a MAF <0.1%.

Humans have two copies of each gene located on autosomal (non-sex) chromosomes, one inherited from the father and the other from the mother. Consider a single nucleotide variant with two alleles, G and T. Thus, the three possible genotypes at this position are GG, GT, and TT. Suppose, in a group of 100 individuals, 81 are found to be homozygous for the G allele (GG), 18 are found to be heterozygous (GT), and 1 is found to be homozygous for the minor allele (TT). Then the frequency of the T allele is estimated as the fraction of all chromosomes in the sample that carry the T allele, that is, $(18 \times 1 + 1 \times 2)/(100 \times 2)$ or 10%. Note that the MAF is not equivalent to the frequency of individuals carrying an allele. For, as in this example, 19 out of 100 (19%) individuals have at least one copy of the minor T allele. Thus, the carrier frequency is 19%, whereas the MAF is 10%.

Variants are also classified by their predicted effect on the RNA transcript or the protein function. Variants in the promoter region of a gene, for example, may cause a change in gene transcription. Variants in the coding region of the protein are predicted to lead to a loss of function of the protein if they change an amino acid residue to a stop codon, if they alter residues in the canonical splice donor ("gu-") or splice acceptor ("-ag") sites, or if they lead to a frameshift and a premature truncation of the protein. The degree of conservation of a particular amino acid across species may predict tolerance to missense variants that change an individual amino acid. In vitro testing of protein function or in silico prediction programs can be used to estimate the effects of missense variants.

Recent genetic sequencing studies have shown that most human genetic variants are rare or extremely rare [13–15]. Rare variants are more likely to affect the structure or function of proteins than common variants. Evolution predicts that deleterious variants responsible for human disease should be uncommon and recent [16]. So, if deleterious variants are extremely rare and each individual has thousands of rare variants, then a major challenge exists to determine which of these potentially deleterious variants are playing a role in disease.

The American College of Medical Genetics and the European Society of Human Genetics have classified genetic variants into five categories: pathogenic, likely pathogenic, variant uncertain significance (VUS), likely benign, and benign (Fig. 8.1) [17, 18]. Variants with a clear causal link to disease are classified as pathogenic, whereas those that have been shown to have no correlation with disease are benign. Many variants, however, fall in the VUS category, which can make the results of genetic testing challenging to interpret. A "novel" variant, or one that is unique to an individual and which has not been reported in a disease-specific database, may be classified as a VUS. If later studies, such as the demonstration of segregation analysis in families or the finding of additional reports of the variant in

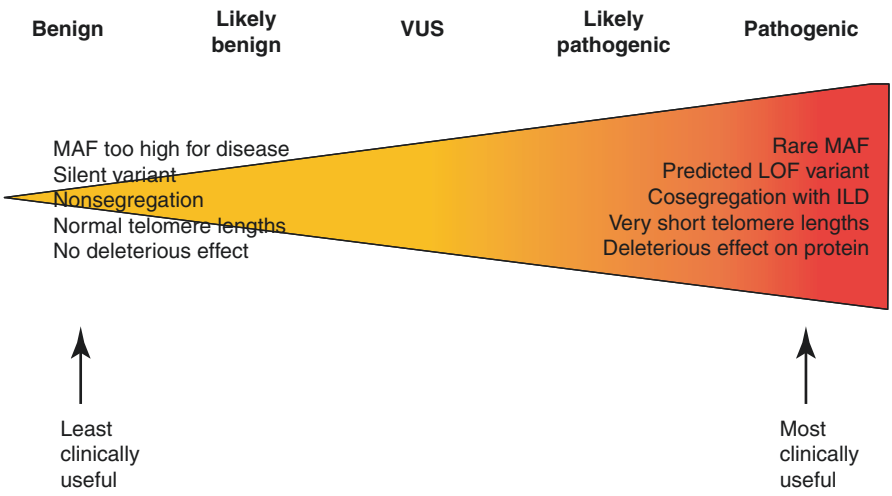

Fig. 8.1 Classification of genetic variants. Genetic variants are classified into one of the five categories: benign, likely benign, variant of uncertain significance (VUS), likely pathogenic, and pathogenic. The type of evidence to support each variant class in ILD patients includes the variant minor allele frequency (MAF) in comparison with disease frequency; the predicted effect of the variant on protein function with silent changes predicting little impact on protein function and loss of function (LOF) variants predicting a null variant in telomere-related genes; and co-segregation of the variant with ILD in multiple affected family members, telomere length with extremely short lengths in support of pathogenic variants in telomere-related genes, and well-established functional studies showing an alteration of protein function. The clinical significance of any sequence variant falls on a gradient, ranging from those that are certainly benign to those that are certainly pathogenic

patients with the same disease, suggest that the variant may be pathogenic, its classification may change over time.

Study Designs to Assess Effects of Common and Rare Variants

Common variants, or single nucleotide polymorphisms (SNPs), are commonly assessed through the use of genome-wide association studies (GWAS). These evaluate for differences in genetic variant frequencies across cohorts of cases and controls. The ability to find a statistical significance in this type of analysis depends on the effect size of an individual variant as well as the sample size of the cohorts. Large sample sizes have more power to detect differences than small sample sizes. GWAS findings that are confirmed in an independent replication cohort are less likely to be due to spurious associations from subtle differences in ancestry between cases and controls.

Exome sequencing, in contrast to GWAS studies, provides sequence information for nearly all bases in the coding region of a gene. While exome sequencing

generates much more data than GWAS, it is generally blind to noncoding regions. Thus, an exome sequencing study may not capture SNPs located in the promoter or introns of genes. In whole genome sequencing, the entire genome, rather than just the coding regions, is sequenced. In sequencing studies, the variants in each gene are compared across cohorts of cases and controls to determine if there are differences between the nature and number of variants per gene.

The cost of genotyping greatly influences study design. Previously, sequencing studies were prohibitively expensive to conduct on large populations. As technological advances have driven down costs, sequencing is now much more feasible. In time, whole genome sequencing may be routinely used to analyze all coding and noncoding variants across the genome.

MUC5B Promoter Polymorphism

MUC5B is a gene that encodes mucin 5B, which is a highly glycosylated protein component of mucus. Mucin 5B lubricates the oral cavity, lung, and cervix; it has a crucial role in innate immune function [19]. In 2011, Seibold and colleagues identified SNP rs35705950, which is located 3 kb upstream of the *MUC5B* transcription start site, as being significantly associated with familial and sporadic IPF [20]. The frequency of this common variant was compared between patients and healthy control groups. The MAF of the rs35705950 variant was 33.8% in FPF patients, 37.5% in sporadic IPF patients, and 9.1% in controls. The ORs for disease was 6.8 (95% CI 3.9–12.0) and 20.8 (95% CI 3.8–113.7) for FPF patients who are heterozygous and homozygous, respectively, for the risk allele. Similarly, the OR for IPF was 9.0 (95% CI 6.2–13.1) and 21.8 (95% CI 5.1–93.5) for sporadic IPF patents who are heterozygous and homozygous, respectively, for this allele.

The rs35705950 variant is associated with upregulation of *MUC5B* expression in healthy lung tissue. Mucin expression is 14-fold higher in lung disease from affected individuals versus unaffected controls [20]. This increased expression occurs primarily in the distal airways rather than in honeycomb cysts [21].

The association between *MUC5B* promoter variant rs35705950 and IPF has been validated in multiple independent patient cohorts collected from different countries and with different ethnicities [20, 22–28]. The MAF of this risk allele varies across populations, ranging from 0.8% to 12% in healthy controls of Japanese and white ethnicity, respectively [25, 29]. Its allele frequency also varies across IPF patient cohorts, from 3.4% to 41.9% in Japanese and French IPF patients, respectively [23, 29]. Despite these wide ranges, it has been found to be statistically associated with IPF in patients identified from the United States, France, Italy, the United Kingdom, Germany, and Japan.

The *MUC5B* promoter variant rs35705950 appears to be a risk factor that is more specific for certain types of pulmonary fibrosis, including IPF and chronic HP [28]. This minor allele was not associated with non-IPF diagnoses of patients banked by

the Lung Tissue Research Consortium [27]. It was not associated with ILD due to systemic sclerosis or sarcoidosis [23, 26].

The rs35705950 variant has also been associated with radiographic interstitial lung abnormalities (ILAs), which are thought to be a precursor of IPF. It has been hypothesized that a proportion of individuals with ILA radiographic findings progress over time to clinical ILD. Those with ILAs demonstrate increased mortality from respiratory causes [30, 31]. The odds for ILAs were 2.8 times higher for each copy of the rs35705950 minor allele in the Framingham Heart Study [32], and ILA progression was associated with increasing age and *MUC5B* promoter phenotype [31].

Other Common Variants in Fibrosing IIP

Several other common variants have been statistically associated with fibrosing IIPs. A GWAS study found an association between rs2736100 in intron 2 of the *TERT* gene and IPF patients from Japan [33]. A GWAS study of sporadic IPF patients revealed associations in common variants in genes encoding the Toll-interacting protein (*TOLLIP*) and signal peptidase-like 2C (*SPPL2C*) [25].

A large GWAS study by Fingerlin and colleagues compared the frequency of common variants in 1616 patients with fibrotic IIP with 4683 controls, with replication analysis using 876 IIP cases and 1890 controls [24]. The study was the largest of its kind and confirmed previously identified genetic associations between fibrotic IIP and SNPs in the *MUC5B* and *TERT* genes. Seven novel loci were also associated with IIP. The common variants identified in this study were found in genes that have a role in host defense (*MUC5B*, *ATP11A*), cell adhesion (*DSP*, *DPP9*), and telomere length (*TERT*, *TERC*, *OBFC1*), which suggests that the pathogenesis of fibrotic IIPs may involve disparate pathways.

Prognostic Information from Common Variants

Common variants may inform prognosis and response to treatment of IPF patients. The *MUC5B* rs35705950 risk variant is associated with improved survival in sporadic IPF compared to subjects without the variant [34]. Similarly, individuals who have the *TOLLIP* rs5743890 risk allele have better outcomes than those individuals with the protective allele [25].

In a retrospective analysis of data from the PANTHER-IPF trial, a significant interaction was seen between a variant in *TOLLIP* (rs3750920) and N-acetylcysteine (NAC) [35]. Those with the rs3750920 TT genotype who were treated with NAC had a decreased risk of the composite outcome of death, transplantation, hospitalization, or greater than a 10% decline in forced vital capacity (FVC). In contrast, those with the CC genotype who received NAC had an increased risk of the composite endpoint. These findings possibly suggest that genotype-stratified patient populations may respond differently to IPF therapies and deserve additional study.

Rare Variants in Pulmonary Fibrosis

Pathogenic rare variants, in comparison with common variants, have much lower allele frequencies and have much greater effect sizes than SNPs in causing pulmonary fibrosis. The inherited predisposition to FPF has been linked to germline rare variants in at least ten different genes. More mutations have been described in *TERT*, the protein component of telomerase, than any other gene. Over 90 different variants in *TERT* have been reported in FPF and sporadic ILD patients (Table 8.1); all are individually rare. Pathogenic rare variants in *TERC*, *RTEL1*, *PARN*, *NAF1*, *DKC1*, *TINF2*, *SFTPC*, *SFTPA1*, and *SFTPA2* are also associated with an increased risk for developing severe adult-onset pulmonary fibrosis. Reduced penetrance is seen in all large kindreds, even those with well-characterized pathogenic variants [2, 36, 37]. These findings underscore the threefold complexity of rare variants linked to pulmonary fibrosis: (1) involvement of multiple genes (locus heterogeneity), (2) multiple different pathogenic variants within each gene (allelic heterogeneity), and (3) reduced penetrance of pulmonary fibrosis for individuals carrying the risk allele.

Figure 8.2 summarizes the genetic locus heterogeneity observed in one FPF cohort. Panel A describes the percentage of FPF kindreds linked to pathogenic variants in seven different genes. Panel B describes the leukocyte telomere lengths of probands of FPF kindreds from this same cohort. It shows that there are a disproportionate number of probands with short telomere lengths: ~30% have telomere lengths <1st percentile (adjusted for age), and an additional ~15% have telomere lengths between the 1st and 10th percentile. Short leukocyte telomere lengths characterize the majority individuals with pathogenic variants in telomere-related genes. This FPF collection can be considered a collection of discrete genetic subtypes. Families can be grouped by gene with ~20% characterized as *TERT*-associated FPF. They can also be grouped by pathway with ~30% characterized as telomere-related FPFs and 3% as surfactant-related FPFs. At this time, most FPF kindreds are currently unexplained.

Table 8.1 demonstrates the degree of allelic heterogeneity seen in one gene, *TERT*. There are nearly 100 different rare variants in this gene that have been linked to FPF or sporadic ILD in the literature. The allele frequency for each is <0.01%. Rare variants in this gene have been found in patients collected across the globe, including the United States, France, Canada, Brazil, and China. Some variants have been found in multiple different unrelated families. For example, the p.Arg865His variant has been described in unrelated families from the United States, Brazil, and Newfoundland [2, 38, 39]. The level of evidence in support of the pathogenicity for each variant varies widely. Data to support the pathogenicity of some variants includes a very rare allele frequency, identification in multiple unrelated individuals with the same phenotype, co-segregation analysis with disease in large kindreds, association with short telomere lengths, and demonstration of decreased in vitro protein activity. For others, the strength of evidence in support of pathogenicity is much less robust. For this latter group, clinical counseling would be ambiguous without additional information to support the pathogenicity of the variant.

Table 8.1 Rare variants in *TERT* reported in association with familial pulmonary fibrosis or sporadic ILD

Gene	DNA change[a]	Impact on protein[a]	Frequency in ExAC database[b]	No. of unrelated families	Leukocyte telomere length percentile (mean)	Co-segregation with ILD[c]?	Decreased protein function?	References
TERT	c.22_43dup22	p.Arg15ProfsX184	Absent	1		Yes		[94]
TERT	c.55C>T	p.Arg19Cys	Absent	1				[94]
TERT	c.97C>T	p.Pro33Ser	Absent	1	<1st			[2, 3]
TERT	c.164T>A	p.Leu55Gln	Absent	1	<10th		Yes	[36]
TERT	c.198_207del	p.Ala67ProfsX8	Absent	1				[94]
TERT	c.219+1G>A	Splicing	Absent	1	<1st	Yes		[36]
TERT	c.228C>A	p.Cys76X	Absent	1				[94]
TERT	c.293C>A	p.Ala98Asp	Absent	1	<1st			[3]
TERT	c.377C>A	p.Thr126Lys	0.00001662	1	<5th			[3]
TERT	c.395G>A	p.Arg132Gln	Absent	1		Yes		[94]
TERT	c.416T>G	p.Leu139Arg	Absent	1	<1st			[3]
TERT	c.430G>A	p.Val144Met	Absent	2	<1st	Yes	Yes	[2, 3, 54]
TERT	c.508G>A	p.Val170Met	0.00002134	2	<1st		Yes	[93]
TERT	c.569C>T	p.Ala190Val	0.00001765	1	<5th			[3]
TERT	c.330delC	p.Pro112ProfsX16	Absent	1				[36]
TERT	c.1002_1004delCTC	p.Ser335del	Absent	1	<1st			[3]
TERT	c.1103C>T	p.Ser368Phe	Absent	1				[96]
TERT	c.1397G>C	p.Arg466Pro	Absent	1	<1st			[3]
TERT	c.1417G>C	p.Val473Leu	Absent	1	<5th			[3]
TERT	c.1456C>T	p.Arg486Cys	Absent	1	<5th	Yes	Yes	[2, 3]
TERT	c.1511C>T	p.Ser504Leu	Absent	1		Yes		[94]
TERT	c.1603C>T	p.Arg535Cys	Absent	1	<1st			[3]
TERT	c.1627A>G	p.Lys543Glu	Absent	1				[94]
TERT	c.1630T>C	p.Phe544Leu	Absent	1		Yes		[94]

TERT	c.1710G>T	p.Lys570Asn	Absent	3	<1st		Yes	[3, 94, 96, 97]
TERT	c.1770-2A>G	Splicing	Absent	1				[42]
TERT	c.1864C>T	p.Arg622Cys	Absent	2				[94]
TERT	c.1865G>A	p.Arg622His	Absent	1				[98]
TERT	c.1885G>C	p.Gly629Arg	Absent	1				[42]
TERT	c.1892G>A	p.Arg631Gln	Absent	2	<1st	Yes	Yes	[3, 38, 54]
TERT	c.1895C>T	p.Pro632Leu	Absent	1	<1st			[3]
TERT	c.1931C>T	p.Thr644Met	0.00005773	1				[98]
TERT	c.1989C>G	p.Ser663Arg	Absent	1				[42]
TERT	c.2006C>T	p.Arg669Gln	Absent	1	<10th			[3]
TERT	c.2011C>T	p.Arg671Trp	Absent	1	<1st		Yes	[3, 54]
TERT	c.2033C>A	p.Ala678Asp	Absent	1	<1st			[3, 99]
TERT	c.2069G>C	p.Trp690Ser	Absent	1				[42]
TERT	c.2080G>A	p.Val694Met	Absent	1	<5th		Yes	[3, 66, 100]
TERT	c.2081T>A	p.Val694Glu	Absent	1				[42]
TERT	c.2093G>A	p.Arg698Gln	Absent	1				[94]
TERT	c.2105C>T	p.Pro702Leu	Absent	2	<1st	Yes	Yes	[3, 66, 94]
TERT	c.2110C>T	p.Pro704Ser[d]	Absent	5	<5th	Yes	Yes	[3, 42, 66, 101]
TERT	c.2146G>A	p.Ala716Thr	Absent	2	<1st	Yes	Yes	[93, 94]
TERT	c.2159T>C	p.Ile720Thr	Absent	1				[94]
TERT	c.2225G>A	p.Arg742His	Absent	1				[42]
TERT	c.2240delT	p.Val747fsX20	Absent	1	<5th	Yes	Yes	[2, 3]
TERT	c.2266C>T	p.Arg756Cys	0.000008238	1	<1st			[3]
TERT	c.2267G>T	p.Arg756Leu	Absent	1				[94]
TERT	c.2287-2A>G	Splicing	Absent	1				[94]

(continued)

Table 8.1 (continued)

Gene	DNA change[a]	Impact on protein[a]	Frequency in ExAC database[b]	No. of unrelated families	Leukocyte telomere length percentile (mean)	Co-segregation with ILD[c]?	Decreased protein function?	References
TERT	c.2383-2A>G	Splicing	Absent	1		Yes		[94, 102]
TERT	c.[2431C>T;2433C>T][e]	p.Arg811Cys	Absent	1	<1st	Yes		[3]
TERT	c.2469-2A>C	Splicing	Absent	1	<10th	Yes	Yes	[36]
TERT	c.2469-2A>T	Splicing	Absent	1	<1st	Yes		[3]
TERT	c.2521C>T	p.Leu841Phe	Absent	2	<5th		Yes	[3, 93]
TERT	c.2539G>A	p.Gly847Ser	Absent	1	<10th			[3]
TERT	c.2572C>T	p.Arg858Trp	Absent	1	<1st			[3]
TERT	c.2593C>T	p.Arg865Cys	Absent	1	<5th		Yes	[2, 3]
TERT	c.2594G>A	p.Arg865His	0.00001278	3	<1st	Yes	Yes	[2, 3, 38, 39]
TERT	c.2371G>A and c.2599G>A	p.Val791Ile and Val867Met	Absent	2	<10th	Yes	Yes	[103]
TERT	c.2599G>A	p.Val867Met	Absent	1	<10th		Yes	[3, 54]
TERT	c.2620A>G	p.Thr874Ala	Absent	1				[42]
TERT	c.2621C>G	p.Thr874Arg	Absent	1	<1st	Yes		[3]
TERT	c.2638G>A	p.Ala880Thr	Absent	4				[94]
TERT	c.2648T>G	p.Phe883Cys	Absent	1	<10th	Yes		[38]
TERT	c.2647T>A	p.Phe883Ile	Absent	1	<1st			[3]
TERT	c.2678A>T	p.Glu893Val	Absent	1				[94]
TERT	c.2705A>G	Lys902Arg	Absent	1	<1st	Yes	Yes	[60, 93]
TERT	c:2768C>T	p.Pro923Leu	Absent	1	<1st			[104]
TERT	c.2775C>A	p.His925Gln	0.00001674	1	~50th[f]		Yes	[3, 54]
TERT	c.2812C>T	p.Arg938Trp	Absent	1				[42]
TERT	c.2843+1G>A	Splicing	Absent	1		Yes		[94]
TERT	c.2849delC	p.Arg951GlyfsX30	Absent	1		Yes		[94]

TERT	c.2851C>T	p.Arg951Trp	0.000008290	1	<10th	Yes	Yes	[3, 54]
TERT	c.2869A>C	p.Ser957Arg	Absent	1	<1st		Yes	[3, 66]
TERT	c.2911C>T	p.Arg971Cys	Absent	3		Yes		[94]
TERT	c.2912G>A	p.Arg971His	Absent	1	<1st			[3]
TERT	c.2935C>T	p.Arg979Trp	Absent	2		Yes		[94]
TERT	c.2945G>A	p.Cys982Tyr	Absent	1				[94]
TERT	c.2947C>T	p.His983Tyr	Absent	1	<1st			[3]
TERT	c.2968C>T	p.Gln990X	Absent	1		Yes		[94]
TERT	c.2991delG	p.Val997Valfs**X**51	Absent	1	<1st	Yes		[95, 105]
TERT	c.3026C>T	p.Ala1009Val	Absent	1				[94]
TERT	c.3055C>T	p.Leu1019Phe	Absent	1	<1st		Yes	[3, 66]
TERT	c.3093_3094deTTlinsCA	p.Phe1032Ile	Absent	1				[98]
TERT	c.3148A>G	p.Lys1050Glu	Absent	1	<5th			[3]
TERT	c.3150G>C	p.Lys1050Asn	0.00008867	1	<5th			[3]
TERT	c.3187G>A	p.Gly1063Ser	Absent	1	<10th	Yes	Yes	[3, 54]
TERT	c.3202G>A	p.Glu1068Lys	Absent	1	<1st	Yes		[3]
TERT	c.3216G>A	p.Trp1072X	Absent	1		Yes		[94]
TERT	c.3251 G>C	Arg1084Pro	Absent	1	<1st		Yes	[62]
TERT	c.3256C>T	p.Arg1086Cys	Absent	1				[42]
TERT	c.3323C>T	p.Pro1108Leu	0.00003088	1				[102]
TERT	c.3329C>T	p.Thr1110Met	0.00005010	1	<10th		Yes	[36]
TERT	c.3346_3522del177	p.Glu1116fsX11	Absent	1	<1st	Yes	Yes	[2, 3]

[a]The positions of the DNA and protein variants are described using TERT NM_198253.2 (isoform 1)
[b]The frequencies (if present) of the variants found in the Exome Aggregation Consortium (ExAC) database [15] version 0.3.1 (www.exac.broadinstitute.org) are listed. Variants with an allele frequency of >1% were not included
[c]Co-segregation in at least two family members with progressive pulmonary fibrosis
[d]Also found in one individual in the control population [42]
[e]Both variants are found in two siblings, suggesting that they in *cis*
[f]This same rare variant was found by directly sequencing lung tissue from an affected sister with IPF, liver dysfunction, leukopenia, and anemia. Telomerase activity as measured by the in vitro telomere repeat amplification protocol (TRAP) was 40% [54]

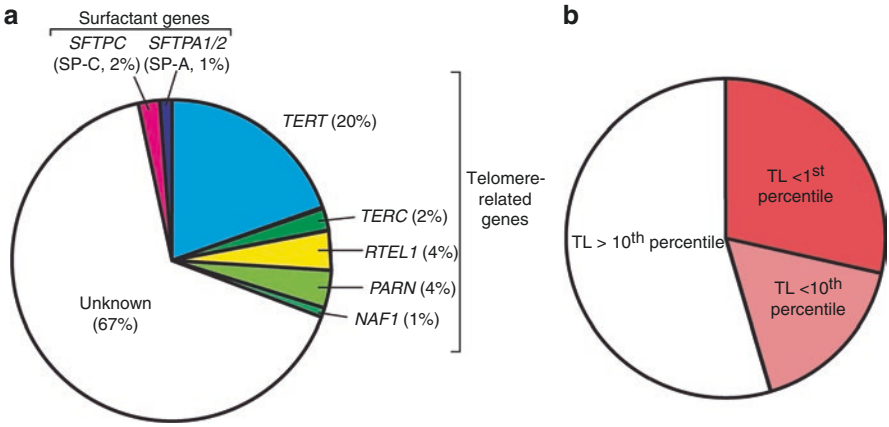

Fig. 8.2 Genetic heterogeneity of familial pulmonary fibrosis (FPF). (**a**) Percentage of variants in different genes found in FPF probands ($n = 228$) [2, 3, 37, 54, 66, 95]. Only variants that are considered pathogenic or likely pathogenic were included; none were found in *DKC1*, *TINF2*, or *SFTPA1* (data not shown). Rare variants were not included if the family analysis demonstrated a lack of cosegregation of the variant and fibrotic ILD or if there was no indication that the variant led to a deleterious effect on protein function. Overall, ~30% of probands have pathogenic variants in telomere-related genes, and ~3% of probands have pathogenic variants in surfactant-related genes. (**b**) Percentage of probands of FPF families ($n = 228$) that demonstrate an age-adjusted leukocyte telomere length of <1st percentile (~30%), <10th percentile (45%), or >10th percentile (55%)

Rare Variants in Telomere Maintenance Genes

Telomeres are repetitive nucleotide sequences at the ends of chromosomes that prevent progressive shortening of the chromosome during cell replication. The overall length of the telomere is influenced by its starting length, the cellular activity of telomerase, the number of cell divisions, and the environment. Most rare variants in telomere-related genes found in patients with FPF or sporadic end-stage lung fibrosis are found in one of four genes, *TERT*, *TERC*, *RTEL1*, and *PARN*, with fewer cases linked to *NAF1*, *DKC1*, and *TINF2* [2, 36, 37, 40–45].

Telomerase is a ribonucleoprotein complex composed of a reverse transcriptase protein (encoded by *TERT*) which catalyzes the addition of repeated DNA sequences to the ends of chromosomes by using a telomerase RNA (*TERC*) template [46–48]. The regulator of telomere length 1 (*RTEL1*) is a DNA helicase that disassembles DNA secondary structures at the end of the chromosome, such as the T-loop and G-quadruplex structures. Polyadenylation-specific RNase (*PARN*) removes adenosine nucleotide tails from the end of *TERC*, allowing it to serve as the template for the telomere repetitive sequence [49]. NAF1 loads dyskerin core complexes onto TERC; loss of function variants in this gene have also been described in FPF patients [43]. Rare variants in two other telomere-related genes, *DKC1* and *TINF2*, have also been described in patients with ILD [44, 45].

Regulation of telomerase activity is important in determining cellular life span. Most somatic human cells have undetectable telomerase activity after birth, and as a result, telomeres in these cells progressively shorten with each round of cell division, eventually leading to cell senescence [50]. Expression of telomerase prevents senescence of stem cells or those with increased replicative potential [51–53].

FPF kindreds with rare pathogenic variants in the telomere-related genes demonstrate an autosomal dominant pattern of inheritance with reduced penetrance. Pulmonary fibrosis is rare for individuals before 40 years of age, except for those with dyskeratosis congenita. Pulmonary fibrosis in telomere-related gene mutations may manifest as an IIP or an ILD of known cause as discussed above, and discordant diagnoses for individuals with an identical mutation can occur in up to 80% of families [3].

Pathogenic rare variants are associated with much higher penetrance of disease than common variants. In contrast with the *MUC5B* allele, in which <1% of individuals who have inherited the risk allele develop IPF, approximately 50–60% of individuals with an inherited pathogenic *TERT* rare variant develop pulmonary fibrosis by 60 years of age [54]. While smoking or exposure to other fibrogenic triggers may be triggers for developing fibrosis, this has not been prospectively studied. The association between short telomere lengths and chronic HP also supports the role of environmental factors in the development of short telomere-associated lung fibrosis. Perhaps cumulative environmental exposures partly explain the increasing incidence of lung fibrosis with age.

Genetic burden of pathogenic variants and genetic anticipation lead to earlier presentations of disease. Patients who inherit two mutations in *TERT*, *RTEL1*, and *PARN* may develop disease as children or young adults, in the form of dyskeratosis congenita (DC) or Hoyeraal-Hreidarsson syndrome [55–57]. Patients with DC often have reticular skin pigmentation, nail dystrophy, and oral mucosal leukoplakia in childhood and bone marrow failure by the second decade of life. Onset of pulmonary fibrosis occurs sooner in DC patients following a bone marrow transplant [58]. Genetic anticipation may occur in families with telomere-related gene mutations due to progressive telomere shortening, leading to earlier and increasingly severe disease in each generation [3, 59]. Earlier presentations of pulmonary fibrosis across subsequent generations have been found in FPF families with *TERT*, *TERC*, and *RTEL1* mutations [3, 38, 60, 61].

Subclinical Pulmonary Disease in Pathogenic Rare Variant Carriers

Asymptomatic rare variant *TERT* carriers may exhibit subclinical signs of pulmonary fibrosis. Asymptomatic carriers may demonstrate increased quantitative tissue volumes on high-resolution computed tomography (HRCT) and reduced diffusion capacity at rest and with exercise [11]. The lag time between subclinical and overt disease is highly variable [62].

Prognosis Related to Pathogenic Rare Variants in Telomere-Related Genes

In a study by Newton and colleagues, patients with mutations in telomere-related genes had a uniformly progressive course [3]. The mean rate of decline in FVC was 300 mL·year^{-1} and the median time to death or transplant was 2.87 years. There was no significant difference in the time to death or transplant for different gene mutations or for patients with a clinical diagnosis of IPF or one of several different non-IPF ILD diagnoses. These data suggest that surgical lung biopsy may be unnecessary in patients with FPF due to telomere-related mutations, as the exact clinical diagnosis does not affect prognosis.

Lung transplantation is the only curative treatment for progressive pulmonary fibrosis. Several retrospective series have reported outcomes in patients with *TERT* and *TERC* mutations undergoing lung transplant [63–65]. Thrombocytopenia, myelodysplastic syndrome, bone marrow failure, acute kidney injury requiring dialysis support, and adjustment of immunosuppression due to hematologic toxicity were commonly observed.

Telomere Length and Clinical Outcomes

Telomere lengths can be measured by several different methods. Most studies investigate the telomere lengths of leukocytes, because these are available with a minimally invasive blood draw. Telomere length studies of lung cells are more difficult because they require surgical samples and may be influenced by regional heterogeneity of an underlying ILD. Leukocyte telomere lengths can be measured by quantitative immunofluorescence or flow-FISH, by Southern blot analysis or terminal restriction fragment length (TRFL) analysis, or by quantitative PCR (QPCR) amplification of the telomere end relative to a single-copy gene. Each technique has inherent limitations. For example, flow-FISH requires a freshly drawn sample of blood, TRFL analysis is very time-consuming, and QPCR can be less reproducible [39].

Sporadic ILD patients with short leukocyte telomere lengths have a worse prognosis than those with normal telomere lengths. Between 23% and 50% of patients with sporadic IPF have age-adjusted telomere lengths less than the 10th percentile [66, 67]. These short telomere sporadic IPF patients have worse transplant-free survival in multiple independent cohorts than sporadic patients with normal telomere lengths [68, 69]. Interestingly, telomere length <10th percentile for age is associated with the degree of fibrosis, histopathologic features of UIP, and reduced survival in patients with chronic HP [28]. Leukocyte telomere length <10th percentile is also associated with worse survival and a shorter time to onset to chronic lung allograft dysfunction in pulmonary fibrosis patients after lung transplantation [70].

Treatment of Pulmonary Fibrosis Associated with Pathogenic Rare Variants

To our knowledge, there have been no studies that have investigated the effects of various antifibrotic medications in patients stratified by rare variants or telomere length. This is an area that is ripe for investigation. A single publication reported that pirfenidone was well-tolerated in 18 patients with *TERT* or *TERC* rare variants, but efficacy was not reported [71]. It is similarly unclear if patients with non-IPF diagnoses (especially chronic HP or CTD-ILD) who have pathogenic rare variants in telomere-related genes respond better to antifibrotic medications or immunosuppression.

Success in treating bone marrow failure syndromes with androgen therapy provides support for sex hormones as a target of future therapies for patients with telomere-related mutations. The primary mechanism for regulating telomerase activity is transcriptional regulation of *TERT* [72], and sex hormones are important regulators of *TERT* transcription. A synthetic androgen has been shown to increase in vitro telomerase activity of lymphocytes isolated from patients with *TERT* or *TERC* mutations [73]. Androgens are a standard therapeutic option for bone marrow failure in patients with dyskeratosis congenita who are unable to undergo hematopoietic stem cell transplantation, but side effects of the treatment need to be closely monitored [74]. Danazol, an androgenic synthetic sex hormone, was recently studied in 27 patients with telomere-related diseases (bone marrow failure and pulmonary fibrosis). Treatment with danazol led to telomere elongation and a positive hematologic response but was limited by toxicities [75]. It remains to be seen if sex hormones may be potential treatments for pulmonary fibrosis patients with telomere-related mutations or short telomere lengths.

Rare Variants in Surfactant Metabolism Genes

Pulmonary surfactant is a lipid- and protein-rich product of type II alveolar epithelial cells that prevents atelectasis and participates in the host immune response. Surfactant proteins (SP)-A and SP-D are hydrophilic proteins that assist in clearance of bacterial and viral pathogens and can dampen immune function of effector cells [76]. SP-B and SP-C are hydrophobic proteins that, along with phospholipids, serve to reduce surface tension in alveoli. Phospholipids are translocated into lamellar bodies for surfactant assembly by *ABCA3*, an ATP-binding cassette transporter [77].

Rare variants in surfactant genes may lead to diverse pulmonary manifestations. SP-B deficiency most commonly leads to neonatal respiratory failure and has an autosomal recessive pattern of inheritance [78, 79]. Biallelic *ABCA3* mutations usually lead to severe neonatal disease or ILD in infancy or childhood, though case reports of adult ILD have been described [80].

Autosomal dominant lung disease due to mutations in the gene encoding SP-C (*SFTPC*) was initially described in a neonate with respiratory distress, but adult-onset disease is also common [81, 82]. The pathogenesis of pulmonary fibrosis attributed to >40 different mutations in the *SFTPC* gene involves protein misfolding and a toxic gain of function of the misfolded protein. Rare variants in the BRICHOS domain lead to misfolding of pre-SP-C, resulting in ER stress and type II alveolar cell toxicity [83], while at least one rare variant (Ile73Thr) within the non-BRICHOS domain leads to alterations in autophagic vacuole maturation [84]. Heterozygous variants in the genes encoding SP-A (*SFTPA1* and *SFTPA2*) are linked with pulmonary fibrosis and lung adenocarcinoma [85–87]. The mechanism of disease for SP-A mutations presumably involves decreased secretion of mature SP-A and increased ER stress [85, 86]. Sporadic IPF has been associated with increased ER stress, suggesting a possible shared mechanism between some patients with familial and sporadic ILDs [88].

ILD patients with rare pathogenic variants in the SP-C gene exhibit incomplete penetrance and phenotypic heterogeneity. *SFTPC* rare variants are found in 1–2% of FPF cohorts [38, 89], though a Dutch cohort reported an incidence of 25% [90]. The most frequent radiographic patterns in ILD patients with *SFTPC* mutations include diffuse ground-glass opacities, septal thickening, and upper lobe-predominant subpleural cysts [90–92]. Histologic patterns associated with these mutations include, most commonly, UIP, followed by NSIP, OP, and desquamative interstitial pneumonia [90]. The effect of antifibrotic therapies in patients with surfactant-related mutations is unknown.

When to Suspect FPF

A detailed family history is essential in the evaluation of all patients with ILD. Because FPF kindreds may demonstrate incomplete penetrance, a thorough family history should include investigation of pulmonary fibrosis in any first, second, and more distantly related family members. In addition, clinicians should ask about a personal or family history of bone marrow failure, liver disease, or early graying of hair, as these features suggest a short telomere syndrome [11, 93]. An earlier age of the onset of ILD in subsequent generations may reflect genetic anticipation, which can be seen in short telomere syndromes.

Clinical Testing for FPF

Clinical evaluation is offered to all first-degree relatives of FPF patients, especially for those in which there are many affected family members. We recommend baseline pulmonary function testing and a HRCT for all at-risk family members over 40 years of age. If the patient is asymptomatic, no additional testing is

recommended. Instead, we recommend avoidance of fibrogenic exposures and regular exercise. For individuals with a cough or exertional dyspnea, we follow serial pulmonary function testing instead of serial CT chest scans to avoid excess radiation exposure. There are no clear guidelines regarding the frequency of testing. Due to the efficacy of antifibrotic therapy, these agents are a therapeutic option for those with evidence of early progressive disease.

Genetic Testing for FPF

Results of genetic testing in a patient with ILD may have implications regarding prognosis or diagnostic workup. Since those with rare telomere-related mutations may have any number of ILD diagnoses, the presence of one of these alleles may affect the physician's and patient's decision to obtain a surgical lung biopsy. Further, identification of a specific genetic cause of disease gives patients a name to a syndrome that may explain divergent phenotypes in themselves or family members. When a pathogenic variant is identified in the index case, specific variant testing to look for the presence or absence of this same variant could be considered for other family members.

Genetic testing for inherited variants is considered a once in a lifetime test. If genetic testing is being considered, we recommend referral to a genetic counselor to give the patient full opportunity to discuss the risks, benefits, and costs of testing. Insurance companies have different policies regarding coverage of these tests. Some patients may be concerned about potential discrimination if the result of genetic testing is positive, especially if transplant-related or long-term care costs are imminent. While federal law prohibits discrimination against patients with known genetic diseases by employers and health insurance companies, life insurance and long-term disability do not carry federal protection against discrimination. Some patients choose to pay out of pocket so that the results are not disclosed in the medical record. If the patient chooses to pay out of pocket, testing may cost approximately $200 for a specific variant found in a family member or up to $1500 for the analysis of a single gene. Given the genetic heterogeneity of FPF, panel testing is generally more cost-effective.

Prior to agreeing to genetic testing, the patient should understand what results are possible. Variants that are reported usually include those that are categorized as a pathogenic variant, likely pathogenic variant or a VUS. A VUS result causes the most confusion and frustration for those expecting a Yes or No result. But it is important to recognize that a VUS result is a relatively common occurrence, especially since so many different novel or ultra-rare variants underlie the genetic architecture of FPF and severe sporadic lung fibrosis (Table 8.1). The report of a VUS may require additional evaluations such as segregation analysis in families, telomere length testing, or in vitro assays of protein function.

Segregation analysis in a family is the best way to assess for pathogenicity of a variant. However, it is often the most difficult analysis to perform. Leukocyte

telomere length testing is often performed to assess the pathogenicity of variants in telomere-related genes. However, it is important to note that some individuals with pathogenic or likely pathogenic variants in telomere-related genes have telomere lengths that are >10th percentile [3, 37, 54, 94]. Longer telomere lengths are more commonly found in individuals with variants in *PARN* rather than *TERC* [3, 37], so this test does not perfectly discriminate between pathogenic and nonpathogenic telomere-related variants.

For all of the above reasons, in our practice we engage our patients in a dialogue prior to ordering CLIA-certified genetic sequencing. Testing is recommended if the identification of a pathogenic variant would influence clinical practice, such as the decision to obtain a surgical lung biopsy. At this point in time, we do not recommend testing for common SNPs such as the *MUC5B* or *TOLLIP* SNPs. We do not recommend genetic testing if the pretest probability of finding a mutation is low or if the information provided would not change clinical management for the patient or family member.

If a pathogenic variant is discovered, a letter is provided to the patient describing the gene variant and its clinical significance, and we also give the patient contact information for a clinician who can provide additional information. The patient is encouraged to share this letter with family members. If a pathogenic variant is discovered in an at-risk family member, they are strongly counseled to avoid smoking, minimize environmental fibrogenic exposures, and avoid medications associated with pulmonary fibrosis (such as nitrofurantoin or amiodarone). Telomere-related mutation carriers are counseled regarding the incidence of bone marrow failure and liver disease. As pulmonary genetics is a rapidly evolving field, patients are notified that specific gene-related or variant-related recommendations may change as more knowledge on this subject accumulates.

Conclusions

There have been numerous advances in the field of pulmonary fibrosis genetics during the last decade. A number of common variants are associated with pulmonary fibrosis, with the *MUC5B* promoter SNP the most replicated of these. Rare variants in telomere-related and surfactant genes have also been found and characterized in patients with familial and sporadic ILD. All of these discoveries have rapidly changed our understanding of the molecular underpinnings of pulmonary fibrosis. While recommendations regarding specific treatments are not yet available, future research may illuminate how genetic information can be best utilized to improve patient care.

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References

1. Steele MP, Speer MC, Loyd JE, Brown KK, Herron A, Slifer SH, et al. Clinical and pathologic features of familial interstitial pneumonia. Am J Respir Crit Care Med. 2005;172(9):1146–52.
2. Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. Proc Natl Acad Sci U S A. 2007;104(18):7552–7.
3. Newton CA, Batra K, Torrealba J, Kozlitina J, Glazer CS, Aravena C, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. Eur Respir J. 2016;48(6):1710–20.
4. Hodgson U, Laitinen T, Tukiainen P. Nationwide prevalence of sporadic and familial idiopathic pulmonary fibrosis: evidence of founder effect among multiplex families in Finland. Thorax. 2002;57(4):338–42.
5. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006;174(7):810–6.
6. Loyd JE. Pulmonary fibrosis in families. Am J Respir Cell Mol Biol. 2003;29(3 Suppl):S47–50.
7. Garcia-Sancho C, Buendia-Roldan I, Fernandez-Plata MR, Navarro C, Perez-Padilla R, Vargas MH, et al. Familial pulmonary fibrosis is the strongest risk factor for idiopathic pulmonary fibrosis. Respir Med. 2011;105(12):1902–7.
8. Lee HY, Seo JB, Steele MP, Schwarz MI, Brown KK, Loyd JE, et al. High-resolution CT scan findings in familial interstitial pneumonia do not conform to those of idiopathic interstitial pneumonia. Chest. 2012;142(6):1577–83.
9. Okamoto T, Miyazaki Y, Tomita M, Tamaoka M, Inase N. A familial history of pulmonary fibrosis in patients with chronic hypersensitivity pneumonitis. Respir Int Rev Thorac Dis. 2013;85(5):384–90.
10. Reddy TL, Tominaga M, Hansell DM, von der Thusen J, Rassl D, Parfrey H, et al. Pleuroparenchymal fibroelastosis: a spectrum of histopathological and imaging phenotypes. Eur Respir J. 2012;40(2):377–85.
11. Diaz de Leon A, Cronkhite JT, Yilmaz C, Brewington C, Wang R, Xing C, et al. Subclinical lung disease, macrocytosis, and premature graying in kindreds with telomerase (TERT) mutations. Chest. 2011;140(3):753–63.
12. Kropski JA, Pritchett JM, Zoz DF, Crossno PF, Markin C, Garnett ET, et al. Extensive phenotyping of individuals at risk for familial interstitial pneumonia reveals clues to the pathogenesis of interstitial lung disease. Am J Respir Crit Care Med. 2015;191(4):417–26.
13. Tennessen JA, Bigham AW, O'Connor TD, Fu W, Kenny EE, Gravel S, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science. 2012;337(6090):64–9.
14. Nelson MR, Wegmann D, Ehm MG, Kessner D, St Jean P, Verzilli C, et al. An abundance of rare functional variants in 202 drug target genes sequenced in 14,002 people. Science. 2012;337(6090):100–4.
15. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285–91.
16. McClellan J, King MC. Genetic heterogeneity in human disease. Cell. 2010;141(2):210–7.
17. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med Off J Am Coll Med Genet. 2015;17(5):405–24.
18. Claustres M, Kozich V, Dequeker E, Fowler B, Hehir-Kwa JY, Miller K, et al. Recommendations for reporting results of diagnostic genetic testing (biochemical, cytogenetic and molecular genetic). Eur J Hum Genet EJHG. 2014;22(2):160–70.
19. Roy MG, Livraghi-Butrico A, Fletcher AA, McElwee MM, Evans SE, Boerner RM, et al. Muc5b is required for airway defence. Nature. 2014;505(7483):412–6.
20. 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.

21. Conti C, Montero-Fernandez A, Borg E, Osadolor T, Viola P, De Lauretis A, et al. Mucins MUC5B and MUC5AC in distal airways and honeycomb spaces: comparison among idiopathic pulmonary fibrosis/usual interstitial pneumonia, fibrotic nonspecific interstitial pneumonitis, and control lungs. Am J Respir Crit Care Med. 2016;193(4):462–4.
22. Zhang GY, Liao T, Gao WY. MUC5B promoter polymorphism and pulmonary fibrosis. N Engl J Med. 2011;365(2):178. author reply -9
23. Borie R, Crestani B, Dieude P, Nunes H, Allanore Y, Kannengiesser C, et al. The MUC5B variant is associated with idiopathic pulmonary fibrosis but not with systemic sclerosis interstitial lung disease in the European Caucasian population. PLoS One. 2013;8(8):e70621.
24. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, Steele MP, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. Nat Genet. 2013;45(6):613–20.
25. Noth I, Zhang Y, Ma S-F, 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):309–17.
26. Stock CJ, Sato H, Fonseca C, Banya WA, Molyneaux PL, Adamali H, et al. Mucin 5B promoter polymorphism is associated with idiopathic pulmonary fibrosis but not with development of lung fibrosis in systemic sclerosis or sarcoidosis. Thorax. 2013;68(5):436–41.
27. Wei R, Li C, Zhang M, Jones-Hall YL, Myers JL, Noth I, et al. Association between MUC5B and TERT polymorphisms and different interstitial lung disease phenotypes. Transl Res. 2014;163(5):494–502.
28. Ley B, Newton CA, Arnould I, Elicker BM, Henry TS, Vittinghoff E, et al. The MUC5B promoter polymorphism and telomere length in patients with chronic hypersensitivity pneumonitis: an observational cohort-control study. Lancet Respir Med. 2017;5(8):639–47.
29. Horimasu Y, Ohshimo S, Bonella F, Tanaka S, Ishikawa N, Hattori N, et al. MUC5B promoter polymorphism in Japanese patients with idiopathic pulmonary fibrosis. Respirology. 2015;20(3):439–44.
30. Putman RK, Hatabu H, Araki T, Gudmundsson G, Gao W, Nishino M, et al. Association between interstitial lung abnormalities and all-cause mortality. JAMA. 2016;315(7):672–81.
31. Araki T, Putman RK, Hatabu H, Gao W, Dupuis J, Latourelle JC, et al. Development and progression of interstitial lung abnormalities in the Framingham Heart Study. Am J Respir Crit Care Med. 2016;194(12):1514–22.
32. Hunninghake GM, Hatabu H, Okajima Y, Gao W, Dupuis J, Latourelle JC, et al. MUC5B promoter polymorphism and interstitial lung abnormalities. N Engl J Med. 2013;368(23):2192–200.
33. Mushiroda T, Wattanapokayakit S, Takahashi A, Nukiwa T, Kudoh S, Ogura T, et al. A genome-wide association study identifies an association of a common variant in TERT with susceptibility to idiopathic pulmonary fibrosis. J Med Genet. 2008;45(10):654–6.
34. Peljto AL, Selman M, Kim DS, Murphy E, Tucker L, Pardo A, et al. The MUC5B promoter polymorphism is associated with idiopathic pulmonary fibrosis in a Mexican cohort but is rare among Asian ancestries. Chest. 2015;147(2):460–4.
35. Oldham JM, Ma SF, Martinez FJ, Anstrom KJ, Raghu G, Schwartz DA, et al. TOLLIP, MUC5B, and the response to N-acetylcysteine among individuals with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2015;192(12):1475–82.
36. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med. 2007;356(13):1317–26.
37. Stuart BD, Choi J, Zaidi S, Xing C, Holohan B, Chen R, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. Nat Genet. 2015;47(5):512–7.
38. Fernandez BA, Fox G, Bhatia R, Sala E, Noble B, Denic N, et al. A Newfoundland cohort of familial and sporadic idiopathic pulmonary fibrosis patients: clinical and genetic features. Respir Res. 2012;13:64.
39. Gutierrez-Rodrigues F, Santana-Lemos BA, Scheucher PS, Alves-Paiva RM, Calado RT. Direct comparison of flow-FISH and qPCR as diagnostic tests for telomere length measurement in humans. PLoS One. 2014;9(11):e113747.

40. Cogan JD, Kropski JA, Zhao M, Mitchell DB, Rives L, Markin C, et al. Rare variants in RTEL1 are associated with familial interstitial pneumonia. Am J Respir Crit Care Med. 2015;191:646–55.
41. Kannengiesser C, Borie R, Menard C, Reocreux M, Nitschke P, Gazal S, et al. Heterozygous RTEL1 mutations are associated with familial pulmonary fibrosis. Eur Respir J. 2015;46:474–85.
42. Petrovski S, Todd JL, Durheim MT, Wang Q, Chien JW, Kelly FL, et al. An exome sequencing study to assess the role of rare genetic variation in pulmonary fibrosis. Am J Respir Crit Care Med. 2017;196(1):82–93.
43. Stanley SE, Gable DL, Wagner CL, Carlile TM, Hanumanthu VS, Podlevsky JD, et al. Loss-of-function mutations in the RNA biogenesis factor NAF1 predispose to pulmonary fibrosis-emphysema. Sci Transl Med. 2016;8(351):351ra107.
44. Alder JK, Stanley SE, Wagner CL, Hamilton M, Hanumanthu VS, Armanios M. Exome sequencing identifies mutant TINF2 in a family with pulmonary fibrosis. Chest. 2015;147(5):1361–8.
45. Kropski JA, Mitchell DB, Markin C, Polosukhin VV, Choi L, Johnson JE, et al. A novel dyskerin (DKC1) mutation is associated with familial interstitial pneumonia. Chest. 2014;146(1):e1–7.
46. Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, et al. The RNA component of human telomerase. Science. 1995;269(5228):1236–41.
47. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell. 1985;43(2 Pt 1):405–13.
48. Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, et al. Telomerase catalytic subunit homologs from fission yeast and human. Science. 1997;277(5328):955–9.
49. Moon DH, Segal M, Boyraz B, Guinan E, Hofmann I, Cahan P, et al. Poly(A)-specific ribonuclease (PARN) mediates 3′-end maturation of the telomerase RNA component. Nat Genet. 2015;47(12):1482–8.
50. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994;266(5193):2011–5.
51. Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB, et al. Telomerase maintains telomere structure in normal human cells. Cell. 2003;114(2):241–53.
52. Weng NP, Granger L, Hodes RJ. Telomere lengthening and telomerase activation during human B cell differentiation. Proc Natl Acad Sci U S A. 1997;94(20):10827–32.
53. Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW. Telomerase activity in human germline and embryonic tissues and cells. Dev Genet. 1996;18(2):173–9.
54. Diaz de Leon A, Cronkhite JT, Katzenstein AL, Godwin JD, Raghu G, Glazer CS, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. PLoS One. 2010;5(5):e10680.
55. Walne AJ, Vulliamy T, Kirwan M, Plagnol V, Dokal I. Constitutional mutations in RTEL1 cause severe dyskeratosis congenita. Am J Hum Genet. 2013;92(3):448–53.
56. Du HY, Pumbo E, Manley P, Field JJ, Bayliss SJ, Wilson DB, et al. Complex inheritance pattern of dyskeratosis congenita in two families with 2 different mutations in the telomerase reverse transcriptase gene. Blood. 2008;111(3):1128–30.
57. Tummala H, Walne A, Collopy L, Cardoso S, de la Fuente J, Lawson S, et al. Poly(A)-specific ribonuclease deficiency impacts telomere biology and causes dyskeratosis congenita. J Clin Invest. 2015;125(5):2151–60.
58. Giri N, Lee R, Faro A, Huddleston CB, White FV, Alter BP, et al. Lung transplantation for pulmonary fibrosis in dyskeratosis congenita: case report and systematic literature review. BMC Blood Disord. 2011;11:3.
59. Armanios M, Blackburn EH. The telomere syndromes. Nat Rev Genet. 2012;13(10):693–704.
60. Armanios M, Chen JL, Chang YP, Brodsky RA, Hawkins A, Griffin CA, et al. Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. Proc Natl Acad Sci U S A. 2005;102(44):15960–4.
61. Vulliamy T, Marrone A, Szydlo R, Walne A, Mason PJ, Dokal I. Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. Nat Genet. 2004;36(5):447–9.

62. El-Chemaly S, Ziegler SG, Calado RT, Wilson KA, Wu HP, Haughey M, et al. Natural history of pulmonary fibrosis in two subjects with the same telomerase mutation. Chest. 2011;139(5):1203–9.
63. Borie R, Kannengiesser C, Hirschi S, Le Pavec J, Mal H, Bergot E, et al. Severe hematologic complications after lung transplantation in patients with telomerase complex mutations. J Heart Lung Transplant. 2015;34(4):538–46.
64. Silhan LL, Shah PD, Chambers DC, Snyder LD, Riise GC, Wagner CL, et al. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. Eur Respir J. 2014;44(1):178–87.
65. Tokman S, Singer JP, Devine MS, Westall GP, Aubert JD, Tamm M, et al. Clinical outcomes of lung transplant recipients with telomerase mutations. J Heart Lung Transplant. 2015;34(10):1318–24.
66. 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.
67. Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. Proc Natl Acad Sci U S A. 2008;105(35):13051–6.
68. 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.
69. Dai J, Cai H, Li H, Zhuang Y, Min H, Wen Y, et al. Association between telomere length and survival in patients with idiopathic pulmonary fibrosis. Respirology. 2015;20(6):947–52.
70. Newton CA, Kozlitina J, Lines JR, Kaza V, Torres F, Garcia CK. Telomere length in patients with pulmonary fibrosis associated with chronic lung allograft dysfunction and post-lung transplantation survival. J Heart Lung Transplant. 2017;36:845–53.
71. Justet A, Borie R, Nunes H, Cottin V, Marchand Adam S, Cadranel J, et al. Safety and efficacy of pirfenidone in patients with lung fibrosis and TERT mutation. Eur Respir J. 2016;48(suppl 60):484.
72. Blackburn EH. Switching and signaling at the telomere. Cell. 2001;106(6):661–73.
73. Calado RT, Yewdell WT, Wilkerson KL, Regal JA, Kajigaya S, Stratakis CA, et al. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. Blood. 2009;114(11):2236–43.
74. Khincha PP, Wentzensen IM, Giri N, Alter BP, Savage SA. Response to androgen therapy in patients with dyskeratosis congenita. Br J Haematol. 2014;165(3):349–57.
75. Townsley DM, Dumitriu B, Liu D, Biancotto A, Weinstein B, Chen C, et al. Danazol treatment for telomere diseases. N Engl J Med. 2016;374(20):1922–31.
76. Han S, Mallampalli RK. The role of surfactant in lung disease and host defense against pulmonary infections. Ann Am Thorac Soc. 2015;12(5):765–74.
77. Weaver TE. Synthesis, processing and secretion of surfactant proteins B and C. Biochim Biophys Acta. 1998;1408(2–3):173–9.
78. Nogee LM, Garnier G, Dietz HC, Singer L, Murphy AM, deMello DE, et al. A mutation in the surfactant protein B gene responsible for fatal neonatal respiratory disease in multiple kindreds. J Clin Invest. 1994;93(4):1860–3.
79. Andersen C, Ramsay JA, Nogee LM, Shah J, Wert SE, Paes B, et al. Recurrent familial neonatal deaths: hereditary surfactant protein B deficiency. Am J Perinatol. 2000;17(4):219–24.
80. Flamein F, Riffault L, Muselet-Charlier C, Pernelle J, Feldmann D, Jonard L, et al. Molecular and cellular characteristics of ABCA3 mutations associated with diffuse parenchymal lung diseases in children. Hum Mol Genet. 2012;21(4):765–75.
81. Nogee LM, Dunbar AE 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. N Engl J Med. 2001;344(8):573–9.
82. Thomas AQ, Lane K, Phillips J 3rd, Prince M, Markin C, Speer M, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med. 2002;165(9):1322–8.

83. Mulugeta S, Nguyen V, Russo SJ, Muniswamy M, Beers MF. A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and caspase 3 activation. Am J Respir Cell Mol Biol. 2005;32(6):521–30.
84. Hawkins A, Guttentag SH, Deterding R, Funkhouser WK, Goralski JL, Chatterjee S, et al. A non-BRICHOS SFTPC mutant (SP-CI73T) linked to interstitial lung disease promotes a late block in macroautophagy disrupting cellular proteostasis and mitophagy. Am J Physiol Lung Cell Mol Physiol. 2015;308(1):L33–47.
85. Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, et al. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. Am J Hum Genet. 2009;84(1):52–9.
86. Nathan N, Giraud V, Picard C, Nunes H, Dastot-Le Moal F, Copin B, et al. Germline SFTPA1 mutation in familial idiopathic interstitial pneumonia and lung cancer. Hum Mol Genet. 2016;25(8):1457–67.
87. van Moorsel CH, Ten Klooster L, van Oosterhout MF, de Jong PA, Adams H, Wouter van Es H, et al. SFTPA2 mutations in familial and sporadic idiopathic interstitial pneumonia. Am J Respir Crit Care Med. 2015;192(10):1249–52.
88. Lawson WE, Crossno PF, Polosukhin VV, Roldan J, Cheng DS, Lane KB, et al. Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. Am J Physiol Lung Cell Mol Physiol. 2008;294(6):L1119–26.
89. Coghlan MA, Shifren A, Huang HJ, Russell TD, Mitra RD, Zhang Q, et al. Sequencing of idiopathic pulmonary fibrosis-related genes reveals independent single gene associations. BMJ Open Respir Res. 2014;1(1):e000057.
90. van Moorsel CH, van Oosterhout MF, Barlo NP, de Jong PA, van der Vis JJ, Ruven HJ, et al. Surfactant protein C mutations are the basis of a significant portion of adult familial pulmonary fibrosis in a Dutch cohort. Am J Respir Crit Care Med. 2010;182(11):1419–25.
91. Mechri M, Epaud R, Emond S, Coulomb A, Jaubert F, Tarrant A, et al. Surfactant protein C gene (SFTPC) mutation-associated lung disease: high-resolution computed tomography (HRCT) findings and its relation to histological analysis. Pediatr Pulmonol. 2010;45(10):1021–9.
92. Cottin V, Reix P, Khouatra C, Thivolet-Bejui F, Feldmann D, Cordier JF. Combined pulmonary fibrosis and emphysema syndrome associated with familial SFTPC mutation. Thorax. 2011;66(10):918–9.
93. Parry EM, Alder JK, Qi X, Chen JJ, Armanios M. Syndrome complex of bone marrow failure and pulmonary fibrosis predicts germline defects in telomerase. Blood. 2011;117(21):5607–11.
94. Borie R, Tabeze L, Thabut G, Nunes H, Cottin V, Marchand-Adam S, et al. Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. Eur Respir J. 2016;48(6):1721–31.
95. Garcia CK. Unpublished data.
96. Calado RT, Regal JA, Kleiner DE, Schrump DS, Peterson NR, Pons V, et al. A spectrum of severe familial liver disorders associate with telomerase mutations. PLoS One. 2009;4(11):e7926.
97. Xin ZT, Beauchamp AD, Calado RT, Bradford JW, Regal JA, Shenoy A, et al. Functional characterization of natural telomerase mutations found in patients with hematologic disorders. Blood. 2007;109(2):524–32.
98. Dai J, Cai H, Zhuang Y, Wu Y, Min H, Li J, et al. Telomerase gene mutations and telomere length shortening in patients with idiopathic pulmonary fibrosis in a Chinese population. Respirology. 2015;20(1):122–8.
99. Chambers DC, Clarke BE, McGaughran J, Garcia CK. Lung fibrosis, premature graying and macrocytosis. Am J Respir Crit Care Med. 2012;186:e8–9. In press.
100. Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med. 2005;352(14):1413–24.

101. Du HY, Pumbo E, Ivanovich J, An P, Maziarz RT, Reiss UM, et al. TERC and TERT gene mutations in patients with bone marrow failure and the significance of telomere length measurements. Blood. 2009;113(2):309–16.
102. Juge PA, Borie R, Kannengiesser C, Gazal S, Revy P, Wemeau-Stervinou L, et al. Shared genetic predisposition in rheumatoid arthritis-interstitial lung disease and familial pulmonary fibrosis. Eur Respir J. 2017;49(5):1602314.
103. Alder JK, Cogan JD, Brown AF, Anderson CJ, Lawson WE, Lansdorp PM, et al. Ancestral mutation in telomerase causes defects in repeat addition processivity and manifests as familial pulmonary fibrosis. PLoS Genet. 2011;7(3):e1001352.
104. Gansner JM, Rosas IO, Ebert BL. Pulmonary fibrosis, bone marrow failure, and telomerase mutation. N Engl J Med. 2012;366(16):1551–3.
105. Maryoung L, Yue Y, Young A, Newton CA, Barba C, van Oers NS, et al. Somatic mutations in telomerase promoter counterbalance germline loss-of-function mutations. J Clin Invest. 2017;127(3):982–6.

Chapter 9
Evolving Genomics of Pulmonary Fibrosis

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Introduction

The central dogma of gene expression in eukaryotic cells assumes a process initiated by a signal that triggers the transcription of a DNA sequence into messenger RNA, which is then translated into a protein. Recent analysis suggests that this initial dogma may have been oversimplified, and many other factors may be included with a significant role for epigenetic modification of DNA, large and small noncoding RNAs, and various posttranslational mechanisms (Fig. 9.1). This new and complex image is a direct result of genomics, a discipline that emerged out of the Human Genome Project [1] and the rapid spread of technologies that endorsed genome-scale transcript profiling and variant calling as well as advanced computational and analytical methods. This discipline, dedicated to study the sequence, expression, and function of multiple genes in parallel with the goal of understanding their biological function and interactions in health and disease, is rapidly becoming a key component of twenty-first-century medical research and an important component of efforts to redesign the practice of medicine as a precise and personalized endeavor. While in practice the discipline of genomics generally includes both genome-scale studies of genetic code (DNA) and transcripts (RNA), we will mainly focus on

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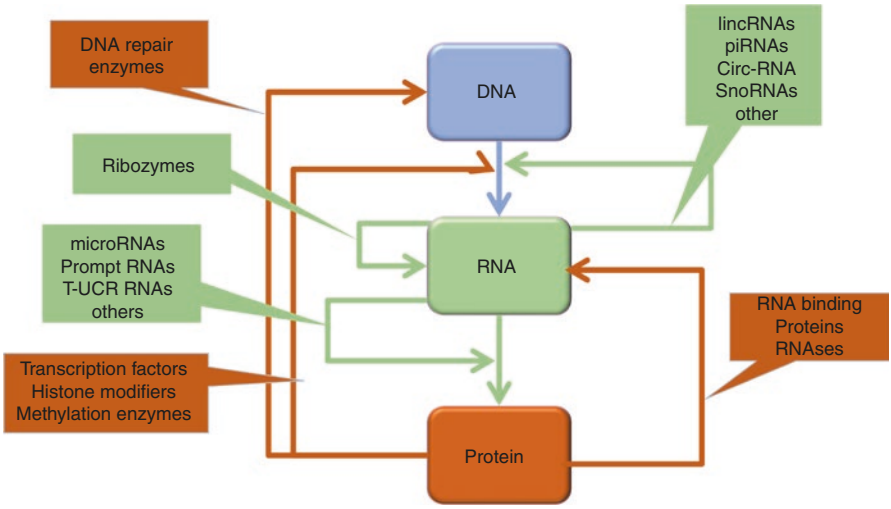

Fig. 9.1 The new dogma of gene expression regulation shifts from a linear view of DNA leading to RNA (transcription) leading to protein (translation), to a complex model in which proteins and nonprotein-coding RNAs act as regulators of the genome expression potential on multiple levels. Abbreviations: T-UCR RNA transcribed ultraconserved regions RNA, LincRNA large intergenic RNA, PIWI-interacting RNA (PiRNA), tRNA-derived stress-induced RNA (TiRNA), Small nucleolar RNA (SnoRNA)

advances in applying genome-scale transcript profiling approaches in this chapter, as the DNA variant profiling approach fits more in a discussion of the genetic sources of disease.

Genome-Scale Transcript Profiling

The concept of genome-scale transcript profiling was initially developed as a slide hybridization-based gene expression detection technology. Gene expression microarrays were originally based on the principle of light-directed, in situ oligonucleotide synthesis developed by Fodor and colleagues [2] and the later development of cDNA and oligonucleotide arrays [3, 4]. More recently, novel methods in next-generation RNA sequencing (RNAseq) that utilize high-throughput sequencing technologies are applied to genome-scale transcript profiling. Such technologies provide transcript-level information combined with gene structure information such as alternative splicing, information about noncoding RNAs and posttranscriptional modifications, and genomic variants at the nucleotide base level of resolution [5]. Therefore, these technologies will soon render arrays obsolete. However, regardless of the technology used, experiments are performed with RNA extracted from the tissue or cell of interest and depend on the purity and integrity of the RNA specimens.

Genome-scale transcript profiling experiments measure the expression of large number of transcripts, typically around 40,000–50,000, generating a large amount of information that has to be preprocessed, analyzed, and validated before the results can be used. Obtaining the right information out of these large datasets represents the major challenge when analyzing large genomic datasets. Before describing the most significant results obtained from genomic studies in lung fibrosis, it is critical to understand the steps required after the completion of microarray and RNAseq experiments. These steps can be summarized in three broad categories: quality assessment, normalization, and statistical analysis. Because quality assessment and normalization approaches greatly vary with technology, they will not be discussed here.

Once the genomic dataset is assessed for quality and normalized and outliers and batch effect (if present) are handled satisfactorily, investigators proceed to perform statistical analyses. Different algorithms for statistical analyses can be used for genome-scale transcriptomic data, and their use depends on the objectives of the study. Typically, the statistical algorithms used for gene expression profiling in human disease can be grouped into four major study objectives as defined by Simon and colleagues [6]: class comparison, class prediction, class discovery, and pathway analysis. We also consider including two additional study objectives in this group: outcome analysis and meta-analysis. Table 9.1 provides a description of the types of transcriptomic study objectives and lists some of the available algorithms that can be applicable to each type. Some of these algorithms can be used independently; be part of a computational software such as GeneSpring GX®, Bioconductor [44], and BRB array tools [45]; or work in a statistical environment, the most widely used being R statistical environment [46]. However, regardless of the tools, attention to addressing the testing of multiple hypotheses and achieving effective visualization is critically important.

After the statistical analysis is completed, the number of differentially expressed transcripts can still be too large to be validated and studied in depth. Traditionally, two different approaches have been used to deal with this issue: the reductionist or "cherry picking" approach and the global or "systems" approach [47]. In the "cherry picking" approach, researchers select a differentially expressed coding or noncoding transcript for which there is prior biological knowledge. This transcript is validated at the RNA and protein level if it is a coding RNA, studied in depth in vitro and in vivo to determine its disease relevance, and finally translated back to humans to confirm the association with the disease of interest and its potential use as a biomarker or as a therapeutic target. When using the global or "systems" approach, researchers try to study gene expression profiles as a unit; this involves using the concept that differentially expressed genes belong to a common pathway that is relevant to disease or can interact with each other depending on their pattern of expression. The global approach has been possible with the use of gene ontology annotations along with using previously published knowledge of gene interactions and pathway analysis focusing on the identification of differentially expressed genes occurring in the same molecular pathway.

Finally, the selection of relevant genes for validation can be facilitated with the integration of patient clinical information to the analysis of gene expression data,

Table 9.1 Summary of the type of transcript profiling experimental objectives and relevant algorithms for statistical analysis

Study objectives	Description	Statistical algorithms for each category
Class comparison	Class comparison analyses focus on the identification of differentially expressed genes among predefined classes of samples	*t*-test
		Analysis of variance (ANOVA) [7]
		Significance analysis of microarrays (SAM) [8, 9]
		Random variance model (RVM) [10]
		Lassoed principal component (LPC) [11]
Class prediction	Class prediction studies are also based on predefined classes of samples, although its goal is to develop a statistical prediction model based on the expression of a group of genes to allow the prediction of the class in each sample	Threshold number of misclassifications (TNoM) [12, 13]
		Compound covariate predictor [14, 15]
		Partial least square [16]
		k-Nearest neighbor (KNN) [17, 18]
		Support vector machine (SVM) [19]
		Nearest shrunken centroid (PAM) [20]
		Top scoring pairs [21]
Class discovery	Class discovery emphasizes the detection of an unidentified class based on the co-expression of genes. Typically these studies are performed to characterize an unknown clinical disease sub-phenotype based on the expression of clusters of genes	K-means clustering [22]
		Hierarchical clustering [23]
		Biclustering [24]
		Self-organizing maps (SOM) [25]
		Model-based clustering [26]
		Gene expression dynamic inspector (GEDI) [27]
Pathway analysis	Pathway analysis studies focus on the identification of differentially expressed genes that occur in the same molecular pathway in predefined classes of samples	Global test for groups of genes [28]
		Gene set enrichment analysis (GSEA) [29]
		SAM-GS [30]
		Gene set analysis (GSA) [31]
		Integrative microarray analysis of pathways (IMAP) [32]
		Gene set expression comparison [33]
Outcome analysis	Outcome analysis studies explore the association of gene expression with a predefined outcome (e.g., survival, transplant-free survival, disease progression)	Cox model [34]
		Partial least squares proportional hazard regression [35]
		Multiple random validation [36]
		Prediction by supervised principal component (SuperPC) [37]
Meta-analysis	Gene expression meta-analysis studies combine multiple and similar gene expression datasets to increase the statistical power and accuracy of the results	Truncated product method for combining P-values [38]
		t-based modeling [39]
		RankProd [40]
		Meta-analysis based on control of false discovery rate [41]
		Predictor-based approach [42]
		MetaOmics [43]

which allows the identification of profiles that characterize a clinical variable of interest. This is typically used to study gene profiles associated with drug responses, disease severity, disease progression, and patient outcomes.

In summary, the analysis of genome-scale transcript profiling experiments requires dedicated quality control, data normalization, and statistical analysis based on the objectives of the study. The selection of gene(s) or noncoding transcripts for validation and potential translation to patient care can be facilitated using a reductionist approach, a global approach, or both. Depending on the ultimate goal of the study, clinical variables could be introduced into the analysis of gene expression to ensure an easier translation to clinical practice.

Contribution of Genomics to Our Mechanistic Understanding of Lung Fibrosis

In contrast to hypothesis-driven experimental approaches that are based on what is known and rarely result in a novel or unexpected result, the results of genome-scale transcript profiling experiments often contain results that were unforeseen or even contrary to currently accepted paradigms. Considering that many breakthroughs in modern medicine were the result of serendipity [48, 49], one could consider large-scale genomic profiling experiments as means to introduce serendipitous discoveries into pulmonary research by identifying new data that provide important insights and facilitate the generation of new hypotheses.

The lung phenotype in IPF is not a result of passive accumulation of extracellular matrix The dominant paradigm explaining fibrosis in the last decade of the twentieth century assumed that fibrosis and accumulation of extracellular matrix were the results of a protease-antiprotease imbalance, in which increase in activity of naturally occurring inhibitors of metalloproteases and reduction of the activity of matrix metalloproteases led to the accumulation of extracellular matrix [50]. This paradigm was supported by observations from a limited set of hypothesis-based, carefully designed experiments, but it was never tested in a global non-biased analysis of the lung environment in IPF.

When Zuo et al. [51] analyzed lung tissue of patients with IPF and compared them with healthy controls, they immediately noticed that multiple members of the matrix metalloproteinase (MMP) family were upregulated at the mRNA and protein level in IPF lungs, including MMPs 1, 7, and 9. Among the overexpressed genes in IPF, MMP-7 was the most informative and was localized to the alveolar epithelium, a finding suggestive of the active role of the alveolar epithelium in lung remodeling that is characteristic of IPF. Interestingly, MMP-7 knockout mice were relatively protected from bleomycin-induced fibrosis suggesting the potential role of this protease as a regulator of fibrosis. Although these original observations were obtained on a very small number of tissue samples, it is impressive that these initial observations have been repeatedly verified [52–54].

MMP-7 has proteolytic effects that target the cleavage of molecules such as collagen type IV, aggrecan, laminin, fibronectin, gelatin, entactin, decorin, tenascin, vitronectin, osteonectin, elastin, and SPP1, among others [55]. In addition, MMP-7 is an example of a metalloprotease that may have regulatory effects that can be inferred by looking at its bioactive substrates that include potentially fibrosis-relevant proteins such as FAS ligand, β4 integrin, E-cadherin, pro-HB-epidermal growth factor, plasminogen, pro-TNF-α, pro-α-defensin, endostatin, syndecan, and insulin growth factor binding protein-3 (IGFBP-3) [56]. While the local effects of MMP-7 overexpression in the alveolar epithelium in humans are not clear, evidence from mice concerning its regulation of neutrophil egress, regulation of dendritic cells, and activation of defensins [57–59] suggest that it may have a significant role in regulating the local inflammatory milieu, as does its effect on SPP1 [60].

Many MMPs, including MMPs 1-3, 8-16, 19, 20, 21, and 23-28 [50, 51, 54, 60–84], have been consistently found to be increased or decreased in IPF lungs, serum, and/or BAL, and some of these MMPs have been shown to be relevant to the pathogenesis of pulmonary fibrosis. As an example, Yamashita et al. demonstrated that rats transfected with an adeno-MMP-3 vector developed transient pulmonary fibrosis, and in vitro treatment of lung epithelial cells with MMP-3 resulted in activation of the β-catenin signaling pathway with subsequent induction of epithelial-to-mesenchymal transition, which is one of the proposed mechanisms for the development of lung fibrosis [62]. More recently, after performing microarray expression studies of the lung microenvironment obtained from laser capture microdissected lung tissue from IPF patients, our group identified MMP-19 overexpression in hyperplastic epithelial cells from IPF individuals when compared with normal-appearing epithelial cells. The presence of MMP-19 was confirmed by immunohistochemistry in hyperplastic epithelial cells overlying fibrotic areas. However, in contrast to what was observed with MMP-7, MMP-19 knockout mice developed worse fibrosis when exposed to bleomycin, suggesting that MMP-19 overexpression was actually a failed effort at protection in this model. Thus, genome-scale transcript profiling studies led to a paradigm shift in the perception of the role of proteases in lung fibrosis, and instead of the simplistic protease-antiprotease imbalance paradigm, we now have a complex view that suggests that proteases have multiple and sometimes opposing roles in lung fibrosis. These roles depend on how MMPs are temporally expressed, the cell type and spatial distribution of MMPs in lung tissues, and the availability of MMP substrates [66].

Genome-scale transcript profiling studies not only generated relevant information regarding the presence and potential role of some of the MMP family members in the pathogenesis of IPF but also opened a new biomarker field for use in IPF diagnosis, disease monitoring, and mortality prediction. Based on our previous findings [51], our group applied a targeted proteomic approach and identified a protein signature including MMP-1, MMP-7, MMP-8, IGFBP-1, and TNFRSA1F [54] that was able to distinguish IPF from healthy controls with a sensitivity of 98.6% and specificity of 98.1%. Two members of this signature, MMP-1 and MMP-7, differentiated IPF patients from those with subacute/chronic hypersensitivity pneumonitis

(HP) with a sensitivity of 96.3% and specificity of 87.2%. Increased concentrations of MMPs, including MMP-7, have also been shown in the peripheral blood and bronchoalveolar lavage of IPF patients [54, 85], confirming that these molecules not only participate in disease pathogenesis but can also be used as makers of disease presence. Indeed, MMP-7 peripheral blood concentrations have been significantly associated with all-cause mortality or transplant-free survival in multiple studies [86, 87]. Xu and collaborators applied single-cell RNAseq that identified different subtypes of epithelial cells in IPF lungs; interestingly, MMP-7 was one of highest differentially expressed genes described in one of the subtypes of epithelial cells found in IPF samples [88].

Thus, the emergence of MMPs as mechanistically important in determining the lung phenotype in IPF and other interstitial lung diseases (as well as their role as new peripheral blood biomarkers candidates) can be fully attributed to unbiased genome-scale transcript profiling.

Role of the Microbiome in the Development, Pathogenesis, and Exacerbations of IPF

Although bacterial infection has only been indirectly implicated in IPF progression and mortality, a number of studies have attempted to evaluate the role of the lung microbiome in the pathogenesis of IPF. Given that previous studies have encountered difficulties elucidating the role of bacteria through culture-dependent microbiological techniques, the advent of the new genome-scale methods has led to significant progress. In microbiome studies bacterial communities are clustered by species using OTUs (operational taxonomic units), which are based on the sequence of their highly conserved regions or the hypervariable regions of their 16S rRNA that is used for sequence-based strain typing to differentiate species among bacterial communities [89].

COMET (Correlating Outcomes with biochemical Markers to Estimate Time-Progression in idiopathic pulmonary fibrosis), a multicenter cohort study, evaluated the role of microbiome in IPF subjects vs a control group. In this study, Han and colleagues pyrosequenced 16s rRNA of 454 BAL samples and found an increased burden of bacteria in IPF [90]. They also found an association between an increased abundance of *Streptococcus* and *Staphylococcus* specific OTU and an increased risk of disease progression [90]. In parallel to the COMET study, Molyneaux et al. [91] studied 65 patients with stable IPF vs 44 control subjects (17 patients with moderate COPD and 27 healthy control subjects). They observed a twofold increase in bacterial burden in IPF patients (quantified by 16S rRNA gene/mL BAL fluid) compared to control subjects, which was associated with a decline of FVC by 10% at 6 months ($P = 0.02$). The species that were more abundantly found were *Veillonella*, *Neisseria*, *Streptococcus*, and *Haemophilus* spp., providing evidence that the bacterial burden, rather than a specific microbiological community, can predict prognosis [91]. They

also studied a different cohort to determine the role of changes in the microbiome in acute exacerbations of IPF, and they demonstrated that IPF patients experiencing an acute exacerbation had a bacterial burden four times higher when compared with stable IPF controls after the two groups were matched for age, sex, smoking history, and baseline lung function [92]. They also found an increased burden of *Proteobacteria* sp. and *Stenotrophomonas* spp. and, interestingly, the gastrointestinal infectious agent *Campylobacter* spp. as compared with controls. This finding opens the door for further studies that investigate the role of prophylactic antibiotic use in individuals with stable disease to see if such an approach diminishes the risk of acute exacerbations in patients with IPF [92].

This same group of researchers also studied the role of the host response to the respiratory microbiome. Gene expression profiles in peripheral blood, using Affymetrix Human Gene 1.1 ST arrays, provide a network analysis of gene expression data using WGCNA. This analysis identified two modules that were associated with the diagnosis of IPF, bacterial burden, specific OTU, and peripheral blood neutrophilia. This included expression of the NLRC4 gene, which encodes a key component of inflammasomes and plays a crucial role in the host response to proteins from pathogenic bacteria and fungi. Also identified was PGLYRP1, which is a gene that encodes a novel antimicrobial protein with bactericidal activity against gram-positive bacteria, as well as MMP-9 and DEFA4, which were previously found to be associated with IPF [93, 94]. In addition, two specific antimicrobial peptides, secretory leukoprotease inhibitor (SLPI), which is a serine protease antagonist, and cathelicidin antimicrobial peptide (CAMP), a molecule with antimicrobial activity, cell chemotaxis, immune mediator induction, and inflammatory response regulation, were found to be expressed. Because transforming growth factor-β (TGF-β) is usually activated by serine proteases, the finding of increased expression of SLPI suggests the possibility of an important role of the SLPI gene in the pathogenesis of IPF. These results further strengthen the relationships among the host peripheral blood transcriptome, microbial signatures, and disease progression [95]. However it needs to be recognized that this correlation does not imply causality between bacterial burden and IPF pathogenesis.

Other groups have evaluated the impact of aerosolized IFN-γ in the lower airway microbiome and the changes it causes in the host immune phenotype in IPF patients [96]. They found only small changes in diversity of the lung microbiome, and these were not significant. This suggests that the lung microbiome is independently associated with host immune status and provides evidence (through a transcriptomic approach) that modulation of the immune response is unlikely to have a critical role on IPF pathogenesis [96].

In summary, as our understanding of the microbiome and its role in the progression and association with acute exacerbations in IPF grows, it is feasible that we can use high-throughput technologies in the lung microbiome of IPF patients to create various computational tools. These may help to develop peripheral biomarkers during exacerbations and microbiome changes to guide prognostication, differentiation, stratification, and diagnostic aids of IPF and potentially other ILDs.

The Wnt Pathway in IPF

As previously mentioned, one of the advantages of the "systems" approach over the "cherry picking approach" for genome-scale transcript profiling is that grouping differentially expressed genes in gene sets allows researchers to identify pathways (and genes within such pathways) that better characterize the differences between the analyzed groups. This approach also allows the generation of new hypotheses regarding disease pathogenesis by focusing on pathways that were not previously perceived as relevant to pathogenesis of the disease, and it also helps researchers focus on differentially expressed genes within a pathway that would have been otherwise missed within a large list of differentially expressed genes.

Following a "systems" approach, unbiased gene expression profiling has consistently validated the recapitulation of developmental pathways, specially Wnt-related pathways and the linkage of this pathway with inflammation [97]. The finding that Wnt signaling pathways reactivated in adult tissues following injury has consistently been validated as contributing to the pathogenesis of IPF. Indeed, canonical Wnt/β-catenin signaling is overexpressed in various cell types in human and experimental pulmonary fibrosis [98]. The Wnt pathways are a network of glycoproteins involved in embryogenesis and lung development that was best characterized after the identification of a mutation in one of its genes, "Wingless," of wingless *Drosophila melanogaster* (fruit fly) [99]. The key player of the Wnt canonical signaling is β-catenin. In the absence of specific Wnt ligands, cytosolic β-catenin is tightly regulated by the so-called β-catenin destruction complex, a multiprotein complex that targets β-catenin via phosphorylation and ubiquitination for proteasomal degradation [100, 101]. Without Wnt signaling, β-catenin is degraded by its destruction complex [102]. Classically, Wnt signaling has been separated into canonical and noncanonical signaling pathways. The canonical Wnt signaling pathway involves β-catenin, and pathways activated by Wnt ligands independently of β-catenin are classified as noncanonical Wnt pathways. New advances and discoveries in this developmental pathway underscore the complexity of this signaling pathway and raise questions about the separation of Wnt signaling in purely canonical and noncanonical signaling pathways [103]. Experiments in mice have demonstrated that β-catenin is required for the normal differentiation of the bronchiolar and alveolar epithelium [104].

In humans, mutations and genetic variances in genes of the Wnt pathway have been associated with different conditions such as cancer, neuropsychiatric disorders, cardiac diseases, and bone disorders, among others [105]. Chilosi et al. demonstrated β-catenin accumulation in fibroblastic foci of IPF lungs and its expression co-localized with two Wnt downstream target genes, cyclin-D1 and MMP-7, in proliferative bronchiolar lesions [106]. This report was followed by the findings of Konigshoff et al. that demonstrated the overexpression of WNT1, WNT7B, WNT10B, FZD2, FZD3, CTNNB1, and LEF1 in lung tissue of individuals with IPF. In particular, the increase in WNT1-inducible signaling protein-1 (WISP1), a gene reported to be involved in the regulation of epithelial cell function and fibroblast differentiation and a downstream regulator of fibrotic markers, suggests that

functional Wnt/β-catenin signaling activity is enhanced in lung tissue of individuals with IPF [98]. Through an unbiased gene expression screen to identify cell-specific mediators of the canonical Wnt pathway in mouse type II alveolar epithelial cells, Aumiller et al. [97] found that IL-1β, a proinflammatory cytokine that has been shown to induce pulmonary fibrosis, was one of the highest upregulated genes induced by WNT3A stimulation. IL-1β has since been confirmed to be upregulated in human IPF lungs. Increased functional Wnt in IPF and the demonstration of reversal of pulmonary fibrosis after the inhibition of Wnt/β-catenin further confirms these results [107, 108]. Interestingly, MMP-7, recently mentioned as both a mechanistically relevant molecule and a peripheral blood biomarker, is a well-described Wnt pathway target gene [109].

In summary, the observation of overexpression of Wnt signaling in IPF suggests an aberrant recapitulation of developmental pathways that are not usually involved in normal lung health as an altered wound healing response. A better understanding of these mechanisms could lead to potential therapeutic strategies for this devastating lung disease.

Apoptosis in lung fibrosis from a genomic perspective Two studies using genomic-based approaches and published only a month apart confirmed the role of apoptosis in IPF pathogenesis. Bridges et al. [110] performed microarray gene expression experiments obtained from normal lung samples and compared them with IPF lungs, including samples obtained from micro-dissected fibroblastic foci. They used class discovery (unsupervised clustering) and class comparison (*t*-test) analyses and identified Twist1 as one of the most consistently upregulated transcription factors in the IPF lung. In this study, researchers determined that overexpression of Twist1 led to increased viability of rat lung fibroblasts when exposed to pro-apoptotic molecules (lipid 4-HNE and thapsigargin), while knockdown of Twist1 resulted in increased activity of caspase-3, a marker of apoptosis, following addition of lower concentrations of these pro-apoptotic stimuli. They also demonstrated that pro-fibrotic growth factors such as platelet-derived growth factor (PDGF) induced Twist1 expression in rat lung fibroblasts, which was necessary to protect these cells from apoptosis, particularly in the continued presence of these pro-fibrotic growth factors. In summary, these investigations demonstrated an anti-apoptotic role of Twist1 by promoting fibroblast viability when this cells where exposed to growth factors.

Our group corroborated these findings and confirmed the role of apoptosis in the pathogenesis of acute exacerbations of IPF [93]. We performed microarray experiments and compared lung tissue of IPF subjects with acute exacerbation, lung tissue from IPF subjects with stable disease, and lungs with normal histology using a class comparison approach (significance analysis of microarrays). A total of 579 genes were found to be differentially expressed between lungs of patients with acute exacerbations of IPF or stable IPF; out of these genes, cyclin A2 (CCNA2), a cell cycle regulatory gene, was one of the top overexpressed genes in this signature, and it was localized to alveolar epithelial cells in subjects with acute exacerbations of IPF. Increased CCNA2 protein expression was localized to proliferating epithelial cells, and these findings suggested accelerated epithelial cell proliferation, potentially as a compensatory response to injured epithelium. More interesting was the

finding that lungs of IPF patients showed widespread apoptosis by in situ TUNEL assay. Taken together, these observations suggest an aberrant proliferative response of the alveolar epithelium in response to apoptosis during IPF acute exacerbations.

Global analysis of IPF lungs reveals dramatic changes in epithelial cell phenotype While the histopathological hallmark of IPF is the presence of fibroblastic foci, there is growing evidence of the role of the alveolar epithelium in IPF pathogenesis [111, 112]. Part of this comes from the observation that a large number of differentially expressed genes in IPF are localized to the alveolar epithelium. We have demonstrated that MMP-1, MMP-7, and MMP-19 localize to the alveolar epithelium as do SPP1, N-cadherin, IGFBP-4, and CCNA2 [113]. Similarly, the Wnt pathway genes WNT1, WNT3a, β-catenin, and Gsk-3β have also been localized to the alveolar and bronchial epithelium as well as HIF1A and VEGF [51, 60, 106, 114–117]. Impressively, a global view of known epithelial cells in IPF (Fig. 9.2)

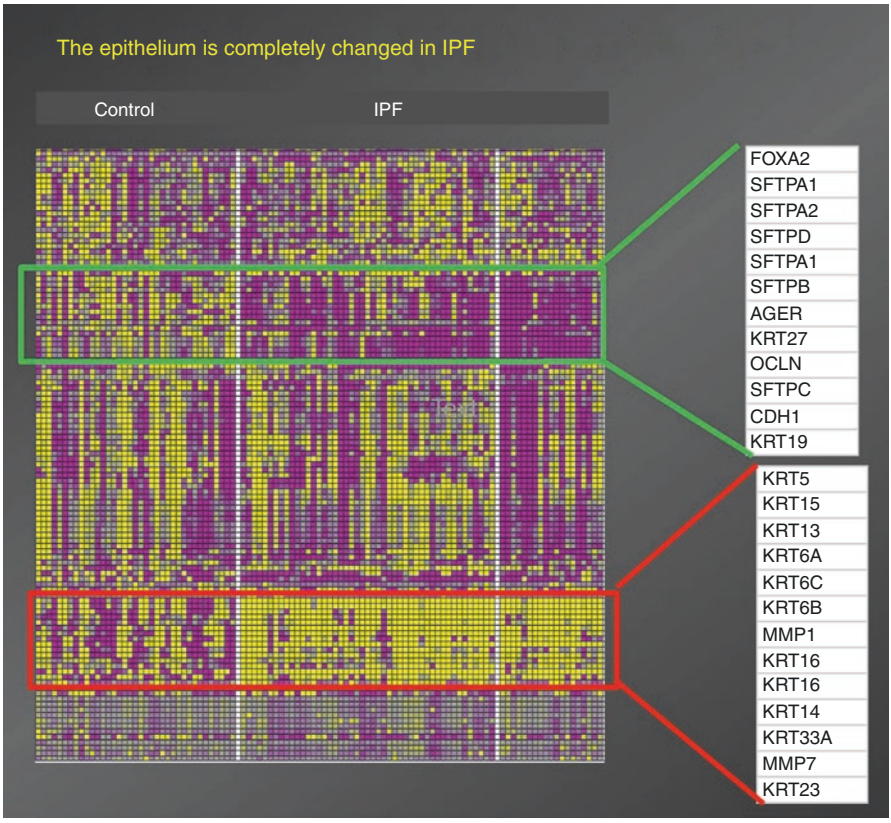

Fig. 9.2 Illustrative figure of changes in epithelial gene expression in IPF lungs. Genes known to be expressed in the epithelium were extracted form a larger microarray dataset. Increased shades of yellow mean increased, gray means unchanged, and increased shades of purple mean decreased gene expression. Note the reduction in genes known to be expressed in type II cells and the change in the cytokeratin profile of IPF lungs

demonstrates a shift in epithelial cell markers with a decline in traditional epithelial markers and increase in markers that are not regularly expressed. Many other genes that may be associated with preservation of a normal epithelial cell phenotype are differentially expressed in IPF, suggesting that key transcriptional events in IPF occur in an injured alveolar epithelium, which in turn responds with the expression of pro-fibrotic markers.

As an example of shifts in epithelial markers, Yang et al. described molecular subtypes of IPF based on differential microarray expression using an analysis of covariance (ANCOVA) model that incorporated clinical variables from patients with varying disease behavior. The molecular signature obtained from transcriptional profiles identified two subtypes of IPF. One was associated with fibrosis (MMPs, osteopontin, keratins) as previously described in the literature; the other molecular phenotype was characterized by cilium gene expression that was associated with more extensive microscopic honeycombing and higher expression of MUC5B and MMP-7. This type of approach is conducive to a more personalized and potentially more precision medicine-based therapeutic approach to future treatments of IPF [118]. In alignment with these results, Xu et al. [88] recently performed single-cell sequencing in epithelial cells from normal lungs and lungs from IPF patients that identified three subsets of epithelial cells related to IPF versus a more homogenous epithelial gene expression profile in the control group. After thorough tissue dissociation and using flow-assisted cell sorting (FACS) to sort epithelial cells from other cell types, they cultured the cells, isolated and enriched the RNA, and used single-cell RNA sequencing (using Hiseq200 Illumina and applying analytic pipeline SINCERA to the generated dataset), which identified four distinct cell clusters. The first cluster was consistent with typical highly differentiated alveolar type 2 (ATII) cell markers that were also present in the signature of the three subtypes of IPF epithelial cells. Individual IPF cells had the ATII gene expression pattern along with three individual signature expression patterns that were consistent with conducting airway epithelial cells (TP63, KRT5, KRT14, BMP7, LAMB3, LAMC2, and ITGB), goblet/club cells (SPDEF, MUC5B, PIGR, AQP3, and SCGB1A1), and indeterminate cells (CTGF, GF11, and FL11, which are key regulators of "activation of myofibroblast," "flux anion," and "T-cell proliferation"). The finding of ATI and ATII transcripts in the IPF epithelial cells and their co-expression of conducting airway and other bronchial cell markers support the hypothesis that epithelial cells of the remodeled distal lung of IPF acquire atypical mixed differentiation states. This is consistent with a diversity of epithelial identities that can be defined by the biological process of the IPF microenvironment influencing the fibrotic lung and partially explains the heterogeneous behavior of this disease over time [88]. New high-throughput sequencing technologies require a very thorough method of isolation of tissue and cells that usually comes from fresh frozen whole lung lysates of tissue biopsies, making them only available in highly specialized academic centers with tissue banking facilities. Our group recently validated the feasibility of performing genome-scale transcript profiling on FFPE (formalin-fixed paraffin-embedded) lung tissue, amplifying the possibility of using genomic techniques to enhance the availability of tissue biopsies for

research in IPF, since this approach allows samples to be acquired from centers that lack biobanking facilities [113].

In summary, the role of epithelial cells in IPF has gained increased attention since the disease paradigm has shifted and pinpoints the repetitive injurious stimuli to the ATII cells as playing a key role in pathogenesis. The increased injury/apoptosis state of epithelial cells alters the normal alveolar structure and drives fibroblast activation and aberrant lung repair that leads to progressive fibrosis. Our understanding of the injurious events and processes involved in aberrant repair of the alveolar epithelium has significantly improved with the new sequencing technologies, and these techniques and strategies hold further promise for providing a better understanding of the pathogenesis of IPF.

Gene Expression Profiling: Classification of Interstitial Lung Diseases and Other Chronic Lung Disease

The diagnosis of interstitial lung diseases in clinical practice can be challenging at times given the fact that some of the patients can present with radiological patterns that are non-conclusive [119–121], and in some cases, lung histology may show discordant patterns such as a usual interstitial pneumonia (UIP) pattern in one lobe and a nonspecific interstitial pneumonia (NSIP) pattern in a different lobe of the same lung [122, 123].

A more common diagnostic dilemma occurs when comparing cases of chronic hypersensitivity pneumonitis (HP), NSIP, and ILD associated with collagen vascular disease from cases of IPF. One of the goals of genomic studies in ILD has been to find transcript profiles that could differentiate among these entities in order to develop more accurate diagnostic strategies. Gene expression studies that address these issues are discussed in the following section.

Differences in gene expression between IPF and HP To study gene expression differences between lung tissues taken from IPF and HP patients, our group performed gene expression microarrays and compared transcript levels using a class comparison (*t*-test) and class prediction (threshold number of misclassifications – TNoM) approach and identified 407 genes that accurately distinguished IPF from HP [114]. The pathway analysis of this signature confirmed the prior knowledge regarding the pathogenesis of these two entities. The HP signature is characterized by enrichment of pathways associated with cytokine and T-cell activation, inflammation, and humoral immune response. In contrast, the IPF signature is characterized by cell adhesion, extracellular matrix, and smooth muscle differentiation as well as genes associated with lung development, heparin binding, enzyme inhibitor activity, and insulin growth factor binding [47]. It is clear after looking at the gene pathway differences between these two conditions that the role of inflammation is more pronounced in HP, while in IPF the role of the matrix and developmental pathways is more characteristic. These findings are concordant with the abundant evidence that inflammation in IPF is not the primary driver of disease pathogenesis as previously thought [124].

Overlapping similarities between COPD and IPF Given that COPD and IPF share risk factors such as cigarette smoking, mechanisms that are common to both diseases have not been clearly elucidated. Kusko et al. [125] identified convergent transcriptomic pathways in emphysema and IPF by applying an integrative transcriptomic approach using RNAseq on emphysema, IPF, and normal lung tissue biopsies. They found 214 genes that were common to IPF and emphysema including many differentially expressed genes from the p53/hypoxia pathway with changes in expression of HIF1a, MDM2, and NFKBIB. Analysis of the RNAseq readouts revealed more differentially spliced genes in IPF and emphysema as compared to normal histology control tissues. These included PDGFA, a gene associated with hypoxic lung injury in murine models of lung disease [126], and NUMB, a gene involved in the prevention of the degradation of TP53 that is involved in the p53/hypoxia pathway.

These authors also integrated miRNA array and RNAseq data with miRconnX, a tool that combines a prior statistical network created from miRNA binding predictions and literature validation with user-submitted data. This allowed them to create a transcriptomic gene regulatory network that identified miR96 (a key microRNA that represses SCL1A1, BTK, and SH3BP5) as a key regulator of the p53/hypoxia pathway in both diseases, and in vitro experiments validate that its overexpression recapitulates components of the shared gene expression network of IPF and emphysema [125]. However, the authors acknowledge certain limitations, including the difficulty in distinguishing the gene expression changes as a cause or a consequence of the disease processes, as well as the transcript origin given the difference in cell-type proportions. Nonetheless this study sheds light on convergent core pathways that initiate chronic lung remodeling in response to environmental injury.

IPF and familial pulmonary fibrosis are unexpectedly different, while IPF and NSIP are unexpectedly similar Yang et al. [127] performed gene expression microarrays of lung tissue from patients with sporadic IPF, familial pulmonary fibrosis, NSIP, and normal controls. They found somewhat disappointing results since the investigators were not able to identify statistically significant differences between IPF and NSIP, and these findings were in agreement with our prior observations [114]. However, they identified genes that were differentially expressed between sporadic IPF and familial pulmonary fibrosis, diseases that otherwise seem to share many more similarities than differences. While the genes distinguishing familial cases from sporadic IPF cases were part of the same functional pathways as genes distinguishing IPF from normal subjects, they seemed to exhibit larger changes. One conclusion was that familial pulmonary fibrosis may represent a more extreme molecular phenotype of the same disease process as sporadic IPF. However, while this is certainly possible, we suggested that harvesting stage in the course of the disease may have played a role in these differences [128], as 50% of the familial samples were obtained from open lung biopsies, whereas 90% of sporadic cases were collected from explant or autopsy specimens.

Different forms of usual interstitial pneumonia share very similar gene expression patterns The usual interstitial pneumonia (UIP) pattern in lung biopsies of patients with systemic sclerosis can be indistinguishable from the UIP pattern of IPF lungs [129], a finding that contrasts with major clinical differences between these two entities. In an attempt to better elucidate the molecular mechanisms behind the differences in systemic sclerosis and IPF, Hsu et al. [130] performed gene expression profiling in patients with systemic sclerosis (SSc) (subclassified as those with a predominant UIP pattern pulmonary fibrosis versus a predominant pulmonary arterial hypertension (PAH) phenotype) and compared gene expression profiles with lung tissues from patients with IPF, patients with idiopathic pulmonary arterial hypertension (IPAH), and normal donors. Using a class comparison approach (efficiency analysis and significance analysis of microarrays), they identified 242 differentially expressed genes between the studied subclasses. The gene expression profile of the UIP lung of IPF patients was very similar to the UIP lung of SSc patients with only 25 genes being uniquely expressed in IPF lung tissues and 20 genes uniquely expressed in the UIP lung tissue of systemic sclerosis patients. The authors of this study acknowledge that one of the limitations in this comparison was the use of explanted lung tissue of patients undergoing lung transplant, which are more likely to represent end-stage disease, and it was suggested that comparisons in gene expression between SSc and IPF lung tissues at earlier stages of disease could potentially provide a better molecular characterization that may distinguish between these two entities.

Kim et al. [131] attempted to develop a molecular test that can distinguish UIP from other ILDs in surgical lung biopsies that could eventually be applied to transbronchial biopsy samples, thereby avoiding the increased risk of performing surgical lung biopsies for the diagnosis of IPF. In this study, they took surgical lung biopsies from 86 patients, and a panel of ILD experts classified them into UIP versus non-UIP (including NSIP, HP, sarcoidosis, respiratory bronchiolitis, organizing pneumonia, and other non-UIP subtypes) diagnoses. They applied gene expression profiling with a machine learning approach and thereby built a model classifier to investigate whether a genomic signal could differentiate UIP from other subtypes that are non-UIP. Using sample-specific pathology labels on biopsy samples, they trained the microarray classifier by logistic regression and identified the top 200 differentially expressed genes to distinguish the UIP from the non-UIP samples, further cross-validating with RNAseq. They found a 92% specificity (95% CI 81–100) and a sensitivity of 82% (64–95) with the microarray classifier, while the RNAseq classifier demonstrated a specificity of 95% (84–100) and a sensitivity of 59% (35–82) in distinguishing UIP from non-UIP. Importantly, they demonstrated a high correlation between the molecular signal built with a classifier algorithm and the expert pulmonary pathologists' diagnoses despite having no clinical or demographic information.

In summary, new gene expression profile tools are undergoing rigorous evaluation to develop new bioinformatics methods that help in the diagnosis of IPF and other ILDs without the need for more invasive diagnostic procedures.

Identification of Gene Expression Profiles Associated with Disease Severity in the IPF Lung and Peripheral Blood

It has been shown that IPF patients have different patterns of disease progression. While some patients can be stable for long period of time, others can quickly deteriorate, have acute exacerbations, and die as a consequence of the disease [132]. The recognition of this erratic clinical behavior of some IPF patients prompted Selman and colleagues [115] to study gene expression profiles of IPF patients with evidence of rapid progression (defined as symptoms starting 6 months prior to initial presentation) and compared them with IPF patients with slow progression (defined as symptoms present for more than 24 months) using a class comparison and class prediction approach. The investigators identified a group of 437 differentially expressed genes between these two groups. When a pathway analysis was performed, patients with evidence of rapid progression had overexpression of genes involved in morphogenesis, cancer, oxidative stress, cell proliferation, apoptosis, and genes from fibroblast/smooth muscle cells. The discovery of overexpression of genes associated with cell proliferation and apoptosis somewhat preceded the findings by Konishi et al. [93], who demonstrated evidence of overexpression of cyclins (cell cycle regulators) along with overwhelming apoptosis in the lung of acute exacerbation patients, again suggesting the potential presence of aberrant proliferative responses in reaction to cellular apoptosis in patients with rapid progression of IPF.

Konishi et al. [93] also made another interesting discovery in the lung tissue of IPF patients with acute exacerbations when they found that alpha defensins, particularly defensin alpha 3 (DEFA3) and 4 (DEFA4), are overexpressed. The authors also demonstrated increased serum levels of these natural antimicrobial peptides, which are part of the innate immune response and participate in host defense [133]. Interestingly, defensins are released in response to microbial invasion and can activate adaptive immunity responses [134], a phenomenon that has been described in IPF [135]. Defensins attract antigen-presenting dendritic cells to the site of invasion and are mostly expressed by neutrophils, epithelial cells, and Paneth cells; interestingly, they are activated by proteolytic cleavage by MMP-7 [59].

These findings complement the recent description of Molyneaux and collaborators where they found an increased burden of respiratory microbiota in acute exacerbations of IPF patients vs stable controls. This highlights a shift in the microbiome composition during acute exacerbations potentially resulting in differential gene expression to a more immune profile [92].

The overexpression of defensins was validated in the peripheral blood transcriptome of IPF patients with evidence of advanced disease, findings recently published by Yang and colleagues [136] who performed gene expression profiling of IPF patients stratified by disease severity. In this study, the investigators defined severe disease as DLCO $\leq$35% or FVC $\leq$50% and compared them with IPF patients with mild disease defined as DLCO $\geq$65% or FVC $\geq$75%. They also compared

these two subclasses of IPF patients with age- and gender-matched healthy controls using a class comparison approach (significance analysis of microarrays). When comparing patients categorized by DLCO ≥65% versus DLCO ≤35%, the authors identified 13 differentially expressed transcripts including once again defensin alpha 3 (DEFA3) and 4 (DEFA4). DEFA3 also differentiated mild and severe IPF cases from healthy controls, confirming the relevance of defensins in IPF progression. These findings provide the notion that defensins are not only surrogates of disease activity and severity but can also be closely associated with IPF pathogenesis.

The functional analysis performed in the study by Yang et al. using the 13 differentially expressed transcripts differentiating mild and severe cases of IPF found overexpression of genes associated with inflammatory responses and immune trafficking in the severe IPF group, a finding that is contradictory to our prior observations in lung tissue from patients with IPF [136]. While this could represent evidence that inflammatory responses are indeed potentially relevant in IPF, it can also represent the presence of a more inflammatory phenotype in the patients with rapid disease progression.

In addition to the aforementioned findings differentiating IPF patients with mild and severe disease, Boon et al. [137] also studied gene expression profiles in lung tissue of IPF patients with evidence of disease progression, defined as a decline of ≥10% and ≥15% over 12 months in FVC% and DLCO%, respectively, and compared them with lung tissue of IPF patients with relatively stable disease (defined as a decline of <10% and <15% over 12 months in FVC% and DLCO%, respectively). The investigators used serial analysis of gene expression (SAGE), a technique that has the same goal as microarrays with the difference that SAGE sampling is based on sequencing of short tags of mRNA, while microarrays are based on hybridization of mRNAs to probes. Using a class comparison (*t*-test) and class discovery (hierarchical clustering) approach, 134 differentially expressed transcripts distinguished the two groups. While this study was limited by the small number of samples (six in each group), it certainly provided interesting findings since some of the overexpressed genes in the group of patients with evidence of IPF progression included surfactant protein A1 (SFTPA1), SPP1, and heat shock 70 KDa protein 1A (HSPA1A), among others. These findings correlate with previous associations of surfactant protein A levels in the serum of IPF patients with the worst survival [138] and with recent observations that IPF patients with autoantibodies against heat shock protein 70 (HSP70) also have increased mortality [139]. With regard to SPP1, we previously reported consistent overexpression of SPP1 when analyzing gene expression profiles of IPF lung tissues compared to normal controls [60] and also demonstrated increased SPP1 levels in bronchoalveolar lavage of IPF patients. We also found evidence suggesting that SPP1 activates MMP-7, co-localizes with this molecule in alveolar epithelial cells of IPF patients, and has a pro-fibrotic effect on lung fibroblasts and epithelial cells. Others have demonstrated the relative protection from bleomycin-induced fibrosis in SPP1 knockout mice and increased SPP1 levels in the serum of patients with ILD. This body of evidence suggests that SPP1

is not only relevant to the pathogenesis of IPF but could also potentially be used as a biomarker of disease progression.

Our group has recently identified a 52-gene signature on peripheral blood mononuclear cells (PBMC) that predicts poor outcomes in IPF patients as determined by transplant-free survival (TFS). This was validated in independent cohorts using microarrays of peripheral blood from IPF and control patients. Significance analysis of microarrays (SAM), an algorithm employing hierarchical clustering to measure expression values clustering samples one gene at a time, was used to estimate Cox scores based on univariate models. We identified changes in the expression of gene pathways in PBMCs that were related to the "costimulatory signal during T-cell activation." Out of this biocarta pathway analysis, CD28, ICOS, LCK, and ITK were the most significantly decreased genes in the patients with the shortest period of TFS. By measuring the DCt expression by qPCR of these aforementioned genes and through combination with the clinical GAP (gender, age, and physiology) score, we showed a better outcome prediction than using the clinical predictor model alone. A decrease in CD28, ICOS, LCK, or ITK expression was associated with a median TFS that ranged from 0.92 to 1.17 years, while increased expression was associated with longer median TFS that ranged from 2.39 to 3.44 years. These results may have clinical utility for predicting poor outcomes, thereby allowing the identification of patients who should be referred for lung transplant evaluation, and these findings have additional implication for subject enrollment and stratification in future drug studies in IPF [140]. We further validated this 52-gene signature in six cohorts using the Scoring Algorithm for Molecular Subphenotypes (SAMS) to classify patients into high- or low-risk groups and predict differences in mortality and TFS. The 52-gene risk profile together with the GAP index resulted in improved predictive accuracy for mortality risk, which supports its potential utility for future clinical drug studies [141].

Our group has now developed a functional genomic model for predicting prognosis in IPF [142], given that our previous results using the gene sets did not provide a weighted score for the gene expression pattern and did not include other genes that we identified in the functional genomic model. By coupling the PBMC gene expression profiling to IPF clinical traits using a WGCNA, we constructed a Prognostic Index (PI) score for each patient, which allowed us to develop a functional genomic model that better identifies those IPF patients with a "good" vs "poor" prognosis as well as those who may be more likely to benefit from IPF-specific therapies.

The fact that the course of IPF is variable and unpredictable has generated substantial interest in finding molecular signatures that help predict outcomes in IPF patients. This need for predictive signatures has opened a new window for prioritizing patients for lung transplantation and for stratification in future studies that evaluate prognosis and drug efficacy. A summary of the most relevant molecules identified in IPF, based on gene expression studies, is provided in Table 9.2.

Table 9.2 Gene Expression in IPF

Gene/ pathway*	Gene name	Direction of expression	Compartment identified	Relevant pathway	References
MMP-7	Matrix metalloproteinase-7	Overexpressed	Lung, peripheral blood, and BAL	Extracellular matrix degradation	[51, 52, 54, 114, 143]
MMP-3	Matrix metalloproteinase-3	Overexpressed	Lung	Extracellular matrix degradation	[62]
MMP-19	Matrix metalloproteinase-19	Overexpressed	Lung – hyperplastic epithelial cells	Extracellular matrix degradation	[63]
SERPINF1 (PEDF)	Pigment epithelium-derived factor	Overexpressed	Lung	Angiogenesis	[144]
SPP1	Osteopontin	Overexpressed	Lung	Extracellular matrix degradation	[60, 137]
HIF1A	Hypoxia-inducible factor-1 alpha	Overexpressed	Lung	Hypoxia	[117]
Wnt*	Wingless and others	Overexpressed	Lung	Wnt signaling	[116, 145]
CXCL12	Chemokine ligand 12	Overexpressed	Lung	Inflammation	[127]
TWIST1	Twist basic helix-loop-helix transcription factor 1	Overexpressed	Lung – fibroblastic foci	Apoptosis	[110]
CCNA2	Cyclin A2	Overexpressed	Lung	Cell cycle regulation	[93]
DEFA3-4	Defensin alpha 3 and 4	Overexpressed	Lung and peripheral blood	Host defense	[93, 136]
CAV1	Caveolin 1	Underexpressed	Lung	Cell cycle regulation	[146]
AGER (RAGE)	Advanced glycosylation end product-specific receptor	Underexpressed	Lung and peripheral blood	Inflammation	[54, 147]
MUC5B	Mucin 5B		Lung		[148]
CCL8	C-C motif chemokine ligand 8	Overexpressed	Fibroblasts derived from lung tissues		[149]
52 gene signature	52 genes	Underexpressed and overexpressed	PBMC	Costimulatory signal during T-cell activation	[140–142]
NLRC4, PGLYRP1, SLPI, and CAMP	4 genes	Upregulated	PBMC	Host defense response	

*Wnt is a pathway (that involves multiple genes)

Noncoding RNAs in IPF (let-7, mir-154, mir-29b, mir-199, and Others)

One of the direct results of the human genome project and the large next-generation sequencing studies that followed including ENCODE [150] was the recognition that noncoding RNAs are critically important in determining cell and organ phenotype through their effects on gene and protein expression (Fig. 9.1). While the data are still emerging, it is already obvious that at least one family of noncoding RNAs, microRNAs, is critically important in IPF. MicroRNAs are small noncoding RNAs (21–25 nucleotides) that bind by base pairing to the 3′ untranslated region of their target mRNAs. In most cases they repress gene expression by increasing mRNA degradation or disrupting the initiation of mRNA translation [151]. In two recent studies that utilized different generations of microRNA profiling technologies, we determined that approximately 10% of the microRNAs measured were differentially expressed in IPF [152, 153]. Our first report focused on let-7d microRNA [152], an epithelial microRNA downregulated in IPF lungs. We found evidence that let7d is a modulator of TGF-β signaling and a sustainer of epithelial cell phenotypes. Thus, when we inhibited let-7 microRNAs in vitro and in vivo, we found a change in epithelial cell phenotype with increased expression of mesenchymal markers and a phenotype consistent with epithelial mesenchymal transition (EMT). We then focused on microRNAs that were increased in IPF lungs and identified 43 significantly upregulated microRNAs [153], over half of which were localized to chromosome 14q32. mir-154 was among the increased microRNAs in IPF fibroblasts and emerged as a regulator of fibroblast proliferation and migration through its permissive effect on Wnt pathway activation in lung fibroblasts, adding to the evidence of aberrant Wnt pathway activation in IPF.

Our group has also described the efficacy of mir-29b blunting pulmonary fibrosis in a murine model. mir-29b is a microRNA that is a key suppressor of many downstream target genes involved in fibrogenesis, including COL1A1, COL3A1, and FBN1, which are usually regulated by the TGF-B/smad3 pathway [154]. Several other studies have suggested roles for mir-21 [155], mir-200 [156], mir-31 [157], and the mir-17-92 cluster [158], and a defect in microRNA processing in IPF has also been suggested [159].

Taken together, these studies indicate a profound dysregulation of microRNAs in IPF that may have significant mechanistic roles and potential therapeutic implications, including a combined analysis of microRNAs and their targets in IPF [160]. The recent recognition of the expression of microRNAs in the peripheral blood in other disease entities [161, 162] in addition to their potential role in the pathogenesis of lung fibrosis should encourage investigators to extend their studies to blood sampling. Finally, considering that microRNAs are only one family of microRNAs, it is highly likely that other noncoding RNAs such as large intergenic noncoding RNAs (lincRNAs) are also aberrantly expressed and functionally relevant to IPF pathogenesis [163, 164]. A growing body of evidence supports the notion that long noncoding RNAs (lncRNAs) act as epigenetic regulators (e.g., by functioning as

sponges or decoys) and play roles in various physiological and pathological conditions. Huang and colleagues [165] used the NONCODE database that contains 33,829 lncRNAs and aligned them with the known dysregulated microRNAs in IPF that included mir-21, mir-31, mir-101, mir-29, mir-199, and let-7d. They identified 34 lncRNAs that have potential binding sites of the dysregulated microRNAs in IPF, out of which 9 lncRNAs were dysregulated in IPF. They further discovered that the CD99 molecule pseudogene (CD99P1) inhibited proliferation and α-smooth muscle actin expression of lung fibroblasts and lncRNA n341773 inhibited collagen expression in lung fibroblasts in in vitro experiments. These results also infer that lncRNA may be involved in IPF. Table 9.3 shows the summary of relevant microRNAs in IPF identified by transcript profiling.

Epigenomic Changes in IPF Lungs

Epigenetic mechanisms, such as DNA methylation and histone modifications, are key adaptive mechanisms by which lasting changes in cell or organism phenotypes are induced in response to environmental or other stresses without changes in DNA sequences or content [175]. In addition to several reports of changes in promoter methylation states in specific genes in IPF [176–178], two recent reports suggested global methylation changes were present in IPF lungs [179, 180]. Rabinovich et al. [180] applied Agilent Human CpG Island Microarrays and methylated DNA immunoprecipitation (MEDIP), a method that applies antibodies to methylated cytosine to identify differential methylation, and identified 625 differentially methylated CpG islands between IPF and control lung tissue samples. Interestingly, they discovered that IPF lungs displayed an intermediate methylation profile between lung cancer and control samples with 402 differentially methylated CpG islands overlapping between IPF and cancer tissues. Sanders et al. [181] utilized the *bisulfite* conversion assay that converts unmethylated cytosine into uracil and determined that 870 genes were differentially methylated. They identified 16 genes with inversely related significant changes in gene methylation and expression, and 8 of these genes were previously shown to be associated with fibrosis. Accordingly, Yang et al. [182] identified DNA methylation changes in IPF using comprehensive high-throughput arrays for relative methylation arrays (CHARM) and later performing an integrative genomic analysis that pinpointed the majority of the changes as occurring outside of CpG islands. This stands in contrast to previous results of Rabinovich et al. [180] and Sanders et al. [181], as there was only a 7% overlap of genes identified previously by these two investigations. Given that recently it has been shown that changes in CpG island shores have more regulatory effects than the ones in the CpG islands themselves, they observed enrichment for gene expression-methylation relationships that were opposite in directionality using QTL mapping, which suggested that gene expression in IPF is at least partially regulated by epigenetic changes of transcription factors that regulate expression of downstream genes. One novel finding in this former study was the identification of CASZ1, a transcription factor with a

Table 9.3 Summary of relevant microRNAs in IPF identified by transcript profiling

MicroRNA	Direction of expression	Compartment identified	Role in IPF	References
miR-92a	Downregulated	Primary IPF fibroblasts	WISP1 regulation in fibroblasts	[166]
miR-210	Upregulated	IPF fibroblasts	Hypoxia induces its expression through HIF2α promoting	[167]
			IPF fibroblast proliferation	
miR-29b	Downregulated	IPF lungs	TGF-β downregulates its expression	[154, 168]
			Target multiple pro-fibrotic markers	
miR-326	Downregulated	IPF lungs	Controls TGF-β signaling and fibrosis-related targets. Such as such as Ets1, Smad3, and MMP-9	[169]
Let-7d	Downregulated	IPF lungs	Transcriptionally inactivated by SMAD3	[152]
			Targets HMGA2, which regulates EMT	
miR-26a	Downregulated			[170]
miR-154	Upregulated	Lung myofibroblasts in IPF	SMAD3 regulation	[153]
			Increase the migration and proliferation of fibroblasts	
miR-29c	Downregulated	IPF epithelial cells	Epithelial integrity and apoptosis regulation	[171]
miR-199a-5p	Upregulated	IPF lungs	Induced by TGF- β	[172]
			Increase migration and invasion of fibroblast	
miR-21	Upregulated	Fibroblastic foci	Target SMAD7	[155]
			SMAD2 phosphorylation induction	
miR-145	Upregulated	NHLF	Activates latent TGF-β	[173]
			Binds to 3′UTR of KLF4 (a-SMA regulator)	
miR-96	Overexpressed	IPF and COPD lungs	Downregulation of glutamate transporter SCL1A1 and BTK inhibitor SH3BP5	[125]
miR-17-92	Downregulated	IPF fibroblasts	Reduction of VEGF, CTGF, COL1A1, and COL3A1	[158]
miR-323a-3p	Downregulated	Lung epithelium	Lowers caspase-3 expression	[174]
			Attenuates TGF-α and TGF-β signaling	
			Modulates inhibitory crosstalk with fibroblasts	

previously unknown role in the pathogenesis of IPF. This transcription factor promotes vascular assembly and morphogenesis by binding to an intronic element in EGFL7 [183]. It is also secreted as an angiogenic factor that binds to ECM, and it has a putative role in Notch signaling. Given that matrix deposition has close relationship with the recapitulation of developmental pathways, this finding may open a door for further studies of the role of CASZ1 in IPF.

While at this stage, it is impossible to draw any final conclusions from the small number of studies performed to date, the findings suggest that changes in gene methylation, one aspect of epigenetics, are indeed relevant to the pathogenesis of IPF. This justifies additional forays into genome-scale profiling of epigenetic changes and opens a window for further exploration of potential epigenetic therapies that may benefit patients with IPF.

Conclusions and Future Directions

The application of genomics to our understanding of fibrotic lung diseases, especially focusing on IPF, the most common and lethal form of idiopathic ILD, will undoubtedly have a significant impact on the identification of novel therapies for treating IPF. Genomic technologies (such as microarrays and RNAseq) have provided significant findings that have led to paradigm shifts with regard to the role of matrix metalloproteases, developmental pathways such as the Wnt pathway, the relevance of the alveolar epithelial changes, and the role of noncoding RNAs.

The contribution of high-throughput technology studies in fibrotic lung disease is not limited to understanding IPF pathogenesis. The transcript profiling findings have also led to the identification of MMP-7, one of the emerging and increasingly validated peripheral blood biomarkers for IPF diagnosis and outcome prediction, as well as many other potentially useful biomarkers. Reviews of differences and similarities in gene expression profiling in lung tissue and the peripheral blood of IPF patients as compared to other forms of ILD and non-ILD diseases like COPD have provided new insights into the identification of gene expression profiles associated with disease severity. Furthermore, these studies have highlighted the depth of disease-relevant information that can be gleaned from genome-scale transcript profiles and should encourage investigators to undertake future studies that are larger in both scope and depth.

While the field of genomics is continuously evolving and new discoveries in ILD are constantly arising, the available data has been extensively analyzed and reviewed; we have now, at least from a “systems” perspective, greatly expanded our ability to characterize the pathology of the IPF lung. It is clear that new studies are required before delving extensively into other less common forms of ILD and to explore the differences between the two physiologic extremes of pulmonary pathologies, the obstructive and restrictive lung diseases. Along these lines, study designs that may

potentially impact the care of patients would include investigations of how genomic phenomena are linked to disease time course as well as large transcriptomic analyses of peripheral blood specimens; such studies could result in the development of biomarkers that provide a closer representation of disease activity and progression at a molecular level [184]. New studies that develop integrative frameworks [185] from large datasets using clinical data as well as changes in regulatory portions of the genome (such as microRNAs and lincRNAs) have shed light on the identification of pathways involved in different forms of ILD. This integration has already been initiated with the financial support of the National Institutes of Health (NIH) through funding of the Lung Tissue Research Consortium (http://www.ltrcpublic.com/) and the Lung Genomics Research Consortium (http://www.lung-genomics.org/), both of which have generated genetic and genomic information from more than 500 lung tissue samples that are available to the public for data analysis. Advanced technologies that allow profiling at the cellular level will undoubtedly have an even greater impact, but funding and resources for data sharing may limit their broader application to the study of IPF pathogenesis.

Finally, it will likely become crucial for investigators and clinicians to integrate genetic and genomic information into patient care. In addition to the findings already described in this chapter, several recent studies have applied genome-wide association studies to the identification of novel variants associated with IPF [148, 186]. However, without a concerted effort, all of the progress achieved in the discipline of genomics may not add significant knowledge to our existing paradigms for the care of patients with ILD. A committed effort from the scientific community, regulatory agencies, and industry is required for the final translation of genetic and genomic information into patient care. This book chapter provides strong evidence of the importance of the genomic field to the study of lung fibrosis, including the potential for translation into the evaluation and treatment of patients with fibrotic lung diseases.

References

1. Yang IV, Schwartz DA. The next generation of complex lung genetic studies. Am J Respir Crit Care Med. 2012;186(11):1087–94.
2. Fodor SP, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D. Light-directed, spatially addressable parallel chemical synthesis. Science. 1991;251(4995):767–73.
3. Lipshutz RJ, Morris D, Chee M, Hubbell E, Kozal MJ, Shah N, et al. Using oligonucleotide probe arrays to access genetic diversity. BioTechniques. 1995;19(3):442–7.
4. Schena M, Heller RA, Theriault TP, Konrad K, Lachenmeier E, Davis RW. Microarrays: biotechnology's discovery platform for functional genomics. Trends Biotechnol. 1998;16(7):301–6.
5. Cloonan N, Grimmond SM. Transcriptome content and dynamics at single-nucleotide resolution. Genome Biol. 2008;9(9):234.
6. Simon R, Korn E, McShane L, Radmacher M, Wright G, Zhao Y. Design and analysis of DNA microarray investigations. New York: Springer-Verlag; 2003.
7. Churchill GA. Using ANOVA to analyze microarray data. BioTechniques. 2004;37(2):173–5, 7.

8. Consortium M, Shi L, Reid LH, Jones WD, Shippy R, Warrington JA, et al. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol. 2006;24(9):1151–61.
9. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A. 2001;98(9):5116–21.
10. Wright GW, Simon RM. A random variance model for detection of differential gene expression in small microarray experiments. Bioinformatics. 2003;19(18):2448–55.
11. Witten DM, Tibshirani R. Testing significance of features by lassoed principal components. Ann Appl Stat. 2008;2(3):986–1012.
12. Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix M, et al. Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature. 2000;406(6795):536–40.
13. Ben-Dor A, Bruhn L, Friedman N, Nachman I, Schummer M, Yakhini Z. Tissue classification with gene expression profiles. J Comput Biol J Comput Mol Cell Biol. 2000;7(3–4):559–83.
14. Tukey JW. Tightening the clinical trial. Control Clin Trials. 1993;14(4):266–85.
15. Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, et al. Gene-expression profiles in hereditary breast cancer. N Engl J Med. 2001;344(8):539–48.
16. Nguyen DV, Rocke DM. Tumor classification by partial least squares using microarray gene expression data. Bioinformatics. 2002;18(1):39–50.
17. Cover TM, Hart PE. Nearest neighbor pattern classification. IEEE Trans Inf Theory. 1967;13:21–7.
18. Parry RM, Jones W, Stokes TH, Phan JH, Moffitt RA, Fang H, et al. k-Nearest neighbor models for microarray gene expression analysis and clinical outcome prediction. Pharmacogenomics J. 2010;10(4):292–309.
19. Brown MP, Grundy WN, Lin D, Cristianini N, Sugnet CW, Furey TS, et al. Knowledge-based analysis of microarray gene expression data by using support vector machines. Proc Natl Acad Sci U S A. 2000;97(1):262–7.
20. Tibshirani R, Hastie T, Narasimhan B, Chu G. Diagnosis of multiple cancer types by shrunken centroids of gene expression. Proc Natl Acad Sci U S A. 2002;99(10):6567–72.
21. Geman D, d'Avignon C, Naiman DQ, Winslow RL. Classifying gene expression profiles from pairwise mRNA comparisons. Stat Appl Gen Mol Biol. 2004;3:19.
22. Hartigan JA, Wong MA. Algorithm AS 136: a K-means clustering algorithm. J Royal Stat Soc Ser C (Appl Stat). 1979;28(1):100–8.
23. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A. 1998;95(25):14863–8.
24. Cheng Y, Church GM. Biclustering of expression data. Proc Int Conf Intell Syst Mol Biol ISMB Int Conf Intell Syst Mol Biol. 2000;8:93–103.
25. Tamayo P, Slonim D, Mesirov J, Zhu Q, Kitareewan S, Dmitrovsky E, et al. Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation. Proc Natl Acad Sci U S A. 1999;96(6):2907–12.
26. Yeung KY, Fraley C, Murua A, Raftery AE, Ruzzo WL. Model-based clustering and data transformations for gene expression data. Bioinformatics. 2001;17(10):977–87.
27. Eichler GS, Huang S, Ingber DE. Gene Expression Dynamics Inspector (GEDI): for integrative analysis of expression profiles. Bioinformatics. 2003;19(17):2321–2.
28. Goeman JJ, van de Geer SA, de Kort F, van Houwelingen HC. A global test for groups of genes: testing association with a clinical outcome. Bioinformatics. 2004;20(1):93–9.
29. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545–50.
30. Dinu I, Potter JD, Mueller T, Liu Q, Adewale AJ, Jhangri GS, et al. Improving gene set analysis of microarray data by SAM-GS. BMC Bioinf. 2007;8:242.
31. Efron B, Tibshirani R. On testing the significance of set of genes. Ann Appl Stat. 2007;1(1):107–29.

32. Setlur SR, Royce TE, Sboner A, Mosquera JM, Demichelis F, Hofer MD, et al. Integrative microarray analysis of pathways dysregulated in metastatic prostate cancer. Cancer Res. 2007;67(21):10296–303.
33. Xu X, Zhao Y, Simon R. Gene set expression comparison kit for BRB-ArrayTools. Bioinformatics. 2008;24(1):137–9.
34. Cox DR. Regression models and life-tables. J Royal Stat Soc Ser B. 1972;34(2):187–220.
35. Nguyen DV, Rocke DM. Partial least squares proportional hazard regression for application to DNA microarray survival data. Bioinformatics. 2002;18(12):1625–32.
36. Michiels S, Koscielny S, Hill C. Prediction of cancer outcome with microarrays: a multiple random validation strategy. Lancet. 2005;365(9458):488–92.
37. Bair E, Hastie T, Paul D, Tibshirani R. Prediction by supervised principal components. J Am Stat Assoc. 2006;101(473):119–37.
38. Zaykin DV, Zhivotovsky LA, Westfall PH, Weir BS. Truncated product method for combining P-values. Genet Epidemiol. 2002;22(2):170–85.
39. Choi JK, Yu U, Kim S, Yoo OJ. Combining multiple microarray studies and modeling interstudy variation. Bioinformatics. 2003;19(Suppl 1):i84–90.
40. Hong F, Breitling R, McEntee CW, Wittner BS, Nemhauser JL, Chory J. RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis. Bioinformatics. 2006;22(22):2825–7.
41. Pyne S, Futcher B, Skiena S. Meta-analysis based on control of false discovery rate: combining yeast ChIP-chip datasets. Bioinformatics. 2006;22(20):2516–22.
42. Fishel I, Kaufman A, Ruppin E. Meta-analysis of gene expression data: a predictor-based approach. Bioinformatics. 2007;23(13):1599–606.
43. Wang X, Kang DD, Shen K, Song C, Lu S, Chang LC, et al. An R package suite for microarray meta-analysis in quality control, differentially expressed gene analysis and pathway enrichment detection. Bioinformatics. 2012;28:2534–6.
44. Reimers M, Carey VJ. Bioconductor: an open source framework for bioinformatics and computational biology. Methods Enzymol. 2006;411:119–34.
45. Simon R, Lam A, Li MC, Ngan M, Menenzes S, Zhao Y. Analysis of gene expression data using BRB-ArrayTools. Cancer Inform. 2007;3:11–7.
46. Raponi M, Zhang Y, Yu J, Chen G, Lee G, Taylor JM, et al. Gene expression signatures for predicting prognosis of squamous cell and adenocarcinomas of the lung. Cancer Res. 2006;66(15):7466–72.
47. Kaminski N, Rosas IO. Gene expression profiling as a window into idiopathic pulmonary fibrosis pathogenesis: can we identify the right target genes? Proc Am Thorac Soc. 2006;3(4):339–44.
48. Ban TA. The role of serendipity in drug discovery. Dialogues Clin Neurosci. 2006;8(3):335–44.
49. Steinberg D. Chance and serendipity in science: two examples from my own career. J Biol Chem. 2011;286(44):37895–904.
50. Selman M, Ruiz V, Cabrera S, Segura L, Ramirez R, Barrios R, et al. TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? Am J Physiol Lung Cell Mol Physiol. 2000;279(3):L562–74.
51. 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.
52. Cosgrove GP, Schwarz MI, Geraci MW, Brown KK, Worthen GS. Overexpression of matrix metalloproteinase-7 in pulmonary fibrosis. Chest. 2002;121(3 Suppl):25S–6S.
53. Fujishima S, Shiomi T, Yamashita S, Yogo Y, Nakano Y, Inoue T, et al. Production and activation of matrix metalloproteinase 7 (matrilysin 1) in the lungs of patients with idiopathic pulmonary fibrosis. Arch Pathol Lab Med. 2010;134(8):1136–42.
54. 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.

55. Pardo A, Selman M. Matrix metalloproteases in aberrant fibrotic tissue remodeling. Proc Am Thorac Soc. 2006;3(4):383–8.
56. Pardo A, Selman M. Role of matrix metaloproteases in idiopathic pulmonary fibrosis. Fibrogenesis Tissue Repair. 2012;5(Suppl 1):S9.
57. Manicone AM, Huizar I, McGuire JK. Matrilysin (matrix metalloproteinase-7) regulates anti-inflammatory and antifibrotic pulmonary dendritic cells that express CD103 (alpha(E)beta(7)-integrin). Am J Pathol. 2009;175(6):2319–31.
58. Swee M, Wilson CL, Wang Y, McGuire JK, Parks WC. Matrix metalloproteinase-7 (matrilysin) controls neutrophil egress by generating chemokine gradients. J Leukoc Biol. 2008;83(6):1404–12.
59. Wilson CL, Schmidt AP, Pirila E, Valore EV, Ferri N, Sorsa T, et al. Differential processing of {alpha}- and {beta}-defensin precursors by matrix metalloproteinase-7 (MMP-7). J Biol Chem. 2009;284(13):8301–11.
60. 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.
61. Checa M, Ruiz V, Montano M, Velazquez-Cruz R, Selman M, Pardo A. MMP-1 polymorphisms and the risk of idiopathic pulmonary fibrosis. Hum Genet. 2008;124(5):465–72.
62. Yamashita CM, Dolgonos L, Zemans RL, Young SK, Robertson J, Briones N, et al. Matrix metalloproteinase 3 is a mediator of pulmonary fibrosis. Am J Pathol. 2011;179(4):1733–45.
63. Yu G, Kovkarova-Naumovski E, Jara P, Parwani A, Kass D, Ruiz V, et al. Matrix metalloproteinase-19 is a key regulator of lung fibrosis in mice and humans. Am J Respir Crit Care Med. 2012;186(8):752–62.
64. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev. 2000;14(2):163–76.
65. Sen AI, Shiomi T, Okada Y, D'Armiento JM. Deficiency of matrix metalloproteinase-13 increases inflammation after acute lung injury. Exp Lung Res. 2010;36(10):615–24.
66. Pardo A, Selman M, Kaminski N. Approaching the degradome in idiopathic pulmonary fibrosis. Int J Biochem Cell Biol. 2008;40(6–7):1141–55.
67. Pardo A, Barrios R, Gaxiola M, Segura-Valdez L, Carrillo G, Estrada A, et al. Increase of lung neutrophils in hypersensitivity pneumonitis is associated with lung fibrosis. Am J Respir Crit Care Med. 2000;161(5):1698–704.
68. McKeown S, Richter AG, O'Kane C, McAuley DF, Thickett DR. MMP expression and abnormal lung permeability are important determinants of outcome in IPF. Eur Respir J. 2009;33(1):77–84.
69. Manicone AM, Harju-Baker S, Johnston LK, Chen AJ, Parks WC. Epilysin (matrix metalloproteinase-28) contributes to airway epithelial cell survival. Respir Res. 2011;12:144.
70. Kaminski N, Allard JD, Pittet JF, Zuo F, Griffiths MJ, Morris D, et al. Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. Proc Natl Acad Sci U S A. 2000;97(4):1778–83.
71. Jara P, Calyeca J, Romero Y, Placido L, Yu G, Kaminski N, et al. Matrix metalloproteinase (MMP)-19-deficient fibroblasts display a profibrotic phenotype. Am J Physiol Lung Cell Mol Physiol. 2015;308(6):L511–22.
72. Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, et al. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell. 1999;99(1):81–92.
73. Hodges RJ, Jenkins RG, Wheeler-Jones CP, Copeman DM, Bottoms SE, Bellingan GJ, et al. Severity of lung injury in cyclooxygenase-2-deficient mice is dependent on reduced prostaglandin E(2) production. Am J Pathol. 2004;165(5):1663–76.
74. Herrera I, Cisneros J, Maldonado M, Ramirez R, Ortiz-Quintero B, Anso E, et al. Matrix metalloproteinase (MMP)-1 induces lung alveolar epithelial cell migration and proliferation, protects from apoptosis, and represses mitochondrial oxygen consumption. J Biol Chem. 2013;288(36):25964–75.
75. Gharib SA, Johnston LK, Huizar I, Birkland TP, Hanson J, Wang Y, et al. MMP28 promotes macrophage polarization toward M2 cells and augments pulmonary fibrosis. J Leukoc Biol. 2014;95(1):9–18.

76. Garcia-Prieto E, Gonzalez-Lopez A, Cabrera S, Astudillo A, Gutierrez-Fernandez A, Fanjul-Fernandez M, et al. Resistance to bleomycin-induced lung fibrosis in MMP-8 deficient mice is mediated by interleukin-10. PLoS One. 2010;5(10):e13242.
77. Garcia-de-Alba C, Becerril C, Ruiz V, Gonzalez Y, Reyes S, Garcia-Alvarez J, et al. Expression of matrix metalloproteases by fibrocytes: possible role in migration and homing. Am J Respir Crit Care Med. 2010;182(9):1144–52.
78. Garcia-Alvarez J, Ramirez R, Sampieri CL, Nuttall RK, Edwards DR, Selman M, et al. Membrane type-matrix metalloproteinases in idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis. 2006;23(1):13–21.
79. Flechsig P, Hartenstein B, Teurich S, Dadrich M, Hauser K, Abdollahi A, et al. Loss of matrix metalloproteinase-13 attenuates murine radiation-induced pulmonary fibrosis. Int J Radiat Oncol Biol Phys. 2010;77(2):582–90.
80. Craig VJ, Quintero PA, Fyfe SE, Patel AS, Knolle MD, Kobzik L, et al. Profibrotic activities for matrix metalloproteinase-8 during bleomycin-mediated lung injury. J Immunol. 2013;190(8):4283–96.
81. Craig VJ, Polverino F, Laucho-Contreras ME, Shi Y, Liu Y, Osorio JC, et al. Mononuclear phagocytes and airway epithelial cells: novel sources of matrix metalloproteinase-8 (MMP-8) in patients with idiopathic pulmonary fibrosis. PLoS One. 2014;9(5):e97485.
82. Cabrera S, Selman M, Lonzano-Bolanos A, Konishi K, Richards TJ, Kaminski N, et al. Gene expression profiles reveal molecular mechanisms involved in the progression and resolution of bleomycin-induced lung fibrosis. Am J Physiol Lung Cell Mol Physiol. 2013;304(9):L593–601.
83. Cabrera S, Gaxiola M, Arreola JL, Ramirez R, Jara P, D'Armiento J, et al. Overexpression of MMP9 in macrophages attenuates pulmonary fibrosis induced by bleomycin. Int J Biochem Cell Biol. 2007;39(12):2324–38.
84. Agnihotri R, Crawford HC, Haro H, Matrisian LM, Havrda MC, Liaw L. Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin). J Biol Chem. 2001;276(30):28261–7.
85. McKeown S, Richter AG, O'Kane C, McAuley DF, Thickett DR. Matrix metalloproteinase expression and abnormal lung permeability are important determinants of outcome in IPF. Eur Respir J. 2009;33(1):77–84.
86. Tzouvelekis A, Herazo-Maya JD, Slade M, Chu JH, Deiuliis G, Ryu C, et al. Validation of the prognostic value of MMP-7 in idiopathic pulmonary fibrosis. Respirology. 2017;22(3):486–93.
87. 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.
88. Xu Y, Mizuno T, Sridharan A, Du Y, Guo M, Tang J, et al. Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis. JCI Insight. 2016;1(20):e90558.
89. Grice EA, Segre JA. The human microbiome: our second genome. Annu Rev Genomics Hum Genet. 2012;13:151–70.
90. Han MK, Zhou Y, Murray S, Tayob N, Noth I, Lama VN, et al. Lung microbiome and disease progression in idiopathic pulmonary fibrosis: an analysis of the COMET study. Lancet Respir Med. 2014;2(7):548–56.
91. Molyneaux PL, Cox MJ, Willis-Owen SA, Mallia P, Russell KE, Russell AM, et al. The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2014;190(8):906–13.
92. Molyneaux PL, Cox MJ, Wells AU, Kim HC, Ji W, Cookson WO, et al. Changes in the respiratory microbiome during acute exacerbations of idiopathic pulmonary fibrosis. Respir Res. 2017;18(1):29.
93. 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.
94. Ramírez G, Hagood JS, Sanders Y, Ramírez R, Becerril C, Segura L, et al. Absence of Thy-1 results in TGF-β induced MMP-9 expression and confers a profibrotic phenotype to human lung fibroblasts. Lab Investig. 2011;91(8):1206–18.

95. Molyneaux PL, Willis-Owen SAG, Cox MJ, James P, Cowman S, Loebinger M, et al. Host-microbial interactions in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2017;195(12):1640–50.
96. Wang J, Lesko M, Badri MH, Kapoor BC, Wu BG, Li Y, et al. Lung microbiome and host immune tone in subjects with idiopathic pulmonary fibrosis treated with inhaled interferon-gamma. ERJ Open Res. 2017;3(3). pii: 00008-2017.
97. Aumiller V, Balsara N, Wilhelm J, Gunther A, Konigshoff M. WNT/beta-catenin signaling induces IL-1beta expression by alveolar epithelial cells in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2013;49(1):96–104.
98. Konigshoff M, Kramer M, Balsara N, Wilhelm J, Amarie OV, Jahn A, et al. WNT1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. J Clin Invest. 2009;119(4):772–87.
99. Sharma RP, Chopra VL. Effect of the Wingless (wg1) mutation on wing and haltere development in *Drosophila melanogaster*. Dev Biol. 1976;48(2):461–5.
100. Rao TP, Kuhl M. An updated overview on Wnt signaling pathways: a prelude for more. Circ Res. 2010;106(12):1798–806.
101. Blankesteijn WM, van de Schans VA, ter Horst P, Smits JF. The Wnt/frizzled/GSK-3 beta pathway: a novel therapeutic target for cardiac hypertrophy. Trends Pharmacol Sci. 2008;29(4):175–80.
102. Baarsma HA, Konigshoff M, Gosens R. The WNT signaling pathway from ligand secretion to gene transcription: molecular mechanisms and pharmacological targets. Pharmacol Ther. 2013;138(1):66–83.
103. Baarsma HA, Konigshoff M. 'WNT-er is coming': WNT signalling in chronic lung diseases. Thorax. 2017;72(8):746–59.
104. Stripp BR, Reynolds SD. Maintenance and repair of the bronchiolar epithelium. Proc Am Thorac Soc. 2008;5(3):328–33.
105. Luo J, Chen J, Deng ZL, Luo X, Song WX, Sharff KA, et al. Wnt signaling and human diseases: what are the therapeutic implications? Lab Investig J Tech Methods Pathol. 2007;87(2):97–103.
106. Chilosi M, Poletti V, Zamo A, Lestani M, Montagna L, Piccoli P, et al. Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. Am J Pathol. 2003;162(5):1495–502.
107. Henderson WR Jr, Chi EY, Ye X, Nguyen C, Tien YT, Zhou B, et al. Inhibition of Wnt/beta-catenin/CREB binding protein (CBP) signaling reverses pulmonary fibrosis. Proc Natl Acad Sci U S A. 2010;107(32):14309–14.
108. Wang C, Zhu H, Sun Z, Xiang Z, Ge Y, Ni C, et al. Inhibition of Wnt/beta-catenin signaling promotes epithelial differentiation of mesenchymal stem cells and repairs bleomycin-induced lung injury. Am J Physiol Cell Physiol. 2014;307(3):C234–44.
109. Schmalhofer O, Spaderna S, Brabletz T. Native promoter reporters validate transcriptional targets. Methods Mol Biol. 2008;468:111–28.
110. Bridges RS, Kass D, Loh K, Glackin C, Borczuk AC, Greenberg S. Gene expression profiling of pulmonary fibrosis identifies Twist1 as an antiapoptotic molecular "rectifier" of growth factor signaling. Am J Pathol. 2009;175(6):2351–61.
111. Selman M, Pardo A. Role of epithelial cells in idiopathic pulmonary fibrosis: from innocent targets to serial killers. Proc Am Thorac Soc. 2006;3(4):364–72.
112. Selman M, Pardo A. Idiopathic pulmonary fibrosis: an epithelial/fibroblastic cross-talk disorder. Respir Res. 2002;3:3.
113. Vukmirovic M, Herazo-Maya JD, Blackmon J, Skodric-Trifunovic V, Jovanovic D, Pavlovic S, et al. Identification and validation of differentially expressed transcripts by RNA-sequencing of formalin-fixed, paraffin-embedded (FFPE) lung tissue from patients with Idiopathic Pulmonary Fibrosis. BMC Pulm Med. 2017;17(1):15.
114. Selman M, Pardo A, Barrera L, Estrada A, Watson SR, Wilson K, et al. Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis. Am J Respir Crit Care Med. 2006;173(2):188–98.

115. Selman M, Carrillo G, Estrada A, Mejia M, Becerril C, Cisneros J, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. PLoS One. 2007;2(5):e482.
116. Konigshoff M, Balsara N, Pfaff EM, Kramer M, Chrobak I, Seeger W, et al. Functional Wnt signaling is increased in idiopathic pulmonary fibrosis. PLoS One. 2008;3(5):e2142.
117. Tzouvelekis A, Harokopos V, Paparountas T, Oikonomou N, Chatziioannou A, Vilaras G, et al. Comparative expression profiling in pulmonary fibrosis suggests a role of hypoxia-inducible factor-1 alpha in disease pathogenesis. Am J Respir Crit Care Med. 2007;176(11):1108–19.
118. Yang IV, Coldren CD, Leach SM, Seibold MA, Murphy E, Lin J, et al. Expression of cilium-associated genes defines novel molecular subtypes of idiopathic pulmonary fibrosis. Thorax. 2013;68(12):1114–21.
119. MacDonald SL, Rubens MB, Hansell DM, Copley SJ, Desai SR, du Bois RM, et al. Nonspecific interstitial pneumonia and usual interstitial pneumonia: comparative appearances at and diagnostic accuracy of thin-section CT. Radiology. 2001;221(3):600–5.
120. Sverzellati N, Wells AU, Tomassetti S, Desai SR, Copley SJ, Aziz ZA, et al. Biopsy-proved idiopathic pulmonary fibrosis: spectrum of nondiagnostic thin-section CT diagnoses. Radiology. 2010;254(3):957–64.
121. Silva CI, Muller NL, Lynch DA, Curran-Everett D, Brown KK, Lee KS, et al. Chronic hypersensitivity pneumonitis: differentiation from idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia by using thin-section CT. Radiology. 2008;246(1):288–97.
122. Flaherty KR, Travis WD, Colby TV, Toews GB, Kazerooni EA, Gross BH, et al. Histopathologic variability in usual and nonspecific interstitial pneumonias. Am J Respir Crit Care Med. 2001;164(9):1722–7.
123. Monaghan H, Wells AU, Colby TV, du Bois RM, Hansell DM, Nicholson AG. Prognostic implications of histologic patterns in multiple surgical lung biopsies from patients with idiopathic interstitial pneumonias. Chest. 2004;125(2):522–6.
124. Selman M, King TE, Pardo A, American Thoracic S, European Respiratory S, American College of Chest P. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med. 2001;134(2):136–51.
125. Kusko RL, Brothers JF 2nd, Tedrow J, Pandit K, Huleihel L, Perdomo C, et al. Integrated genomics reveals convergent transcriptomic networks underlying chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2016;194(8):948–60.
126. Mizuno S, Bogaard HJ, Kraskauskas D, Alhussaini A, Gomez-Arroyo J, Voelkel NF, et al. p53 gene deficiency promotes hypoxia-induced pulmonary hypertension and vascular remodeling in mice. Am J Physiol Lung Cell Mol Physiol. 2011;300(5):L753–61.
127. Yang IV, Burch LH, Steele MP, Savov JD, Hollingsworth JW, McElvania-Tekippe E, et al. Gene expression profiling of familial and sporadic interstitial pneumonia. Am J Respir Crit Care Med. 2007;175(1):45–54.
128. Rosas IO, Kaminski N. When it comes to genes – IPF or NSIP, familial or sporadic – they're all the same. Am J Respir Crit Care Med. 2007;175(1):5–6.
129. Lamblin C, Bergoin C, Saelens T, Wallaert B. Interstitial lung diseases in collagen vascular diseases. Eur Respir J Suppl. 2001;32:69s–80s.
130. Hsu E, Shi H, Jordan RM, Lyons-Weiler J, Pilewski JM, Feghali-Bostwick CA. Lung tissues in patients with systemic sclerosis have gene expression patterns unique to pulmonary fibrosis and pulmonary hypertension. Arthritis Rheum. 2011;63(3):783–94.
131. Kim SY, Diggans J, Pankratz D, Huang J, Pagan M, Sindy N, et al. Classification of usual interstitial pneumonia in patients with interstitial lung disease: assessment of a machine learning approach using high-dimensional transcriptional data. Lancet Respir Med. 2015;3(6):473–82.
132. Martinez FJ, Safrin S, Weycker D, Starko KM, Bradford WZ, King TE Jr, et al. The clinical course of patients with idiopathic pulmonary fibrosis. Ann Intern Med. 2005;142(12 Pt 1):963–7.
133. Oppenheim JJ, Biragyn A, Kwak LW, Yang D. Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. Ann Rheum Dis. 2003;62(Suppl 2):ii17–21.

134. Lillard JW Jr, Boyaka PN, Chertov O, Oppenheim JJ, McGhee JR. Mechanisms for induction of acquired host immunity by neutrophil peptide defensins. Proc Natl Acad Sci U S A. 1999;96(2):651–6.
135. Feghali-Bostwick CA, Tsai CG, Valentine VG, Kantrow S, Stoner MW, Pilewski JM, et al. Cellular and humoral autoreactivity in idiopathic pulmonary fibrosis. J Immunol. 2007;179(4):2592–9.
136. Yang IV, Luna LG, Cotter J, Talbert J, Leach SM, Kidd R, et al. The peripheral blood transcriptome identifies the presence and extent of disease in idiopathic pulmonary fibrosis. PLoS One. 2012;7(6):e37708.
137. Boon K, Bailey NW, Yang J, Steel MP, Groshong S, Kervitsky D, et al. Molecular phenotypes distinguish patients with relatively stable from progressive idiopathic pulmonary fibrosis (IPF). PLoS One. 2009;4(4):e5134.
138. 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.
139. Kahloon RA, Xue J, Bhargava A, Csizmadia E, Otterbein L, Kass DJ, et al. Idiopathic pulmonary fibrosis patients with antibodies to heat shock protein 70 have poor prognoses. Am J Respir Crit Care Med. 2013;187(7):768–75.
140. Herazo-Maya JD, Noth I, Duncan SR, Kim S, Ma SF, Tseng GC, et al. Peripheral blood mononuclear cell gene expression profiles predict poor outcome in idiopathic pulmonary fibrosis. Sci Transl Med. 2013;5(205):205ra136.
141. 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;5:857–68.
142. Huang Y, Ma SF, Vij R, Oldham JM, Herazo-Maya J, Broderick SM, et al. A functional genomic model for predicting prognosis in idiopathic pulmonary fibrosis. BMC Pulm Med. 2015;15:147.
143. 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. 2011;184:459–66.
144. Cosgrove GP, Brown KK, Schiemann WP, Serls AE, Parr JE, Geraci MW, et al. Pigment epithelium-derived factor in idiopathic pulmonary fibrosis: a role in aberrant angiogenesis. Am J Respir Crit Care Med. 2004;170(3):242–51.
145. Studer SM, Kaminski N. Towards systems biology of human pulmonary fibrosis. Proc Am Thorac Soc. 2007;4(1):85–91.
146. Wang XM, Zhang Y, Kim HP, Zhou Z, Feghali-Bostwick CA, Liu F, et al. Caveolin-1: a critical regulator of lung fibrosis in idiopathic pulmonary fibrosis. J Exp Med. 2006;203(13):2895–906.
147. Englert JM, Hanford LE, Kaminski N, Tobolewski JM, Tan RJ, Fattman CL, et al. A role for the receptor for advanced glycation end products in idiopathic pulmonary fibrosis. Am J Pathol. 2008;172(3):583–91.
148. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, Steele MP, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. Nat Genet. 2013;45(6):613–20; advance online publication.
149. Lee JU, Cheong HS, Shim EY, Bae DJ, Chang HS, Uh ST, et al. Gene profile of fibroblasts identify relation of CCL8 with idiopathic pulmonary fibrosis. Respir Res. 2017;18(1):3.
150. Consortium EP, Dunham I, Kundaje A, Aldred SF, Collins PJ, Davis CA, et al. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012; 489(7414):57–74.
151. Bazzini AA, Lee MT, Giraldez AJ. Ribosome profiling shows that miR-430 reduces translation before causing mRNA decay in zebrafish. Science. 2012;336(6078):233–7.
152. Pandit KV, Corcoran D, Yousef H, Yarlagadda M, Tzouvelekis A, Gibson KF, et al. Inhibition and role of let-7d in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2010;182(2):220–9.

153. Milosevic J, Pandit K, Magister M, Rabinovich E, Ellwanger DC, Yu G, et al. Profibrotic role of miR-154 in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2012;47(6):879–87.
154. Montgomery RL, Yu G, Latimer PA, Stack C, Robinson K, Dalby CM, et al. MicroRNA mimicry blocks pulmonary fibrosis. EMBO Mol Med. 2014;6(10):1347–56.
155. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. J Exp Med. 2010;207(8):1589–97.
156. Yang S, Banerjee S, de Freitas A, Sanders YY, Ding Q, Matalon S, et al. Participation of miR-200 in pulmonary fibrosis. Am J Pathol. 2012;180(2):484–93.
157. Yang S, Xie N, Cui H, Banerjee S, Abraham E, Thannickal VJ, et al. miR-31 is a negative regulator of fibrogenesis and pulmonary fibrosis. FASEB J. 2012;26(9):3790–9.
158. Dakhlallah D, Batte K, Wang Y, Cantemir-Stone CZ, Yan P, Nuovo G, et al. Epigenetic regulation of miR-17~92 contributes to the pathogenesis of pulmonary fibrosis. Am J Respir Crit Care Med. 2013;187(4):397–405.
159. 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.
160. Pandit KV, Milosevic J, Kaminski N. MicroRNAs in idiopathic pulmonary fibrosis. Transl Res. 2011;157(4):191–9.
161. Dai Y, Huang YS, Tang M, Lv TY, Hu CX, Tan YH, et al. Microarray analysis of microRNA expression in peripheral blood cells of systemic lupus erythematosus patients. Lupus. 2007;16(12):939–46.
162. Hausler SF, Keller A, Chandran PA, Ziegler K, Zipp K, Heuer S, et al. Whole blood-derived miRNA profiles as potential new tools for ovarian cancer screening. Br J Cancer. 2010;103(5):693–700.
163. Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev. 2011;25(18):1915–27.
164. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem. 2012;81:145–66.
165. Huang C, Yang Y, Liu L. Interaction of long noncoding RNAs and microRNAs in the pathogenesis of idiopathic pulmonary fibrosis. Physiol Genomics. 2015;47(10):463–9.
166. Berschneider B, Ellwanger DC, Baarsma HA, Thiel C, Shimbori C, White ES, et al. miR-92a regulates TGF-beta1-induced WISP1 expression in pulmonary fibrosis. Int J Biochem Cell Biol. 2014;53:432–41.
167. Bodempudi V, Hergert P, Smith K, Xia H, Herrera J, Peterson M, et al. miR-210 promotes IPF fibroblast proliferation in response to hypoxia. Am J Physiol Lung Cell Mol Physiol. 2014;307(4):L283–94.
168. Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F, et al. miR-29 is a major regulator of genes associated with pulmonary fibrosis. Am J Respir Cell Mol Biol. 2011;45(2):287–94.
169. Das S, Kumar M, Negi V, Pattnaik B, Prakash YS, Agrawal A, et al. MicroRNA-326 regulates profibrotic functions of transforming growth factor-beta in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2014;50(5):882–92.
170. Liang H, Gu Y, Li T, Zhang Y, Huangfu L, Hu M, et al. Integrated analyses identify the involvement of microRNA-26a in epithelial-mesenchymal transition during idiopathic pulmonary fibrosis. Cell Death Dis. 2014;5:e1238.
171. Xie T, Liang J, Geng Y, Liu N, Kurkciyan A, Kulur V, et al. MicroRNA-29c prevents pulmonary fibrosis by regulating epithelial cell renewal and apoptosis. Am J Respir Cell Mol Biol. 2017;57:721–32.
172. Lino Cardenas CL, Henaoui IS, Courcot E, Roderburg C, Cauffiez C, Aubert S, et al. miR-199a-5p is upregulated during fibrogenic response to tissue injury and mediates TGFbeta-induced lung fibroblast activation by targeting caveolin-1. PLoS Genet. 2013;9(2):e1003291.
173. Yang S, Cui H, Xie N, Icyuz M, Banerjee S, Antony VB, et al. miR-145 regulates myofibroblast differentiation and lung fibrosis. FASEB J. 2013;27(6):2382–91.
174. Ge L, Habiel DM, Hansbro PM, Kim RY, Gharib SA, Edelman JD, et al. miR-323a-3p regulates lung fibrosis by targeting multiple profibrotic pathways. JCI Insight. 2016;1(20):e90301.

175. Yang IV. Epigenomics of idiopathic pulmonary fibrosis. Epigenomics. 2012;4(2):195–203.
176. Sanders YY, Pardo A, Selman M, Nuovo GJ, Tollefsbol TO, Siegal GP, et al. Thy-1 promoter hypermethylation: a novel epigenetic pathogenic mechanism in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2008;39(5):610–8.
177. Coward WR, Watts K, Feghali-Bostwick CA, Jenkins G, Pang L. Repression of IP-10 by interactions between histone deacetylation and hypermethylation in idiopathic pulmonary fibrosis. Mol Cell Biol. 2010;30(12):2874–86.
178. Cisneros J, Hagood J, Checa M, Ortiz-Quintero B, Negreros M, Herrera I, et al. Hypermethylation-mediated silencing of p14ARF in fibroblasts from idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2012;303(4):L295–303.
179. Sanders YY, Tollefsbol TO, Varisco BM, Hagood JS. Epigenetic regulation of thy-1 by histone deacetylase inhibitor in rat lung fibroblasts. Am J Respir Cell Mol Biol. 2011;45(1):16–23.
180. Rabinovich EI, Kapetanaki MG, Steinfeld I, Gibson KF, Pandit KV, Yu G, et al. Global methylation patterns in idiopathic pulmonary fibrosis. PLoS One. 2012;7(4):e33770.
181. Sanders YY, Ambalavanan N, Halloran B, Zhang X, Liu H, Crossman DK, et al. Altered DNA methylation profile in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186:525–35.
182. Yang IV, Pedersen BS, Rabinovich E, Hennessy CE, Davidson EJ, Murphy E, et al. Relationship of DNA methylation and gene expression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2014;190(11):1263–72.
183. Charpentier MS, Christine KS, Amin NM, Dorr KM, Kushner EJ, Bautch VL, et al. CASZ1 promotes vascular assembly and morphogenesis through the direct regulation of an EGFL7/RhoA-mediated pathway. Dev Cell. 2013;25(2):132–43.
184. Herazo-Maya JD, Kaminski N. Personalized medicine: applying 'omics' to lung fibrosis. Biomark Med. 2012;6(4):529–40.
185. Kim S, Herazo-Maya JD, Kang DD, Juan-Guardela BM, Tedrow J, Martinez FJ, et al. Integrative phenotyping framework (iPF): integrative clustering of multiple omics data identifies novel lung disease subphenotypes. BMC Genomics. 2015;16:924.
186. Noth I, Zhang Y, Ma S-F, Flores C, Barber M, Huang Y, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. Lancet. 2013;1:309–17.
187. Ardini-Poleske ME, Clark RF, Ansong C, Carson JP, Corley RA, Deutsch GH, et al. LungMAP: the molecular atlas of lung development program. Am J Physiol Lung Cell Mol Physiol. 2017;313(5):L733–40.

Chapter 10
Biomarkers in Idiopathic Pulmonary Fibrosis

Shweta Sood, Tonya D. Russell, and Adrian Shifren

Introduction: The Current State of IPF

Diagnosing idiopathic pulmonary fibrosis (IPF) is challenging. The exclusion of other causes of interstitial lung disease (ILD) together with high-resolution computed tomography (HRCT) imaging showing a usual interstitial pneumonia (UIP) pattern of subpleural and basal predominant reticulations, honeycombing (with or without traction bronchiectasis), and absence of inconsistent features together with biopsy specimens (in select cases) demonstrating temporally and spatially heterogeneous fibrosis in the presence of fibroblastic foci form the current practice paradigm for diagnosing IPF [1]. Current guidelines also support the use of clinical, radiological, and physiologic evaluations to estimate IPF disease severity and predict disease progression [2]. 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.

However, patient questionnaires, pulmonary function testing, HRCT imaging, and surgical lung biopsies all have limitations [3]. Symptoms or quality of life surveys are often nonspecific. On clinical questionnaires, patients with both stable IPF and rapidly progressive IPF often report similar levels of impairment. Both pulmonary function testing (PFT) and HRCTs are time-consuming and costly. Frequent imaging exposes patients to radiation. Surgical lung biopsy may exacerbate IPF and is not a diagnostic option for patients with advanced disease at the time of initial presentation. In some patients, IPF cannot be definitively diagnosed even when both HRCT and surgical lung biopsy are obtained. Yet, with the recent Food and Drug Administration (FDA) approval of pirfenidone and nintedanib for IPF, distinguishing

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IPF from other ILDs has become increasingly vital, as it significantly modifies patient management. Equally important, current testing cannot accurately predict mortality with a high degree of certainty in most IPF patients. Thus, patients are often told that the average lifespan is 2.5–3.5 years after initial diagnosis. However, clinicians recognize that many patients barely survive 1 year after diagnosis, while others will live with their disease for several years following the diagnosis [4].

Overview of Existing Physiologic IPF Biomarkers

The term "biomarker" is a portmantologism of the words "biological" and "marker." Biomarkers are quantifiable indicators of biological processes, disease states, or disease responses to an intervention that can be objectively measured both accurately and reproducibly [5, 6]. Unlike symptoms, biomarkers are independent of a patient's subjective appreciation of well-being and are ideally free from recall bias. As such, biomarkers provide the potential to impartially identify high-risk individuals with predisposition to (or risk factors for) a disease, diagnose disease, predict and measure responses to disease-specific therapies, and gauge disease prognosis [7].

Although pharmacologic therapies are now available for patients with IPF, the overall prognosis continues to be poor. Furthermore, the clinical course of patients with IPF can be highly variable. Thus far, physiologic parameters are the most well-studied potential biomarkers, but the data regarding the utility of these biomarkers are conflicting, and no definitive biomarker yet exists. In clinical practice, demographic data such as age and gender and physiologic parameters such as percent-predicted FVC (%FVC), change in %FVC, percent-predicted diffusing capacity for carbon monoxide (%DLCO), 6-min walk distance, oxygen saturations, and oxygen requirements have been the easiest to obtain noninvasively and follow serially. In most cases, these biomarkers have been directed at helping determine prognosis in individual patients.

Du Bois et al. retrospectively reviewed data from IPF patients enrolled in the interferon-γ 1b clinical trials to ascertain the impact of physiologic parameters on mortality. They demonstrated that changes in %FVC over a 24-week period were highly predictive of mortality during the subsequent 1-year period. Decreases in FVC as small as 5–10% at 24 weeks were associated with more than twofold higher mortality risk in IPF patients [8].

The ability of the distance-saturation product (DSP) to establish 12-month survival in IPF patients was evaluated in a retrospective review by Lettieri et al. The DSP was calculated by multiplying the room air walk distance (in meters) by the oxygen saturation nadir. Walks were not performed if the patient could not maintain a resting room air saturation ≥88%, and the walks were terminated if the saturation fell to <80%. A DSP of 200 m% was determined to be the best threshold for separating 12-month survivors from non-survivors. Patients with a DSP of <200 m% were 6.5 times more likely to die within 12 months. The sensitivity, specificity, positive predictive value, and negative predictive value were 78.8%, 91.7%, 86.7%, and 86.3%, respectively [9].

The composite physiologic index (CPI) was formulated to closely reflect the extent of pulmonary fibrosis observed on CT scan. It is derived by fitting measures of pulmonary function against disease extent on CT and calculated using the formula: CPI = 91 – (0.65 × % DLCO) – (0.53 × % FVC) – (0.34 × % FEV_1). The CPI score had a greater correlation with mortality than individual PFT parameters in patients with IPF, including a subgroup of IPF patients with concomitant emphysema. In patients without emphysema, it appeared equivalent to the %DLCO [10].

The gender, age, and physiology (GAP) index is a multidimensional risk prediction model and staging system for IPF developed by Ley et al. [11]. The model assigns points to four baseline variables: gender, age, %FVC, and %DLCO. Higher points are given for male gender, older age, lower %FVC, and lower %DLCO. Three stages are defined: stage I (0–3 points), stage II (4–5 points), and stage III (6–8 points). The 3-year mortality for stage I disease is 16.3% compared to 76.8% for stage III. The GAP index is, therefore, potentially helpful in assessing the risk-benefit for lung transplantation in patients with IPF [11].

Kishaba and colleagues have proposed a novel scoring system for predicting mortality in IPF patients using BMI, %FVC, and respiratory hospitalizations [12]. Points are assigned based on the degree of change in the %FVC and BMI within the year following the diagnosis of IPF as well as the occurrence of respiratory-related hospitalizations within that year. Stage III (≥6 points) patients demonstrated a mean survival of 14.8 months compared to 43.9 months for stage II (3–5 points) and 77.9 months for stage I (0–2 points) patients [12].

Sharp and colleagues applied the CPI, DSP, GAP, and Du Bois score to 167 patients in their IPF clinic. Among independent variables, only the baseline %DLCO showed a significant association with mortality in a multivariate analysis. The CPI was the only multidimensional index that performed as well as baseline %DLCO in their hands [13].

Ideal Biomarkers

The ideal biomarker is one that is easily obtainable, sensitive, specific, predictive, and robust. Sample materials should be collected with a minimum of discomfort or risk to the patient. Examples include venous blood, urine, sputum, or cheek swabs for genetic material. Testing should be inexpensive and highly reproducible. Ideal biomarkers demonstrate a high sensitivity (≥0.9), ideally with a zero baseline during health. They should be detectable early in disease, preferably in the disease's preclinical stages. Ideal biomarkers also need to exhibit a high degree of specificity for the disease in question (e.g., ≥0.9) to minimize the potential for false-positive results. Test results should be rapidly available to facilitate early and prompt initiation of treatment. Finally, to allow for both prognostication and for monitoring of therapeutic effectiveness, the ideal biomarker should be associated with a known disease mechanism, correlate with the severity of target organ damage, and demonstrate the repeated ability to accurately predict clinically relevant outcomes across different patient phenotypes in particular disease states [5].

Novel Biomarkers in IPF

Making the diagnosis of IPF and providing accurate prognostic information are difficult using the currently available tools, namely, clinical, radiological, and physiologic data. Thus, researchers have begun to focus on identifying novel IPF biomarkers. These include biomarkers for diagnosing IPF in a manner that allows for identification of IPF patients and differentiation from normal controls or patients with other lung diseases such as hypersensitivity pneumonitis (HP), chronic obstructive pulmonary disease (COPD), or sarcoidosis. Others are evaluating biomarkers for monitoring disease progression. Fluctuations in these biomarkers would, over time, herald worsening of IPF or the onset of an acute exacerbation. Other biomarkers may allow a more accurate prediction of mortality. In patients with known IPF, elevations of these biomarkers at the time of diagnosis would suggest a better or worse prognosis. Lastly, biomarkers that predict or assess responses to drug therapy are also being sought. Ultimately, the ideal IPF biomarker would encapsulate all these constraints in a single, easily available test. In this chapter, we review existing clinical biomarkers for IPF and highlight a select collection of clinical studies facilitating the identification of potential IPF biomarkers. While several basic science studies have used in vitro and murine models to isolate potential biomarkers, these are beyond the scope of this chapter. We instead focus on studies evaluating potential biomarkers in human blood, sputum, bronchoalveolar lavage fluid (BALF), and urine from patients with IPF.

IPF Pathophysiology and Understanding the Challenges in Discovery of a New IPF Biomarker

The ideal biomarker for IPF has been difficult to identify because the etiology and pathogenesis of IPF remains so elusive. A fundamental understanding of basic IPF pathogenesis aids in evaluating emerging biomarkers. At the most basic level, IPF is characterized by an unknown injury (aging, viral infection, and inhalation injury are all possible causes) to the alveolar epithelial cells (AECs) [4]. This injury may be a single insult but is more likely a series of recurrent injuries over time [14]. The epithelial injury causes AECs to become abnormally activated [15]. This results in a proliferation of type II AECs in an attempt to repair the epithelial damage. However, for unclear reasons, epithelial damage persists.

The exact role of type II AECs in IPF pathogenesis is unclear. Some hypothesize that as type II AECs fail to enact epithelial repair, they release mediators that recruit circulating fibroblasts to help control the epithelial injury [14, 16]. Others suspect that type II AECs undergo epithelial-mesenchymal transition by losing their polarity and cell-cell adhesion, gaining migratory and invasive properties to become mesenchymal stem cells [14, 16]. Lastly, type II AECs become dysfunctional and lose control over local fibroblasts which then undergo uncontrolled proliferation [16].

Other pathologic features of IPF lungs include (a) aberrant T-cell, B-cell, and plasma cell populations that may contribute to excess extracellular matrix (ECM) formation [17, 18], (b) dysregulation of the coagulation cascade, (c) altered angiogenesis with a paucity of capillaries within fibroblastic foci and an excess of blood vessels in the surrounding lung tissue [15, 19], and (d) inflammation confined to regions within fibroblastic foci that may recruit additional inflammatory cells into the lungs [14]. The exact interplay of various cells and inflammatory cytokines in IPF remains nebulous. Regardless of the exact mechanisms, the end result is dysregulated wound repair with excess ECM deposition and pulmonary parenchymal remodeling [4, 20].

Any of the inflammatory cells or mediators involved in epithelial dysfunction or excess ECM deposition can potentially serve as a biomarker for IPF. Several studies have suggested that some of these cells or mediators have the potential of being validated biomarkers.

Current State of Novel IPF Biomarkers

The field of biomarkers in IPF is a novel field. Often it is challenging to appreciate the phenomenal research successes in this field over the last several decades. To the veteran ILD physician, the advances in IPF biomarker research are easily identifiable. However, the young ILD physician might only see an "alphabet soup" of various unconfirmed biomarkers that further complicate their understanding of ILD pathophysiology. For this reason, we have included Table 10.1 which summarizes some of the findings in notable papers on IPF biomarkers. This list is not comprehensive, and there are many more studies that contributed significantly in finding IPF biomarkers. Furthermore, only a limited number of findings are highlighted from each study. However, we hope that this table allows the reader to appreciate the decades of research these investigators and others have performed in order to advance discoveries in the field of IPF biomarkers.

This chapter does not cover genetic mutations predisposing to IPF, including surfactant protein A, surfactant protein C, telomerase RNA component, telomerase reverse transcriptase, and MUC5B. These mutations play a role in disease pathogenesis in approximately 20% of pulmonary fibrosis cases and are covered in Chap. 8.

Diagnostic Biomarkers

Surfactant Proteins

In IPF, type II AEC numbers are increased and contribute to pathologic remodeling. Some researchers suspect that as type II AEC numbers increase, more surfactant is produced. This excess surfactant, along with other type II AEC products, can be

Table 10.1 Timeline of selected IPF biomarker studies

Year	Authors	Biomarker	Suspected normal Physiological role	Selected results	Diagnostic marker candidate	Prognostic marker candidate	References
1995	McCormack et al.	SP-A	SP-A (surfactant protein A)	BAL SP-A/PL (SP-A to total phospholipid ratio) lower in IPF patients than healthy controls	Yes	Yes	[21]
			All surfactant proteins (A, B, C, D) made by type II AECs	This lower ratio predicted 5-year survival			
			Elevated surfactant proteins may indicate alveolar injury				
1997	Ziegenhagen et al.	IL-8	Chemoattractant	IL-8 significantly elevated in serum and BALF in IPF patients vs. controls	Yes		[22]
			Recruits neutrophils	Elevated IL-8 levels were associated with BALF neutrophilia			
				Elevated IL-8 inversely correlated with TLC, DLCO, and PaO2 values			
1998	Yokoyama et al.	KL-6/MUC1	KL-6 is a glycoprotein expressed in type II AECs	Levels of KL-6/MUC-1 higher in IPF non-survivors vs. IPF survivors		Yes	[23]
			Increased KL-6/MUC1 expression in ILD				
2000	Takahashi et al.	SP-A and SP-D	As above	SP-A and SP-D levels higher in IPF patients who died within 3 years versus IPF survivors		Yes	[24]
2002	Greene et al.	SP-A and SP-D	As above	Serum SP-A and SP-D elevated in both IPF and systemic sclerosis patients	Yes	Yes	[25]
				Serum levels of SP-A and SP-D predicted survival in IPF patients			
				SP-D associated with increased parenchymal disease in IPF patients			

2002	Ishii et al.	KL-6, SP-A, SP-D	As above	Serum SP-A, SP-D, and KL-6 significantly higher in ILD patients (UIP, NSIP, BOOP, and sarcoid) than in healthy volunteers	Yes		[26]
				Serum SP-A significantly higher in UIP patients than NSIP patients			
2002	Ohnishi et al.	KL-6, SP-A, SP-D, MCP-1	As above	Serum KL-6 elevated in ILD (IPF and CT-ILD) patients compared to patients with pneumonia and controls	Yes		[27]
			MCP-1 is monocyte chemoattractant protein-1	SP-A, SP-D, and MCP-1 elevated in both ILD and pneumonia patients but patients with ILD tended to have higher levels than pneumonia group			
2005	Tsoutsou et al.	IL-2, IL-8, IL-10, IL-12 (P40)	IL-8 recruits neutrophils	Serum IL-2, IL-8, IL-10, and IL-12 (p40) elevated in IPF patients	Yes		[28]
			IL-2, IL-10, and IL-12 play role in Th2 immunity				
2006	Satoh et al.	KL-6	As above	IPF and CTD-ILD patients with higher KL-6 levels had increased risk for subsequent mortality		Yes	[29]
2008	Rosas et al.	MMP1, MMP7	Matrix metalloproteinases (MMP) are enzymes that help degrade ECM.	Serum MMP1 and MMP7 higher in IPF [vs. controls, COPD, sarcoid, and chronic HP patients]	Yes		[30]
				Higher MMP7 levels negatively correlated with %FVC and %DLCO			
2009	Gilani et al.	T cells subgroup with CD4+/CD28+ expression	CD28 is a costimulatory marker for T cells that promotes their growth	IPF patients had increased levels of abnormal CD4+/CD28null T cells [lose CD28 expression]	Yes	Yes	[31]
			Absence of CD28 costimulatory factor may contribute to inflammation	1-year survival decreased in patients with higher CD4+/CD28null cells			

(continued)

Table 10.1 (continued)

Year	Authors	Biomarker	Suspected normal Physiological role	Selected results	Diagnostic marker candidate	Prognostic marker candidate	References
2009	Kinder et al.	SP-A, SP-D	As above	Increased serum SP-A level predicted early mortality in IPF patients		Yes	[32]
2009	Kotsianidis et al.	Regulatory T cells (Tregs)	Tregs [CD4+CD25+FOXP3+] cells, play a role in immune tolerance (preventing autoimmunity)	BAL and serum T-regulatory cells reduced in patients with IPF compared to CTD-ILD and controls	Yes		[33]
				These reductions correlated with %FVC and %TLC			
2009	Moeller et al.	Fibrocytes	Circulating fibrocytes [CD45+ and collagen1+] may differentiate into myofibroblasts, produce cytokines and growth factors, deposit excess ECM, and promote angiogenesis	Serum circulating fibrocytes significantly elevated in IPF patients versus controls	Yes	Yes	[34]
				Fibrocyte levels rose in IPF patients during acute exacerbations			
				Elevated fibrocytes predicted short-term mortality but did not correlate with PFTs			
2009	Prasse et al.	CCL18	CCL18, or CC-chemokine 18, effects adaptive immunity and may upregulate collagen production by fibroblasts	Higher mortality in IPF patients with elevated serum CCL18		Yes	[35]
2010	Ando et al.	Serum VEGF	Promotes angiogenesis	Serum VEGF increased in IPF patients with high A/a gradients compared to healthy volunteers or IPF patients with low A/a gradients	Yes		[36]
				Serum VEGF levels also elevated in lung cancer patients			
				Serum VEGF inversely correlated with FVC			

2010	Collard et al.	KL-6, SP-D, vWF, Protein C, thrombomodulin, PAI-1, IL-6	As above	All listed biomarkers significantly elevated in patients with acute IPF exacerbation versus stable IPF patients		Yes	[37]
			von Willebrand factor (vWF) is possible endothelial cell injury marker				
			Thrombomodulin, protein C, and plasminogen activator inhibitor 1 (PAI-1) levels may represent dysregulated coagulation cascade				
			IL-6 may have proinflammatory role				
2010	Furuhashi et al.	YKL-40	YKL-40, also called human cartilage glycoprotein 39 (HCgp-39) and chitinase 3-like 1, is a glycoprotein	Serum and BALF levels of YKL-40 levels significantly higher in IPF patients than in control subjects	Yes		[38]
			Its function is unknown, but it may be a growth factor for mesenchymal cells and play a role in angiogenesis	Higher YKL-40 levels correlated positively with serum KL-6 concentrations and elevated A/a gradients			
				Higher YKL-40 levels negatively correlated with DLCO and PaO2			
2011	Korthagen et al.	YKL-40	As above	Serum and BALF YKL-40 levels were significantly higher in IPF patients versus healthy controls	Yes	Yes	[39]
				IPF patients with high serum or BALF YKL-40 levels had significantly shorter survival than those with low YKL-40 levels in serum or BALF			

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Table 10.1 (continued)

Year	Authors	Biomarker	Suspected normal Physiological role	Selected results	Diagnostic marker candidate	Prognostic marker candidate	References
2011	Okamoto et al.	Periostin	Released from monocytes and fibrocytes	Serum levels of periostin were significantly high in IPF patients, moderately elevated in fNSIP patients, slightly elevated in COP patients compared to control subjects	Yes		[40]
			Promotes cell proliferation and migration, increases epithelial-mesenchymal transition	Elevated periostin levels correlated inversely with VC and DLCO			
2011	Seibold et al.	MUC5B promoter	Unclear role	MUC5B expression in the lung 14 times higher in IPF subjects (both familial and non-familial IPF) versus healthy volunteers	Yes		[41]
2011	Song et al.	MMP-7 and SP-A	As above	Patients with elevated MMP-7 and SP-A had shorter 1-year survival and more rapid reduction in %FVC		Yes	[42]
2012	Cha et al.	cCK-18	Caspase-cleaved cytokeratin-18 (cCK-18) is a degradation product found in epithelial cells after apoptosis	Serum cCK-18 increased in IPF subjects vs. controls but not associated with disease severity or outcome	Yes		[43]
2012	Fahim et al.	CEA	Carcinoembryonic antigen (CEA) is a glycoprotein involved in cell adhesion. Mostly produced during fetal development but elevated in several gastrointestinal malignancies and in tobacco users	Half of IPF subjects in this study had elevated serum CEA levels	Yes		[44]
				Elevated CEA levels inversely correlated %FVC and %DLCO			

2012	Naik et al.	Periostin	As above	Fibroblasts from IPF patients produced more periostin than controls	Yes	Yes	[45]
				Serum periostin levels at baseline in IPF patients correlated with clinical progression at 48 weeks			
2012	Richards et al.	MMP-7, ICAM-1, VCAM-1, IL-8, S100A12	MMP as per above	MMP-7, ICAM-1, IL-8, VCAM-1, and S100A12 associated with mortality or disease progression in IPF patients		Yes	[46]
			ICAM and VCAM (intracellular and vascular cellular adhesion molecules)				
			IL-8 and S100A12 may cause neutrophil recruitment				
2012	Samukawa et al.	Napsin A, KL-6, SP-A	Napsin A is a proteinase expressed in AEC II and alveolar macrophages	Serum napsin A, KL-6, and SP-A were elevated in IPF but not in patients with adenocarcinoma or kidney disease or healthy control	Yes		[47]
				Serum napsin A inversely correlated with %FVC			
2013	Chien et al.	Lysyl oxidase-like 2 (LOXL2)	LOXL2 is secreted by fibroblasts and promotes cross-linking of ECM molecules, including fibrillar collagens	Higher sLOXL2 level associated with increased risk for IPF disease progression		Yes	[48]
2013	Herazo-Maya et al.	Peripheral blood mononuclear cell (PBMC) gene expression	PBMCs include T cells, B cells, NK cells, and monocytes. This paper investigated gene expression in these cells	Decreased expression of genes for CD28, ICOS, LCK, ITK found in IPF patients	Yes	Yes	[49]
				These decreases in T-cell gene predicted shorter transplant-free survival			
				These decreases also correlated with reduced CD4/CD28 T-cell expression			

(continued)

Table 10.1 (continued)

Year	Authors	Biomarker	Suspected normal Physiological role	Selected results	Diagnostic marker candidate	Prognostic marker candidate	References
2013	Jaffar et al.	Fibulin-1	Fibulin-1 is a glycoprotein (produced by lung fibroblasts) in ECM and blood necessary for alveolar septa formation especially during embryogenesis	Serum fibulin-1 increased in IPF patients vs. healthy volunteers	Yes	Yes	[50]
				Serum fibulin-1 nonsignificantly increased in IPF subjects vs. patients with other ILDs (like HP)			
				Higher initial serum fibulin-1 levels predicted IPF progression 1 year later			
2013	Kahloon et al.	Heat shock protein (HSP) 70	HSP help ensure proper folding (and thus function) of proteins. HSP also activate various immune cells. In IPF, authors postulate HSP IgG autoantibodies elevated in IPF patients	Anti-HSP-70 IgG autoantibodies found in 3% of controls, 25% of IPF patients, 50% of IPF patients who died, and 70% of IPF with acute exacerbations	Yes	Yes	[17]
				IPF patients with anti-HSP70 autoantibodies had higher levels of proinflammatory cytokines (IL-4, increased IL-8 from monocytes), higher lymphocyte counts, greater FVC reductions, and reduced 1-year survival			
2013	Nathan et al.	Red cell distribution width (RDW)	RDW is a measure RBC size variability reported of routine CBCs and can be elevated in other pulmonary diseases	IPF patients with normal RDW values had a median survival of 43.1 months compared with 16.3 months for those whose RDW was greater than 15		Yes	[51]

2013	Xue et al.	B cells and BLyS	BLyS is a B-cell survival factor	In IPF patients, there were elevated serum levels of antigen differentiated circulating B cells compared to healthy volunteers	Yes	Yes	[52]
				Plasma concentrations of BLyS were higher in IPF patients versus control and COPD patients			
				BLyS levels correlated positively with pulmonary artery pressures and negatively with 1-year survival			
2014	Ohshimo et al.	KL-6/MUC1	As above	Baseline serum KL-6 levels significantly higher in patients who developed acute exacerbations than in stable IPF patients		Yes	[53]
2014	Stuart et al.	Telomeres	Telomeres protect chromosomal ends from degradation	Shorter mean telomere length (measured from peripheral blood samples) in IPF and other ILD types versus controls	Yes	Yes	[54]
				Mean telomere length shortest in patients with familial pulmonary fibrosis			
				Shorter telomere length associated with transplant-free survival and worse survival only in IPF patients			
2014	Vuga et al.	CXCL13	C-X-C motif chemokine 13 (CXCL13) promotes B-cell trafficking to inflammatory foci	CXCL13 gene expression (mRNA) higher in IPF versus controls and COPD patients	Yes		[55]
2015	De Biasi et al.	Endothelial cells (CEC)	Circulating endothelial cells (CEC)	IPF patients had low circulating fibroblasts, low circulating endothelial cells (CEC), and normal levels of endothelial progenitor cells (EPC)	Yes	Yes-therapy response	[18]
			Endothelial progenitor cells (EPC)	Nintedanib and pirfenidone further decreased CEC levels			

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Table 10.1 (continued)

Year	Authors	Biomarker	Suspected normal Physiological role	Selected results	Diagnostic marker candidate	Prognostic marker candidate	References
2015	DePianto et al.	MMP3 and CXCL13	As above	Serum MMP3 levels nonsignificantly elevated in IPF vs. controls	Yes	Yes	[56]
				CXCL13 significantly elevated in IPF vs. controls			
				Higher MMP3 negatively correlated with %FVC			
				Higher CXCL13 levels negatively correlated with %DLCO			
				Both biomarkers negatively correlated with survival over 3-year period			
2015	Jenkins et al.	Neo-epitopes	MMP degrade ECM and release by-products called neo-epitopes	Rate of change in concentration of 6 neo-epitopes (C1M, C3M, C5M, C6M, CRPM, and BGM) predictive of progression and survival	Yes	Yes	[57]
2015	Sokai et al.	MMP-10	As above	Serum MMP-7 and MMP-10 negatively correlated with %FVC and %DLCO	Yes	Yes	[58]
				MMP-10 elevations predicted survival			
2016	Bauer et al.	MMP-7	As above	Serum MMP-7 elevated in IPF patients	Yes	Yes	[59]
				MMP-7 levels also increased over time			
				Baseline levels of MMP-7 predicted disease progression			
				Baseline serum MMP-7 concentration could predict changes in FVC as early as month 4			

2016	Buendia-Roldan et al.	CC16	CC16 (club cell secretory protein 16) produced primarily by bronchiolar club cells may have been anti-inflammatory	Serum and BAL CC16 significantly higher in IPF subjects compared to non-IPF patients and healthy controls	Yes		[60]
2016	Cao et al.	Leptin	May play a role in fibrogenesis	Plasma leptin higher in patients with IPF versus controls	Yes	Yes	[61]
				Plasma leptin also negatively associated with survival			
2016	Fernandez et al.	Myeloid-derived suppressor cells (MDSC)	MDSCs have strong immunosuppressive activity, thought to contribute to tumor-immune cell evasion	Increased MDSC levels found in IPF and non-IPF ILD compared with controls	Yes		[62]
				Circulating MDSC inversely correlated with maximum vital capacity in IPF, but not in COPD or non-IPF ILD			
2016	Guiot et al.	Insulin growth factor binding proteins (IGFBP)	The role of IGFBP and IGF in pulmonary fibrosis remains unclear. Thought to contribute cell growth and metabolism and promote fibrosis in IPF	Elevated IGFBP-1 and IGFBP-2 levels in IPF patients	Yes	Yes-therapy response	[63]
				IGFBP-2 decreases in patients on anti-fibrotic therapy			
2016	Hamai et al.	MMP-7. KL-6	As above	Serum MMP-7 and KL-6 levels independent predictors in IPF patients		Yes	[64]
				Elevated levels of both KL-6 and MMP-7 resulted in poorer survival rates in IPF patients			

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Table 10.1 (continued)

Year	Authors	Biomarker	Suspected normal Physiological role	Selected results	Diagnostic marker candidate	Prognostic marker candidate	References
2016	Horikiri et al.	Prostaglandin E-major urinary metabolite (PGE-MUM)	Prostaglandins have anti-fibrotic effects	PGE-MUM levels elevated in patients with chronic fibrosing interstitial pneumonia (both IPF and fibrotic NSIP patients) versus healthy volunteers	Yes		[65]
				PGE-MUM levels correlate positively with HRCT fibrotic scores			
				PGE-MUM levels correlate negatively with %DLCO in patients with CFIP			
2016	Kono et al.	Mac-2 binding protein (M2BP)	M2BP2 is a glycoprotein that helps mediate cell adhesion and promotes fibrosis. *Wisteria floribunda* agglutinin positive-M2BP (WFA+-M2BP) assay can detect fibrosis-related glycoalteration	Serum WFA+-M2BP levels in IPF patients were significantly higher than in controls	Yes	Yes	[66]
				In IPF there was a positive correlation with serum WFA+-M2BP levels and age, KL-6 levels, BAL neutrophilia, HRCT honeycombing scores, and fibrotic foci scores on biopsies			
				Negative correlation was seen with FVC, %DLCO, and survival			
2016	White et al.	SP-D, MMP-7, osteopontin	As above	Combined plasma SP-D, MMP-7, and osteopontin levels higher in IPF versus other ILD patients (except RA-ILD)	Yes		[67]
			Osteopontin (made by macrophages) is a protein inducing migration, proliferation, and adhesion of fibroblasts				

2017	Guiot et al.	Sputum cell counts. Sputum IGFBP-2, IL-8, and MMP-7	As above	IPF patients had increased sputum neutrophils, eosinophils, macrophages, and epithelial cells compared to controls	Yes		[68]
				MMP-7, IL-8, and IGFBP-2 uniquely elevated in IPF patients compared to both controls and COPD subjects			
				KL-6 and MMP-7 both inversely correlated with %TLC			
2017	Schuliga et al.	Urokinase plasminogen activator (uPA)	Unclear role. Historically, uPA thought to be protective against fibrosis. These authors argue uPA mediates plasminogen activation by human lung fibroblasts in association with increased IL-6 production and proliferation	Serum uPA levels and activity higher in IPF patients than controls	Yes		[69]
				Serum uPA levels correlated negatively with %FVC			

detected in both BALF and serum, especially as alveolar injury leads to increased permeability in the lung [70]. Type II AECs secrete four types of surfactant proteins (SP): SP-A, SP-B, SP-C, and SP-D [24, 71]. SP-A and SP-D are hydrophilic, while SP-B and SP-C are hydrophobic molecules. Surfactant protein A and surfactant protein D (SP-D) are part of the collectin subgroup of the C-type lectin superfamily. They are also secreted by Clara cells [26].

Multiple studies of small groups of IPF patients have shown that serum levels of SP-A and SP-D are higher in patients with a UIP pattern compared to healthy controls (Table 10.1). However, both SP-A and SP-D levels are also elevated in other chronic ILDs and may therefore not be able to distinguish UIP from other interstitial pneumonias (e.g., NSIP) or sarcoidosis [25, 26, 72]. Furthermore, elevated levels of SP-A and SP-D and are also seen in lung adenocarcinoma [71]. While studies have demonstrated higher serum SP-A and SP-D levels in IPF subjects compared to patients with sarcoidosis and chronic beryllium disease [25], patients with ILD secondary to systemic sclerosis have shown similar levels of serum surfactant proteins to those seen in IPF subjects.

In some models, high serum levels of surfactant proteins appear to be associated with worse survival [24, 25]. Kinder and colleagues found that serum SP-A, but not serum SP-D, was an independent predictor of mortality. After controlling for known clinical predictors of mortality, each increase in the baseline serum SP-A level of 49 ng/mL was accompanied by a 3.3 times increased risk of death within the 1st year after presentation. While their group did not note an association between serum SP-D and mortality, addition of both serum SP-A and SP-D levels to regression models improved the 1-year prediction for risk of death compared to clinical predictors alone [32]. The utility in using these serum levels to predict mortality is again variable. Greene et al. noted that when SP-A and SP-D serum levels were used in a multivariate analysis, they did not improve mortality prediction beyond clinical variables [25].

Studies of BALF levels of SP-A and SP-D have been mixed. Ishii and colleagues demonstrated lower levels of SP-A and SP-D in UIP. However, other diffuse lung diseases also demonstrated lower levels of these surfactant proteins in BALF [26]. McCormack et al. demonstrated lower ratios of SP-A to total phospholipid levels in BALF of IPF patients compared to healthy volunteers, and SP-A to total phospholipid levels were lower in IPF patients who died within 2 years compared to those who survived [21]. It is unclear if lower surfactant protein levels in BALF are due to the solubility properties of SP-A that impair detection in BALF or if alveolar damage causes “leakage” of SP-A out of the alveolar space into the bloodstream, thus lowering airspace concentrations of SP-A while increasing serum concentrations of SP-A [24]. Complicating matters even further is the fact that Phelps and colleagues demonstrated higher levels of SP-A in BALF from IPF patients compared to healthy controls. In their study, BALF SP-A levels were even higher in HP patients than IPF patients [73]. It is clear, therefore, that further research is required before surfactant proteins can be deemed to be valid diagnostic IPF biomarkers.

Mucins

Krebs von den Lungen-6 (KL-6), also known as Mucin 1 (MUC1), is a glycoprotein expressed on the surface of type II AEC and bronchiolar epithelial cells [74]. The exact role of KL-6/MUC1 is unclear, but it may have a role in fibroblast recruitment and survival [70, 75]. It is one of the most extensively studied biomarkers in ILD.

IPF patients have higher baseline KL-6 levels than healthy controls [53, 75, 76] with serum levels of KL-6 >500 U/mL being described in 70–100% of patients with IPF. However, similar elevations in serum levels of KL-6 are found in connective tissue disease (CTD)-related ILD patients [75], and serum KL-6 levels are unable to distinguish UIP patients from patients with a pathologic pattern of NSIP [26]. Furthermore, KL-6 levels may be elevated in chronic HP, sarcoidosis, acute respiratory distress syndrome (ARDS), pulmonary alveolar proteinosis, tuberculosis, and cancer [26, 75]. Thus, it appears that elevations in serum KL-6 are anything but specific for IPF.

Patients who experience an exacerbation of IPF demonstrate even higher levels of serum KL-6 when compared to stable IPF patients [53]. Elevated KL-6 levels at initial presentation of an acute exacerbation were associated with increased mortality in a small study of 14 IPF patients, 8 of whom survived. However, this paper was published several years before the most recent American Thoracic Society (ATS) consensus statement on IPF diagnosis. Therefore, it is possible that some of the 14 patients classified as IPF may indeed have had other types of ILD [23].

A high serum level of KL-6 has been demonstrated to be an independent predictor of mortality [64]. However, in a larger study of 118 patients, baseline KL-6 levels alone did not improve prediction of mortality beyond clinical parameters [42]. Furthermore, correlation between baseline KL-6 levels and pulmonary function tests is variable with some data showing that KL-6 levels are inversely correlated with pulmonary function testing parameters, while other data demonstrate no correlation [40, 53]. Serial changes in KL-6 levels might impart more accurate prognostic information. In this regard, serial increases in KL-6 levels >51.8 U/mL/year have been shown to portend a worse prognosis. In addition, patients with baseline levels >1000 U/mL who exhibited significant increases in serial measurements over time experienced both greater declines in FVC and a worse prognosis [77, 78].

While the ATS does not endorse the use of biomarker testing for diagnostic or prognostic purposes in IPF, KL-6 has been approved in Japan as a diagnostic biomarker for ILD since 1999, and KL-6 levels are obtained over 2 million times annually [75]. Based on conflicting data and lack of specificity, more research is needed to validate KL-6 as a useful IPF biomarker.

Matrix Metalloproteinases (MMPs) and Neo-epitopes

Accumulation of excess ECM is a cardinal feature of IPF. Matrix metalloproteinases (MMP) are a family of calcium-dependent, zinc-containing endopeptidases responsible for regulation of extracellular matrix remodeling [58, 79]. However, this

is an oversimplification of MMP physiology, since MMPs also process bioactive molecules, cleave cell surface receptors, release apoptotic ligands, and result in chemokine and cytokine inactivation. Therefore, MMPs are thought to be involved in cell migration, differentiation, apoptosis, and host defense [80]. Furthermore, as MMPs degrade matrix, they generate de novo sites of fragmented matrix proteins referred to as neo-epitopes [81]. Both MMPs and their neo-epitopes can be quantitatively measured in biologic fluids, and their role as biomarkers in IPF has been researched extensively.

In a study of 20 patients diagnosed with IPF, BALF levels of MMP-2, 3, 7, 8, and 9 were elevated in IPF patients compared to controls. Furthermore, MMP-8 and 9 levels in particular were significantly elevated in IPF patients exhibiting a rapid decline in lung function (>10% decline in FVC or DLCO at 1 year) compared to those IPF patients with relatively stable lung function. Although BALF levels of MMP-3, 8, and 9 were higher in the patients who died during the 3 years of follow-up compared to survivors, the levels of these matrix metalloproteinases did not predict time to death [82].

A study of 74 IPF patients by Rosas et al. demonstrated peripheral blood levels of MMPs could be used to diagnose IPF. In this study, plasma levels of MMP-1, 3, 7, 8, and 9 were overexpressed in IPF patients compared to healthy controls. High serum concentrations of MMP-7 correctly classified 93.2% of all IPF patients, but 9.4% of controls were incorrectly identified as having disease. In combination however, elevated serum levels of MMP-7 and MMP-1 correctly excluded all controls from an IPF diagnosis and differentiated IPF from controls with a sensitivity of 89.2% and specificity of 95.0%. The elevated serum levels of MMP-7 and MMP-1 also correlated with elevated BALF levels of both matrix metalloproteinases [30]. The authors also demonstrated that MMP-7 and MMP-1 concentrations were significantly higher in the serum of IPF patients compared to patients with HP, and the combination of high plasma MMP7 and MMP1 concentrations distinguished IPF from HP with a sensitivity of 96.3% and a specificity of 87.2%. While this study demonstrated good evidence for a peripheral blood protein signature in IPF patients that could distinguish IPF patients from normal controls and those with HP, it did not, however, include patients with other more common interstitial pneumonias such as NSIP and organizing pneumonia (OP).

Other studies have attempted to evaluate outcomes in IPF using MMP levels but have shown the need for multiple biomarkers to improve mortality prediction. Peripheral blood levels of MMP-7 alone have been shown to be an independent predictor of mortality in IPF [42, 64]. In a study of 118 South Korean IPF patients, an MMP-7 level >12.1 ng/mL was associated with a risk of death during follow-up more than twice that of patients with lower plasma levels. However, high levels of MMP-7 and SP-A in combination predicted shorter survival and greater lung function decline compared with those with high levels of one biomarker. Furthermore, high baseline levels of both MMP-7 and SP-A were associated with a risk of death during follow-up that was 3.8 times that of patients with low levels of both biomarkers. Unfortunately, the addition of these two biomarkers to clinical parameters (age,

%FVC, %DLCO, and change in FVC in 6 months) did not improve prognostication beyond clinical parameters alone [42].

The PROFILE study measured baseline serum neo-epitope levels in 189 IPF patients [57, 81]. Mean concentrations of 7 of the 11 measured neo-epitope levels were significantly higher in IPF patients compared to age-matched and gender-matched healthy controls. The elevated neo-epitopes were C1M, C3M, C6M, CRPM, VICM, BGM, and ELM2. When assessed longitudinally, IPF patients with progressive disease at 6 months showed higher baseline concentrations of six neo-epitopes (C1M, C3A, C3M, C6M, CRPM, and VICM) compared to patients with stable disease. Baseline concentrations of two neo-epitopes (C1M, C3A) were associated with increased mortality. Interestingly, the rate of change from baseline to 3 months of six neo-epitope concentrations (C1M, C3M, C5M, C6M, CPRM, BGM) was predictive of overall survival, with increased risk proportional to the magnitude of change in neo-epitope concentrations. This is notable considering that IPF disease progression prediction via FVC decline, as described earlier in this chapter, currently requires PFT monitoring over a 6-month period [81]. It highlights the concept that future validated biomarkers not only will be able to diagnose IPF more accurately but also predict progression more quickly than current testing.

These studies and others suggest an important potential role of MMPs and neo-epitopes as possible diagnostic and prognostic biomarkers in IPF. However, larger studies are needed to validate their role as clinically useful biomarkers.

Other Potential Biomarkers

Periostin is an extracellular matrix protein associated with pathologic fibrotic processes like myelofibrosis and scar formation after myocardial infarction [83, 84]. In a study evaluating the role of periostin in 51 patients with IPF, periostin was not present in the pulmonary cells of five healthy control patients, including epithelial cells and alveolar macrophages. Among the 25 IPF patients in the study with surgical lung biopsies, there was significant expression of periostin in areas of active fibrosis, such as fibroblastic foci, but not in more established areas of fibrosis. Serum levels of periostin were also elevated in IPF patients compared to healthy controls and patients with cryptogenic organizing pneumonia (COP). In addition, serum periostin levels correlated with 6-month change in FVC and DLCO among IPF patients as well as extent of honeycombing on HRCT [40, 85].

Club cell secretory protein 16 (CC16) is a putative anti-inflammatory protein produced by “club” cells, which are small dome-shaped cells located in the simple ciliated epithelium of distal airways. Serum and BALF samples from IPF patients show significantly higher levels of CC16 compared to non-IPF ILD subjects (HP and CTD-related ILD) and healthy volunteers. Although the exact role of CC16 is unclear in IPF pathophysiology, its upregulation in IPF patients may allow it to serve as a potential biomarker once its function becomes clear [60, 86].

Heat shock protein 70 (HSP70) is one of a family of conserved, ubiquitously expressed proteins that ensure proper protein function. Their expression is induced in response to a variety of physiological injuries. Nearly 25% of IPF patients were noted to have IgG autoantibodies against HSP70 compared with only 3% of healthy controls. Furthermore, IgG autoantibody levels against HSP-70 rose during times of IPF exacerbation. Higher autoantibody levels were also associated with increased concentrations of proinflammatory cytokines, higher lymphocyte counts, and greater reductions in FVC over time [17]. However, further studies are needed to elucidate the role of HSP70 and HSP70 autoantibodies before they can be considered as potential biomarkers.

Circulating Cells

Fibrocytes are inactive mesenchymal cells lacking evidence of protein synthesis (in contrast to fibroblasts that are characterized by active matrix protein synthesis). Fibrocytes are found in peripheral blood, where they function as circulating mesenchymal cell progenitors that are targeted to sites for tissue repair and fibrosis. IPF patients have been shown to have significantly higher serum levels of circulating fibrocytes during stable disease. Furthermore, circulating fibrocyte concentrations rose during periods of IPF exacerbation [34]. While fibrocyte numbers did not correlate with lung function or radiologic severity scores, they appeared to independently predict early mortality when levels exceeded 5% of total blood leukocytes. Given the limited number of studies on circulating fibrocytes, they are still only considered to be potential IPF biomarkers.

Circulating endothelial cells (CEC) are endothelial cells that have been shed from the lining of the vascular wall into the bloodstream, and their entry into the circulation is thought to reflect vascular injury [87]. Endothelial progenitor cell (EPC) is a term applied to multiple cell types responsible for regenerating the endothelial lining of blood vessels. The numbers of CEC and EPC may indicate a balance between vascular injury and vascular repair. A recent study showed that IPF patients had low blood levels of CEC along with normal levels of EPC. Treatment with either pirfenidone or nintedanib further reduced the percentage of CEC [18]. These data suggest CEC and EPC play a role in IPF pathogenesis, and these cells should be explored as potential predictive biomarkers in IPF.

$CD4^+/CD28^+$ T cells, a subset of T cells expressing cell differentiation markers CD4 and CD28, have been evaluated in patients with IPF. CD4 cells, or T-helper cells, function to signal other immune cells to coordinate immune responses. CD28 is a costimulatory factor that promotes T-cell growth and development. IPF patients have been shown to have a lowered $CD4^+/CD28^+$ population along with increased circulating $CD4^+/CD28^{null}$ cells [31]. These aberrant T cells have been implicated in promoting inflammation in diseases such as rheumatoid arthritis. Furthermore, it appears that $CD4^+/CD28^{null}$ T cells display natural killer (NK) T-cell activity that can cause endothelial cell damage and apoptosis in vitro [88].

T-regulatory cells, formerly known as suppressor T cells, are a subpopulation of T cells responsible for maintaining tolerance to self-antigens and preventing autoimmune disease. Researchers have discovered decreased levels of circulating T-regulatory cells in BALF and serum from IPF patients compared to patients with CTD-related ILD and healthy controls. Decreased T-regulatory concentrations have also been correlated with FVC and TLC. The depleted T-regulatory populations in IPF patients suggest that they play a role in IPF pathophysiology [33].

Myeloid-derived suppressor cells (MDSC) are a heterogeneous group of myeloid-derived immune cells that possess strong immunosuppressive activities. They expand significantly under pathological conditions like cancer or chronic infections. In cancers, MDSCs help tumor cells evade immune detection and portend both resistance to therapy and a poor prognosis [89]. More recently, increased concentrations of MDSC have been found in multiple ILDs, including IPF, when compared to COPD patients. Furthermore, elevated MDSCs correlated inversely with FVC in IPF patients, but not in non-IPF ILD and COPD patients [62].

Although there have been profound insights into the role of various cell types in IPF, further clarification of each cell's unique contribution to advancing IPF pathogenesis is required. More clinical trials in diverse groups of patients are needed to isolate appropriate cellular biomarkers in IPF. The increased levels of $CD4^{+}/CD28^{null}$ T cells and MDSC, along with decreased levels of T-regulatory cells in IPF patients, suggest that each may have potential as a blood biomarker, and the possibility that a multivariate model incorporating all three can be useful should be explored.

Chemoattractants

Several types of cells play important roles in the pathologic changes associated with IPF. However, the mechanisms of cell recruitment in IPF are poorly understood. Chemoattractants are molecules that induce cells to migrate toward them, and they are thought to play a role in the recruitment and targeting of both inflammatory and noninflammatory cells to sites of injury in IPF lungs.

Interleukin 8 (IL-8) is a chemokine produced by lung macrophages, epithelial cells, and airway smooth muscle cells. It was one of the first cytokines studied in IPF patients. It induces chemotaxis in neutrophils (and to a lesser extent other granulocytes) and stimulates phagocytosis. Elevated levels of IL-8 were detected in BALF from IPF subjects and associated with decreased TLC, DLCO, and PaO2 levels [22, 28, 46]. An association between increased concentrations of peripheral blood IL-8 and worse outcomes in IPF, including decreased transplant-free survival, has been described. However, this result did not persist after adjustment for clinical parameters (age, sex, and baseline FVC) [46].

CCL-18 is a chemokine involved primarily with recruitment of the adaptive immune system. CCL-18 levels are elevated in IPF patients and may promote macrophage activation and collagen deposition. In one study, elevated baseline serum concentrations of CCL18 predicted changes in TLC and FVC and were

associated with a higher incidence of disease progression in IPF patients at 6 months of follow-up [35].

Other chemoattractants have also been shown to be elevated in patients with IPF. CXCL13 is a B-cell chemokine whose gene expression is higher in IPF lungs compared to both control and COPD lungs [55]. Plasma CXCL13 concentrations are also elevated compared to healthy controls and COPD patients, while circulating CXCL13 levels were highest in patients with IPF exacerbations. Serum levels of IL-2, IL-10, and IL-12 are all increased in IPF patients, and these three interleukins are thought to play a role in Th2 responses characterized by IL-5 release. Elevated IL-6 activity has also been detected in select IPF patients [37]. These data suggest a potential role for chemoattractants as biomarkers in patients with IPF. Once again, further research into the role of each chemoattractant in the pathogenesis of IPF as well as larger clinical trials in diverse ILD populations are needed.

Other Biomarkers

Table 10.1 is a more extensive list of biomarkers previously evaluated in clinical studies. Pro-angiogenesis factors, such as serum vascular endothelial growth factor (VEGF), have been found to be elevated in IPF [55], while glycoproteins such as chitinase-3-like protein 1 (CHI3L1), (which is also known as YKL-40 and may play a role in mesenchymal cell growth and angiogenesis), are also elevated in serum and BALF from IPF patients [38, 39]. Various tumor markers such as carcinoembryonic antigen (CEA) have also been found to be higher in select groups of IPF patients [44]. Osteopontin, a protein secreted from macrophages and which may stimulate fibroblast growth, has also been shown to be elevated in IPF patients [67]. Recently, insulin growth factor binding proteins (IGFBP) and insulin-like growth factor (IGF) serum concentrations have been investigated. IPF patients have elevated IGFBP-1 and IGFBP-2 concentrations. Intriguingly, IGFBP-2 levels decreased in patients on anti-fibrotic therapy, suggesting that IGFBP-2 could serve as a biomarker for both IPF diagnosis and response to drug therapy [63].

Sputum Biomarkers, Urine Biomarkers, and Biomarker Panels

More recently, there has been a shift toward investigating novel IPF biomarkers obtained from sputum or urine (two sources of noninvasive biological samples). Guiot et al. showed that sputum samples from IPF subjects displayed higher concentrations of neutrophils, eosinophils, macrophages, and epithelial cells compared to healthy volunteers [68]. Furthermore, sputum levels of MMP-7, IL-8, and IGFBP-2 were uniquely elevated in IPF patients compared to both healthy controls and COPD subjects [68]. Sputum MMP-7 concentrations alone inversely correlated with %TLC in IPF patients.

Other studies are beginning to isolate urinary biomarkers such as urine prostaglandin E metabolites. Prostaglandins are thought to have anti-fibrotic effects, and higher urine concentrations of their metabolites may reflect a compensatory response in IPF, although the function of prostaglandins in IPF patients is still unclear [65].

Finally, as already touched on in this chapter, investigators are resorting to panels of multiple biomarkers to more effectively differentiate IPF patients from healthy volunteers and patients with other pulmonary diseases. Biomarker panels consisting of two or more suspected diagnostic biomarkers may indicate a higher likelihood of IPF than any single biomarker. For example, White et al. showed that a combined serum biomarker panel combining SP-D, MMP-7, and osteopontin differentiated IPF patients from other types of ILD (except for RA-ILD) more readily than each individual biomarker [67]. As IPF pathophysiology is clarified in the future, biomarker panels with high specificity may be able to diagnose disease, identify responses to therapy, or define prognosis at the time of diagnosis.

Limitations of Novel Biomarker Studies and Future Directions

This chapter highlights only a few of the many biomarkers previously investigated. It should be noted that data on all these biomarkers are limited. The most recent ATS consensus guidelines referenced several notable clinical trials that identified biomarkers discussed in this chapter including KL-6/MUC1, surfactant proteins A and D, CCL18, MMP-1, MMP-7, and circulating fibrocytes [1]. Some of these biomarkers are routinely used in clinical practice in countries like Japan, most notably KL-6/MUC1. However, very few physicians worldwide currently use biomarkers in their daily clinical IPF practice.

There are several limitations to the current literature on novel IPF biomarkers. Biomarkers from all sources can be elevated in multiple pulmonary diseases and are often not specific for IPF. Furthermore, current trials have tended to study biomarkers in small homogenous populations of IPF patients, and studies that evaluate biomarkers in a prospective fashion are limited. Older studies published prior to release of the 2011 ATS/ERS/JRS/ALAT Consensus Statement and 2018 ATS/ERS/JRS/ALAT Clinical Practice Guideline used a classification for IPF that is now outdated. Indeed, some of the IPF patient cohorts included in biomarker studies may have contained patients we would now classify with other types of ILD. Since biopsies are not available for all patients with IPF and not all patients have undergone multidisciplinary review by a panel of pulmonologists, radiologists, and pathologists, it is challenging to compare novel biomarkers to a single, well-defined gold standard [3]. Only a limited number of trial results have been reproduced, and many others require further validation [90]. Finally, because IPF pathogenesis remains only partly elucidated, it is unclear how these biomarkers are involved in IPF pathogenesis and disease progression, further complicating their clinical utility. To effectively identify IPF biomarkers, standardization of clinical trials is needed.

Biomarkers must be correlated with PFTs, HRCT, and surgical lung biopsies [3]. Finally, the effects of currently approved FDA drugs, pirfenidone and nintedanib, on novel biomarker levels should be evaluated in all biomarker studies.

It is entirely possible that no single definitive serum, urine, BALF, or sputum biomarker for IPF exists, and a panel of multiple biomarkers from multiple sources may be needed to diagnose, manage, and prognosticate in cases of IPF. Worldwide, there are dozens of clinical trials either in progress or with unpublished results evaluating potential IPF biomarkers [91]. In the next few years, further insights into novel IPF biomarkers are likely to contribute to our understanding of IPF pathophysiology and optimize IPF disease management.

References

1. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE Jr, Kondoh Y, Myers J, Müller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schünemann HJ, 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;183:788–824.
2. Doyle TJ, Pinto-Plata V, Morse D, Celli BR, Rosas IO. The expanding role of biomarkers in the assessment of smoking-related parenchymal lung diseases. Chest. 2012;142:1027–34.
3. van den Blink B, Wijsenbeek MS, Hoogsteden HC. Serum biomarkers in idiopathic pulmonary fibrosis. Pulm Pharmacol Ther. 2010;23:515–20.
4. King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet. 2011;378:1949–61.
5. Travel 2010 1Strimbu K, Travel JA. What are biomarkers? Curr Opin HIV AIDS. 2010;5(6):463–6.
6. Naylor S. Biomarkers: current perspectives and future prospects. Expert Rev Mol Diagn. 2003;3:525–9.
7. Mayuex R. Biomarkers: potential uses and limitations. NeuroRx. 2004;1(2):182–8.
8. du Bois RM, Weycker D, Albera C, Bradford WZ, Costabel U, Kartashov A, Lancaster L, Noble PW, Raghu G, Sahn SA, Szwarcberg J, Thomeer M, Valeyre D, King TE Jr. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184:459–66.
9. Lettieri CJ, Nathan SD, Browning RF, Barnett SD, Ahmad S, Shorr AF. The distance-saturation product predicts mortality in idiopathic pulmonary fibrosis. Respir Med. 2006;100:1734–41.
10. Wells AU, Desai SR, Rubens MB, Goh NS, Cramer D, Nicholson AG, Colby TV, du Bois RM, Hansell DM. Idiopathic pulmonary fibrosis: a composite physiologic index derived from disease extent observed by computed tomography. Am J Respir Crit Care Med. 2003;167:962–9.
11. Ley B, Ryerson CJ, Vittinghoff E, Ryu JH, Tomassetti S, Lee JS, Poletti V, Buccioli M, Elicker BM, Jones KD, King TE Jr, Collard HR. A multidimensional index and staging system for idiopathic pulmonary fibrosis. Ann Intern Med. 2012;156:684–91.
12. Kishaba T, Nagano H, Nei Y, Yamashiro S. Body mass index-percent forced vital capacity-respiratory hospitalization: new staging for idiopathic pulmonary fibrosis patients. J Thorac Dis. 2016;8:3596–604.
13. Sharp C, Adamali HI, Millar AB. A comparison of published multidimensional indices to predict outcome in idiopathic pulmonary fibrosis. ERJ Open Res. 2017;3:pii00096-2016.
14. Tighe RM, Meltzer EB, Noble PW. Chapter 56: Idiopathic pulmonary fibrosis. In: Fishman's pulmonary diseases and disorders. 5th ed. New York: McGraw-Hill; 2015.

15. Hambly N, Shimbori C, Kolb M. Molecular classification of idiopathic pulmonary fibrosis: personalized medicine, genetics and biomarkers. Respirology. 2015;20:1010–22.
16. Gunther A, Korfei M, Mahavadi P, von der Beck D, Ruppert C, Markart P. Unravelling the progressive pathophysiology of idiopathic pulmonary fibrosis. Eur Respir Rev. 2012;21:152–60.
17. Kahloon RA, Xue J, Bhargava A, Csizmadia E, Otterbein L, Kass DJ, Bon J, Soejima M, Levesque MC, Lindell KO, Gibson KF, Kaminski N, Banga G, Oddis CV, Pilewski JM, Sciurba FC, Donahoe M, Zhang Y, Duncan SR. Patients with idiopathic pulmonary fibrosis with antibodies to heat shock protein 70 have poor prognoses. Am J Respir Crit Care Med. 2013;187:768–75.
18. De Biasi S, Cerri S, Bianchini E, Gibellini L, Persiani E, Montanari G, Luppi F, Carbonelli CM, Zucchi L, Bocchino M, Zamparelli AS, Vancheri C, Sgalla G, Richeldi L, Cossarizza A. Levels of circulating endothelial cells are low in idiopathic pulmonary fibrosis and are further reduced by anti-fibrotic treatments. BMC Med. 2015;13:277.
19. Hanumegowda C, Farkas L, Kolb M. Angiogenesis in pulmonary fibrosis: too much or not enough? Chest. 2012;142:200–7.
20. Borensztajn K, Crestani B, Kolb M. Idiopathic pulmonary fibrosis: from epithelial injury to biomarkers – insights from the bench side. Respiration. 2013;86:441–52.
21. McCormack FX, King TE Jr, Bucher BL, Nielsen L, Mason RJ. Surfactant protein A predicts survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1995;152:751–9.
22. Ziegenhagen MW, Zabel P, Zissel G, Schlaak M, Muller-Quernheim J. Serum level of interleukin 8 is elevated in idiopathic pulmonary fibrosis and indicates disease activity. Am J Respir Crit Care Med. 1998;157:762–8.
23. Yokoyama A, Kohno N, Hamada H, Sakatani M, Ueda E, Kondo K, Hirasawa Y, Hiwada K. Circulating KL-6 predicts the outcome of rapidly progressive idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998;158:1680–4.
24. Takahashi H, Fujishima T, Koba H, Murakami S, Kurokawa K, Shibuya Y, Shiratori M, Kuroki Y, Abe S. 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;162:1109–14.
25. Greene KE, King TE Jr, Kuroki Y, Bucher-Bartelson B, Hunninghake GW, Newman LS, Nagae H, Mason RJ. Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis. Eur Respir J. 2002;19:439–46.
26. Ishii H, Mukae H, Kadota J, Kaida H, Nagata T, Abe K, Matsukura S, Kohno S. High serum concentrations of surfactant protein A in usual interstitial pneumonia compared with non-specific interstitial pneumonia. Thorax. 2003;58:52–7.
27. Ohnishi H, Yokoyama A, Kondo K, Hamada H, Abe M, Nishimura K, Hiwada K, Kohno N. Comparative study of KL-6, surfactant protein-A, surfactant protein-D, and monocyte chemoattractant protein-1 as serum markers for interstitial lung diseases. Am J Respir Crit Care Med. 2002;165:378–81.
28. Tsoutsou PG, Gourgoulianis KI. Role of interleukin-10 in idiopathic pulmonary fibrosis. Eur Respir J. 2004;23:179–80.
29. Satoh H, Kurishima K, Ishikawa H, Ohtsuka M. Increased levels of KL-6 and subsequent mortality in patients with interstitial lung diseases. J Intern Med. 2006;260:429–34.
30. Rosas IO, Richards TJ, Konishi K, Zhang Y, Gibson K, Lokshin AE, Lindell KO, Cisneros J, Macdonald SD, Pardo A, Sciurba F, Dauber J, Selman M, Gochuico BR, Kaminski N. MMP1 and MMP7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. PLoS Med. 2008;5:e93.
31. Gilani SR, Vuga LJ, Lindell KO, Gibson KF, Xue J, Kaminski N, Valentine VG, Lindsay EK, George MP, Steele C, Duncan SR. CD28 down-regulation on circulating CD4 T-cells is associated with poor prognoses of patients with idiopathic pulmonary fibrosis. PLoS One. 2010;5:e8959.
32. Kinder BW, Brown KK, McCormack FX, Ix JH, Kervitsky A, Schwarz MI, King TE Jr. Serum surfactant protein-A is a strong predictor of early mortality in idiopathic pulmonary fibrosis. Chest. 2009;135:1557–63.

33. Kotsianidis I, Nakou E, Bouchliou I, Tzouvelekis A, Spanoudakis E, Steiropoulos P, Sotiriou I, Aidinis V, Margaritis D, Tsatalas C, Bouros D. Global impairment of CD4+CD25+FOXP3+ regulatory T cells in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;179:1121–30.
34. Moeller A, Gilpin SE, Ask K, Cox G, Cook D, Gauldie J, Margetts PJ, Farkas L, Dobranowski J, Boylan C, O'Byrne PM, Strieter RM, Kolb M. Circulating fibrocytes are an indicator of poor prognosis in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;179:588–94.
35. Prasse A, Probst C, Bargagli E, Zissel G, Toews GB, Flaherty KR, Olschewski M, Rottoli P, Müller-Quernheim J. Serum CC-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;179:717–23.
36. Ando M, Miyazaki E, Ito T, Hiroshige S, Nureki SI, Ueno T, Takenaka R, Fukami T, Kumamoto T. Significance of serum vascular endothelial growth factor level in patients with idiopathic pulmonary fibrosis. Lung. 2010;188:247–52.
37. Collard HR, Calfee CS, Wolters PJ, Song JW, Hong SB, Brady S, Ishizaka A, Jones KD, King TE Jr, Matthay MA, Kim DS. Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2010;299:L3–7.
38. Furuhashi K, Suda T, Nakamura Y, Inui N, Hashimoto D, Miwa S, Hayakawa H, Kusagaya H, Nakano Y, Nakamura H, Chida K. Increased expression of YKL-40, a chitinase-like protein, in serum and lung of patients with idiopathic pulmonary fibrosis. Respir Med. 2010;104:1204–10.
39. Korthagen NM, van Moorsel CH, Barlo NP, Ruven HJ, Kruit A, Heron M, van den Bosch JM, Grutters JC. Serum and BALF YKL-40 levels are predictors of survival in idiopathic pulmonary fibrosis. Respir Med. 2011;105:106–13.
40. Okamoto M, Hoshino T, Kitasato Y, Sakazaki Y, Kawayama T, Fujimoto K, Ohshima K, Shiraishi H, Uchida M, Ono J, Ohta S, Kato S, Izuhara K, Aizawa H. Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. Eur Respir J. 2011;37:1119–27.
41. Seibold MA, Wise AL, Speer MC, Steele MP, Brown KK, Loyd JE, Fingerlin TE, Zhang W, Gudmundsson G, Groshong SD, Evans CM, Garantziotis S, Adler KB, Dickey BF, du Bois RM, Yang IV, Herron A, Kervitsky D, Talbert JL, Markin C, Park J, Crews AL, Slifer SH, Auerbach S, Roy MG, Lin J, Hennessy CE, Schwarz MI, Schwartz DA. A common MUC5B promoter polymorphism and pulmonary fibrosis. N Engl J Med. 2011;364:1503–12.
42. 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;143:1422–9.
43. Cha SI, Ryerson CJ, Lee JS, Kukreja J, Barry SS, Jones KD, Elicker BM, Kim DS, Papa FR, Collard HR, Wolters PJ. Cleaved cytokeratin-18 is a mechanistically informative biomarker in idiopathic pulmonary fibrosis. Respir Res. 2012;13:105.
44. Fahim A, Crooks MG, Wilmot R, Campbell AP, Morice AH, Hart SP. Serum carcinoembryonic antigen correlates with severity of idiopathic pulmonary fibrosis. Respirology. 2012;17:1247–52.
45. Naik PK, Bozyk PD, Bentley JK, Popova AP, Birch CM, Wilke CA, Fry CD, White ES, Sisson TH, Tayob N, Carnemolla B, Orecchia P, Flaherty KR, Hershenson MB, Murray S, Martinez FJ, Moore BB, COMET Investigators. Periostin promotes fibrosis and predicts progression in patients with idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2012;303:L1046–56.
46. Richards TJ, Kaminski N, Baribaud F, Flavin S, Brodmerkel C, Horowitz D, Li K, Choi J, Vuga LJ, Lindell KO, Klesen M, Zhang Y, Gibson KF. Peripheral blood proteins predict mortality in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;185:67–76.
47. Samukawa T, Hamada T, Uto H, Yanagi M, Tsukuya G, Nosaki T, Maeda M, Hirano T, Tsubouchi H, Inoue H. The elevation of serum napsin A in idiopathic pulmonary fibrosis, compared with KL-6, surfactant protein-A and surfactant protein-D. BMC Pulm Med. 2012;12:55.
48. Chien JW, Richards TJ, Gibson KF, Zhang Y, Lindell KO, Shao L, Lyman SK, Adamkewicz JI, Smith V, Kaminski N, O'Riordan T. Serum lysyl oxidase-like 2 levels and idiopathic pulmonary fibrosis disease progression. Eur Respir J. 2014;43:1430–8.
49. Herazo-Maya JD, Noth I, Duncan SR, Kim S, Ma SF, Tseng GC, Feingold E, Juan-Guardela BM, Richards TJ, Lussier Y, Huang Y, Vij R, Lindell KO, Xue J, Gibson KF, Shapiro SD,

Garcia JG, Kaminski N. Peripheral blood mononuclear cell gene expression profiles predict poor outcome in idiopathic pulmonary fibrosis. Sci Transl Med. 2013;5:205ra136.
50. Jaffar J, Unger S, Corte TJ, Keller M, Wolters PJ, Richeldi L, Cerri S, Prêle CM, Hansbro PM, Argraves WS, Oliver RA, Oliver BG, Black JL, Burgess JK. Fibulin-1 predicts disease progression in patients with idiopathic pulmonary fibrosis. Chest. 2014;146:1055–63.
51. Nathan SD, Reffett T, Brown AW, Fischer CP, Shlobin OA, Ahmad S, Weir N, Sheridan MJ. The red cell distribution width as a prognostic indicator in idiopathic pulmonary fibrosis. Chest. 2013;143:1692–8.
52. Xue J, Kass DJ, Bon J, Vuga L, Tan J, Csizmadia E, Otterbein L, Soejima M, Levesque MC, Gibson KF, Kaminski N, Pilewski JM, Donahoe M, Sciurba FC, Duncan SR. Plasma B lymphocyte stimulator and B cell differentiation in idiopathic pulmonary fibrosis patients. J Immunol. 2013;191:2089–95.
53. Ohshimo S, Ishikawa N, Horimasu Y, Hattori N, Hirohashi N, Tanigawa K, Kohno N, Bonella F, Guzman J, Costabel U. Baseline KL-6 predicts increased risk for acute exacerbation of idiopathic pulmonary fibrosis. Respir Med. 2014;108:1031–9.
54. Stuart BD, Lee JS, Kozlitina J, Noth I, Devine MS, Glazer CS, Torres F, Kaza V, Girod CE, Jones KD, Elicker BM, Ma SF, Vij R, Collard HR, Wolters PJ, Garcia CK. Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. Lancet Respir Med. 2014;2:557–65.
55. Vuga LJ, Tedrow JR, Pandit KV, Tan J, Kass DJ, Xue J, Chandra D, Leader JK, Gibson KF, Kaminski N, Sciurba FC, Duncan SR. C-X-C motif chemokine 13 (CXCL13) is a prognostic biomarker of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2014;189:966–74.
56. DePianto DJ, Chandriani S, Abbas AR, Jia G, N'Diaye EN, Caplazi P, Kauder SE, Biswas S, Karnik SK, Ha C, Modrusan Z, Matthay MA, Kukreja J, Collard HR, Egen JG, Wolters PJ, Arron JR. Heterogeneous gene expression signatures correspond to distinct lung pathologies and biomarkers of disease severity in idiopathic pulmonary fibrosis. Thorax. 2015;70:48–56.
57. Jenkins RG, Simpson JK, Saini G, Bentley JH, Russell AM, Braybrooke R, Molyneaux PL, McKeever TM, Wells AU, Flynn A, Hubbard RB, Leeming DJ, Marshall RP, Karsdal MA, Lukey PT, Maher TM. Longitudinal change in collagen degradation biomarkers in idiopathic pulmonary fibrosis: an analysis from the prospective, multicentre PROFILE study. Lancet Respir Med. 2015;3:462–72.
58. Sokai A, Handa T, Tanizawa K, Oga T, Uno K, Tsuruyama T, Kubo T, Ikezoe K, Nakatsuka Y, Tanimura K, Muro S, Hirai T, Nagai S, Chin K, Mishima M. Matrix metalloproteinase-10: a novel biomarker for idiopathic pulmonary fibrosis. Respir Res. 2015;16:120.
59. Bauer Y, White ES, de Bernard S, Cornelisse P, Leconte I, Morganti A, Roux S, Nayler O. MMP-7 is a predictive biomarker of disease progression in patients with idiopathic pulmonary fibrosis. ERJ Open Res. 2017;3:pii00074-2016.
60. Buendia Roldán I, Sierra P, Montes M, Ruiz V, Ramírez R, Vega A, Salgado A, Vargas MH, Mejía M, Pardo A, Selman M. Club cell secretory protein-CC16 is increased in idiopathic pulmonary fibrosis. Eur Respir J. 2016;48:PA3098.
61. Cao M, Swigris JJ, Wang X, Cao M, Qiu Y, Huang M, Xiao Y, Cai H. Plasma leptin is elevated in acute exacerbation of idiopathic pulmonary fibrosis. Mediat Inflamm. 2016;2016:6940480.
62. Fernandez IE, Greiffo FR, Frankenberger M, Bandres J, Heinzelmann K, Neurohr C, Hatz R, Hartl D, Behr J, Eickelberg O. Peripheral blood myeloid-derived suppressor cells reflect disease status in idiopathic pulmonary fibrosis. Eur Respir J. 2016;48:1171–83.
63. 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;16:86.
64. Hamai K, Iwamoto H, Ishikawa N, Horimasu Y, Masuda T, Miyamoto S, Nakashima T, Ohshimo S, Fujitaka K, Hamada H, Hattori N, Kohno N. 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.
65. Horikiri T, Hara H, Saito N, Araya J, Takasaka N, Utsumi H, Yanagisawa H, Hashimoto M, Yoshii Y, Wakui H, Minagawa S, Ishikawa T, Shimizu K, Numata T, Arihiro S, Kaneko Y, Nakayama K, Matsuura T, Matsuura M, Fujiwara M, Okayasu I, Ito S, Kuwano K. Increased

levels of prostaglandin E-major urinary metabolite (PGE-MUM) in chronic fibrosing interstitial pneumonia. Respir Med. 2017;122:43–50.
66. Kono M, Nakamura Y, Oyama Y, Mori K, Hozumi H, Karayama M, Hashimoto D, Enomoto N, Fujisawa T, Inui N, Yamada M, Hamada E, Colby TV, Maekawa M, Suda T. Increased levels of serum Wisteria floribunda agglutinin-positive Mac-2 binding protein in idiopathic pulmonary fibrosis. Respir Med. 2016;115:46–52.
67. White ES, Xia M, Murray S, Dyal R, Flaherty CM, Flaherty KR, Moore BB, Cheng L, Doyle TJ, Villalba J, Dellaripa PF, Rosas IO, Kurtis JD, Martinez FJ. 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;194:1242–51.
68. Guiot J, Henket M, Corhay JL, Moermans C, Louis R. Sputum biomarkers in IPF: evidence for raised gene expression and protein level of IGFBP-2, IL-8 and MMP-7. PLoS One. 2017;12:e0171344.
69. Schuliga M, Jaffar J, Harris T, Knight DA, Westall G, Stewart AG. The fibrogenic actions of lung fibroblast-derived urokinase: a potential drug target in IPF. Sci Rep. 2017;7:41770.
70. Vij R, Noth I. Peripheral blood biomarkers in idiopathic pulmonary fibrosis. Transl Res. 2012;159:218–27.
71. Tzouvelekis A, Kouliatsis G, Anevlavis S, Bouros D. Serum biomarkers in interstitial lung diseases. Respir Res. 2005;6:78.
72. Doubkova M, Karpisek M, Mazoch J, Skrickova J, Doubek M. Prognostic significance of surfactant protein A, surfactant protein D, Clara cell protein 16, S100 protein, trefoil factor 3, and prostatic secretory protein 94 in idiopathic pulmonary fibrosis, sarcoidosis, and chronic pulmonary obstructive disease. Sarcoidosis Vasc Diffuse Lung Dis. 2016;33:224–34.
73. Phelps DS, Umstead TM, Mejia M, Carrillo G, Pardo A, Selman M. Increased surfactant protein-A levels in patients with newly diagnosed idiopathic pulmonary fibrosis. Chest. 2004;125:617–25.
74. Ley B, Brown KK, Collard HR. Molecular biomarkers in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2014;307:L681–91.
75. Ishikawa N, Hattori N, Yokoyama A, Kohno N. Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases. Respir Investig. 2012;50:3–13.
76. Kennedy B, Branagan P, Moloney F, Haroon M, O'Connell OJ, O'Connor TM, O'Regan K, Harney S, Henry MT. Biomarkers to identify ILD and predict lung function decline in scleroderma lung disease or idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis. 2015;32:228–36.
77. Wakamatsu K, Nagata N, Kumazoe H, Oda K, Ishimoto H, Yoshimi M, Takata S, Hamada M, Koreeda Y, Takakura K, Ishizu M, Hara M, Ise S, Izumi M, Akasaki T, Maki S, Kawabata M, Mukae H, Kawasaki M. Prognostic value of serial serum KL-6 measurements in patients with idiopathic pulmonary fibrosis. Respir Investig. 2017;55:16–23.
78. Yokoyama A, Kondo K, Nakajima M, Matsushima T, Takahashi T, Nishimura M, Bando M, Sugiyama Y, Totani Y, Ishizaki T, Ichiyasu H, Suga M, Hamada H, Kohno N. Prognostic value of circulating KL-6 in idiopathic pulmonary fibrosis. Respirology. 2006;11:164–8.
79. Pardo A, Cabrera S, Maldonado M, Selman M. Role of matrix metalloproteinases in the pathogenesis of idiopathic pulmonary fibrosis. Respir Res. 2016;17:23.
80. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92:827–39.
81. Crooks MG, Hart SP. Biomarkers in idiopathic pulmonary fibrosis: picking the winners for trials. Lancet Respir Med. 2015;3:421–2.
82. McKeown S, Richter AG, O'Kane C, McAuley DF, Thickett DR. MMP expression and abnormal lung permeability are important determinants of outcome in IPF. Eur Respir J. 2009;33:77–84.
83. Oku E, Kanaji T, Takata Y, Oshima K, Seki R, Morishige S, Imamura R, Ohtsubo K, Hashiguchi M, Osaki K, Yakushiji K, Yoshimoto K, Ogata H, Hamada H, Izuhara K, Sata M, Okamura T. Periostin and bone marrow fibrosis. Int J Hematol. 2008;88:57–63.

84. Oka T, Xu J, Kaiser RA, Melendez J, Hambleton M, Sargent MA, Lorts A, Brunskill EW, Dorn GW 2nd, Conway SJ, Aronow BJ, Robbins J, Molkentin JD. Genetic manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. Circ Res. 2007;101:313–21.
85. Tajiri M, Okamoto M, Fujimoto K, Johkoh T, Ono J, Tominaga M, Azuma K, Kawayama T, Ohta S, Izuhara K, Hoshino T. Serum level of periostin can predict long-term outcome of idiopathic pulmonary fibrosis. Respir Investig. 2015;53:73–81.
86. Buendía-Roldán I, Ruiz V, Sierra P, Montes E, Ramírez R, Vega A, Salgado A, Vargas MH, Mejía M, Pardo A, Selman M. Increased expression of CC16 in patients with idiopathic pulmonary fibrosis. PLoS One. 2016;11:e0168552.
87. Damani S, Bacconi A, Libiger O, Chourasia AH, Serry R, Gollapudi R, Goldberg R, Rapeport K, Haaser S, Topol S, Knowlton S, Bethel K, Kuhn P, Wood M, Carragher B, Schork NJ, Jiang J, Rao C, Connelly M, Fowler VM, Topol EJ. Characterization of circulating endothelial cells in acute myocardial infarction. Sci Transl Med. 2012;4(126):126ra33.
88. Dumitriu IE, Araguas ET, Baboonian C, Kaski JC. CD4+ CD28 null T cells in coronary artery disease: when helpers become killers. Cardiovasc Res. 2009;81:11–9.
89. Mantovani A. The growing diversity and spectrum of action of myeloid-derived suppressor cells. Eur J Immunol. 2010;40:3317–20.
90. Spagnolo P, Tzouvelekis A, Maher TM. Personalized medicine in idiopathic pulmonary fibrosis: facts and promises. Curr Opin Pulm Med. 2015;21:470–8.
91. ClinicalTrials.gov. https://clinicaltrials.gov/ct2/results?term=idiopathic+pulmonary+fibrosis+biomarker&Search=Search.

Chapter 11
Idiopathic Pulmonary Fibrosis: Phenotypes and Comorbidities

Christopher S. King, Shambhu Aryal, and Steven D. Nathan

Introduction

This chapter will tackle two distinct but related topics that have garnered significant interest among idiopathic pulmonary fibrosis (IPF) caregivers in the past decade. Given the marked variability in clinical progression of IPF, clinicians have attempted to categorize IPF patients based upon clinical characteristics in an effort to gain additional information on diagnosis, prognosis, or response to therapy. Such categorization of patients is termed phenotyping and will be the initial topic of discussion in this chapter. Two of the earliest recognized clinical phenotypes observed in IPF were combined pulmonary fibrosis and emphysema (CPFE) and idiopathic pulmonary fibrosis with associated pulmonary hypertension (PH-IPF). Both of these comorbid conditions are associated with increased morbidity and mortality [1]. Perhaps the prognostic importance of these conditions led to the recent interest in IPF comorbidities. There is a significantly increased prevalence of a number of comorbid conditions in the IPF population, including cardiac disease, lung cancer, venous thromboembolism (VTE), sleep-disordered breathing, and gastroesophageal reflux disease (GERD). It is hoped that aggressive management of these comorbid conditions may lead to improved outcomes of IPF. A discussion of common pulmonary and extrapulmonary comorbidities of IPF forms the latter portion of this chapter.

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Phenotyping in Idiopathic Pulmonary Fibrosis

Significant variability or heterogeneity exists with regard to a numerous aspects in IPF. Radiographic appearance on high-resolution CT may demonstrate a definitive usual interstitial pneumonia (UIP) pattern or a possible (UIP) pattern, but CT appearance can be inconsistent with UIP in some cases [2]. The clinical course can be highly variable as well with some patients rapidly declining while others slowly progress. Others may suffer very rapid deterioration in the form of an acute exacerbation [3]. These differences in presentation, course, and outcomes have provided a foundation for the concept of distinct IPF phenotypes.

Phenotyping: Value Added?

The word phenotype is derived from the Greek word, *phanein,* meaning "to show." It is the observable characteristics or traits of an individual – the cumulative result of one's genetic makeup after exposure to the environment. In medicine, the term is often applied to patients with shared demographic, morphologic, biochemical, or physiologic properties. The ultimate utility of clinical phenotyping would be realized if directly observable clinical manifestations provide insight into the disease course and/or specific treatment options. However, molecular phenotyping based on distinct genetic or molecular fingerprinting would only be relevant if adaptable and applicable to clinical utility (Fig. 11.1). IPF patients have been divided into clinical

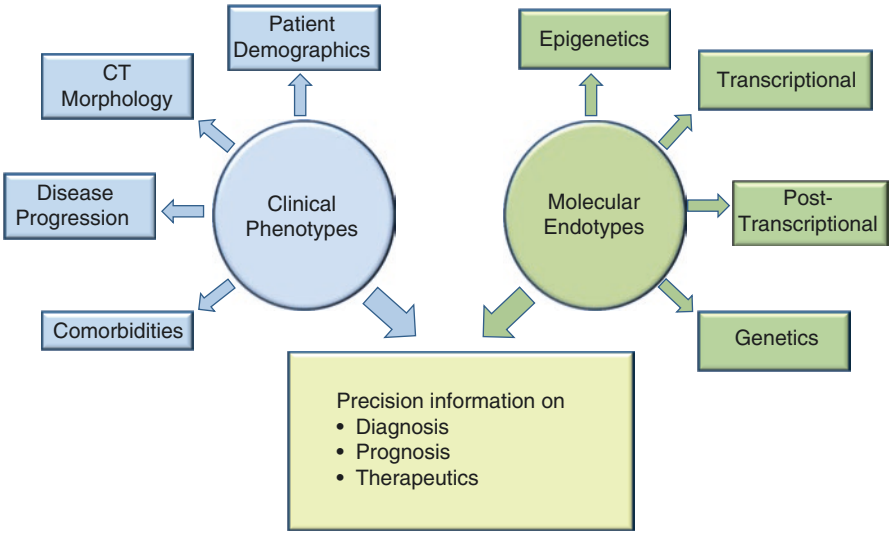

Fig. 11.1 Various means of clinical phenotyping and molecular endotyping

phenotypes in numerous ways including basic demographics, disease progression, comorbidities (CPFE and PH-IPF), and radiographic morphology. In the following sections, we will detail the merits and limitations of various means of IPF phenotyping.

Clinical Phenotypes

Demographic and Physiologic Data

Basic demographics [age, sex, smoking status, body mass index (BMI)], physical exam findings (clubbing), and pulmonary function parameters provide some prognostic information in IPF [3]. Older age has been associated with reduced survival in multiple studies [4, 5]. Female gender is associated with better survival (HR 0.63, CI 0.41–0.97) after adjusting for confounding variables [6]. Various demographic and physiologic variables have been combined to produce several different predictive indices which provide some degree of prognostic information in IPF patients. The best known of these is the Gender-Age-Physiology (GAP) index that incorporates age, gender, forced vital capacity (FVC), and diffusing capacity of carbon dioxide (DLCO) into a single number that enables the grouping of patients into three distinct categories of mortality risk [7]. However, the prognostic value of this score is limited by its inability to account for progression of disease over time. To correct this deficiency, Ley and colleagues added respiratory hospitalizations and 24-week change in FVC to the GAP index variables to create the longitudinal GAP model [8]. The composite physiologic index (CPI) and du Bois model are alternative scoring systems that can be utilized to provide estimates of mortality based on pulmonary function and clinical variables. However, both are cumbersome to calculate and not widely used in clinical practice [9, 10]. In summary, demographic and physiologic variables can be combined in predictive equations to provide some limited prognostic information on individuals with IPF. However, these variables do not represent a true "phenotype" and also fail to offer insight into the molecular mechanisms underlying the disease. Additionally, these clinical variables do not appear to affect treatment efficacy, as nintedanib and pirfenidone both performed equally well across a range of IPF cohorts in subgroup analyses of the respective phase 3 studies [11, 12].

Disease Progression

The clinical course of patients with IPF is difficult to predict. Although the median survival is approximately 3 years from the time of diagnosis, there is wide temporal variation in disease progression and outcomes. Whether there are distinct clinical phenotypes within this spectrum of outcomes is uncertain.

Rapid Progressors

There appears to be a subgroup of patients with a more accelerated course who are at greater risk of succumbing within the 1st year from the time of diagnosis [13–15]. These patients tend to present earlier after the onset of symptoms than those who have a more protracted course [13]. This might be due to the rapidity of progression of their symptoms. At-risk patients are more likely to be males and smokers [13]. However, they remain a difficult group to discern at baseline due to a lack of specific demographic, radiographic, physiologic, or characteristic pathologic features [13, 14]. However, rapid progressors appear to have a distinct genomic fingerprint with a propensity for the overexpression of genes involved in morphogenesis, oxidative stress, migration/proliferation, and genes from fibroblasts/smooth muscle cells [13, 16]. Toll-like receptor 9 (TLR9), an innate immune sensor that is released in response to certain infections, has also been shown to be upregulated in IPF patients with a more accelerated course [14, 17]. It appears that TLR9 can also drive the fibrotic process, raising the interesting possibility that occult infection may play a role, which is consistent with the multiple-hit theory that is commonly invoked in IPF pathogenesis. This may also help to explain the unpredictable course seen in most patients. Aberrant processing of microRNA may also play a role in disease progression in IPF. Analysis of surgical lung biopsies from patients with IPF found variations in microRNA expression in patients with rapid versus slowly progressive variants of the disease [18].

Slow Progressors

On the other end of the spectrum are those patients who survive 5 years and beyond from their initial diagnosis and whose disease course appears to follow a more attenuated trajectory [15]. These patients are also difficult to distinguish at presentation, but as a group they have higher values for body mass index (BMI), FVC % predicted, forced expiratory volume in 1 s (FEV_1) % predicted, total lung capacity (TLC) % predicted, and DLCO% predicted, and they have lower FEV_1/FVC ratios and mean pulmonary artery pressure (mPAP) [15]. Rather than being a distinct subgroup of patients, however, these patients likely represent the protracted extreme of a continuous spectrum of outcomes. There are data to suggest that ongoing survival portends a better prognosis and that "the longer IPF patients live, the more likely they will live longer" [15].

Acute Exacerbators

Acute exacerbations (AEs) of IPF are the topic of Chap. 17, and readers are encouraged to refer to this. Whether those patients who develop AEs should be regarded as a distinct phenotype or whether this should be regarded as an IPF complication is

uncertain. The idiopathic nature of AEs makes this distinction difficult. Specifically, if occult aspiration, viral infections, or any other intercurrent insults were the precipitating factor, then this would qualify AEs as a complication. Elevated pepsin levels have been described in one-third of cases of IPF AEs, attesting to aspiration as possibly playing a role in at least some cases [19]. However, if a specific milieu or genomic phenotype that predisposes patients to develop an AE is eventually identified, this patient subset would be more appropriately classified as a distinct IPF phenotype. Indeed, it has been shown that IPF-AE lung tissues have a distinct genomic profile with upregulation of stress response genes such as heat shock proteins, alpha defensins, and mitosis-related genes including histones and CCNA2 [20]. This gene dysregulation was localized mostly to the alveolar epithelial cells, rather than fibroblasts. Interestingly, from the same gene analysis of IPF-AE lung tissue, there did not appear to be upregulation of genes that are typically associated with infection or inflammation. Although the epithelial cell has been shown to be a potential precursor cell for fibroblasts through epithelial-mesenchymal transition, why these cells should alter their expression to that of a more acute lung injury phenotype remains uncertain.

There do not appear to be any distinct clinical features that can reliably identify those patients at risk for an acute exacerbation. However, it has been reported that patients with lower FVC and non-smokers are at higher risk. Additionally, male gender and undergoing surgical lung biopsy may also represent risk factors [21, 22]. Elevated serum KL-6 levels, a glycoprotein present in MUC1 mucin which increases with type II alveolar epithelial cell injury or proliferation, have been demonstrated to be a sensitive predictor for the development of AE-IPF. However, this biomarker is not routinely available in clinical practice [23]. It is possible that the available antifibrotic therapies for IPF affect the underlying molecular milieu leading to AE-IPF differently, as nintedanib has been demonstrated to reduce the incidence of AE-IPF. Whether this holds true for pirfenidone remains uncertain, although there is evidence to suggest a similar effect. Specifically, an earlier Japanese study of pirfenidone was stopped early due to an increased incidence of AE-IPF in the placebo arm [24]. However, AE-IPF was not an end point in the phase 3 program that resulted in the approval of pirfenidone [25]. In a post hoc analysis, pirfenidone has been shown to reduce respiratory-related hospitalizations, which might be a surrogate for IPF-AEs. Perhaps in the future, specific antifibrotics might be used preferentially in patients found to be at increased risk of AE-IPFs as we further sub-phenotype AE-IPF.

Morphologic

Given the fundamental role that HRCT plays in the diagnostic evaluation of IPF, it is natural that clinicians have attempted to phenotype disease category based on CT morphology. A definitive UIP pattern on CT is defined as the presence of subpleural, lower lung predominant fibrosis with honeycombing and an absence of

inconsistent features (mosaicism, ground-glass opacities, profuse micronodules, peribronchovascular predominance). This pattern obviates the need for biopsy to arrive at a confident diagnosis of IPF in the absence of an identifiable cause for pulmonary fibrosis [2]. The presence of honeycombing on HRCT, which distinguishes a definitive UIP pattern from a probable UIP pattern, has been associated with decreased median survival in multiple studies [26–28]. The prognostic significance of honeycombing has also been established in unclassifiable interstitial lung disease (ILD), as patients diagnosed as having unclassifiable ILD who have honeycombing and greater HRCT fibrosis scores have a worse prognosis that mirroring that of IPF patients [29].

Whether the presence of honeycombing represents a discrete phenotype or simply a more advanced case along the spectrum of the same disease is unclear. Perhaps the discrepancy in survival is solely attributable to lead-time bias, with patients whose HRCTs demonstrate honeycombing having more advanced disease. A post hoc subgroup analysis of the INPULSIS trial of nintedanib found that patients with possible UIP and traction bronchiectasis on HRCT (who did not undergo surgical biopsy for confirmation of diagnosis) had disease that progressed similarly to those with honeycombing on CT and/or UIP confirmed by surgical biopsy. They also appeared to respond similarly to therapy with nintedanib. Since this subgroup of patients with a "possible IPF diagnosis" have the same natural history and response to therapy as IPF patients, perhaps they should then be phenotyped and considered as having a diagnosis of IPF [30].

While the value of morphologic phenotyping via HRCT imaging when applied to IPF alone is questionable, perhaps morphologic phenotyping will be useful when applied to the full spectrum of ILDs. Traditionally, ILD has been divided into distinct diagnoses based on suspected etiology. Two patients may both have a UIP pattern on HRCT, yet if one has rheumatoid arthritis and the other does not, they will be labeled as separate disease entities (rheumatoid-associated ILD and IPF, respectively) and treated with different therapies (immunosuppression and antifibrotic agents, respectively). However, it is currently unclear if this artificial division based upon etiology is appropriate. Solomon and colleagues demonstrated that patients with rheumatoid-associated UIP have a similarly poor prognosis when compared with matched controls with IPF [31]. It seems plausible that all patients with a UIP pattern on HRCT or surgical lung biopsy share similar underlying pathophysiologic molecular mechanisms and should be treated similarly. Studies are ongoing to determine if patients with fibrotic lung diseases other than IPF will respond to treatment with antifibrotic medications [32, 33]. If positive, these studies could lead to a major paradigm shift in the classification and treatment of ILD.

Coexistent Pulmonary Disease: Phenotype or Comorbidity?

CPFE and PH-ILD were two of the earliest recognized clinical phenotypes. However, coexistent emphysema and PH could also be regarded as comorbidities,

although this distinction is probably semantic. CPFE affects a distinct population, which is comprised predominantly of elderly males with a smoking history [34]. The diagnosis is established by the characteristic CT findings of lower lung predominant fibrosis in combination with upper lung emphysema (Fig. 11.2). Pulmonary function testing (PFTs) is typified by preserved lung volumes with a severely reduced diffusing capacity [34]. Patients with CPFE have an increased risk for lung cancer and PH in comparison to IPF alone [1]. There is debate over whether CPFE increases the risk of death over IPF alone. Some suggest the heterogeneity in reported outcomes is due to inclusion in some series of other chronic interstitial pneumonias with a more favorable prognosis than IPF [34].

PH commonly complicates IPF with a reported prevalence of approximately 30–50% in most series [1]. Generally, PH complicating IPF is mild with a mean pulmonary artery pressure (mPAP) of 25–30 mmHg; however, a minority (approximately 10%) have severe PH with mPAP >35 mmHg or a low cardiac index [35, 36]. This phenotype has prognostic significance as PH-IPF is associated with increased morbidity (increased need for supplemental oxygen, decreased quality of life, and lower exercise tolerance) and increased mortality [35, 37, 38]. Both CPFE and PH-IPF are discussed in greater detail below.

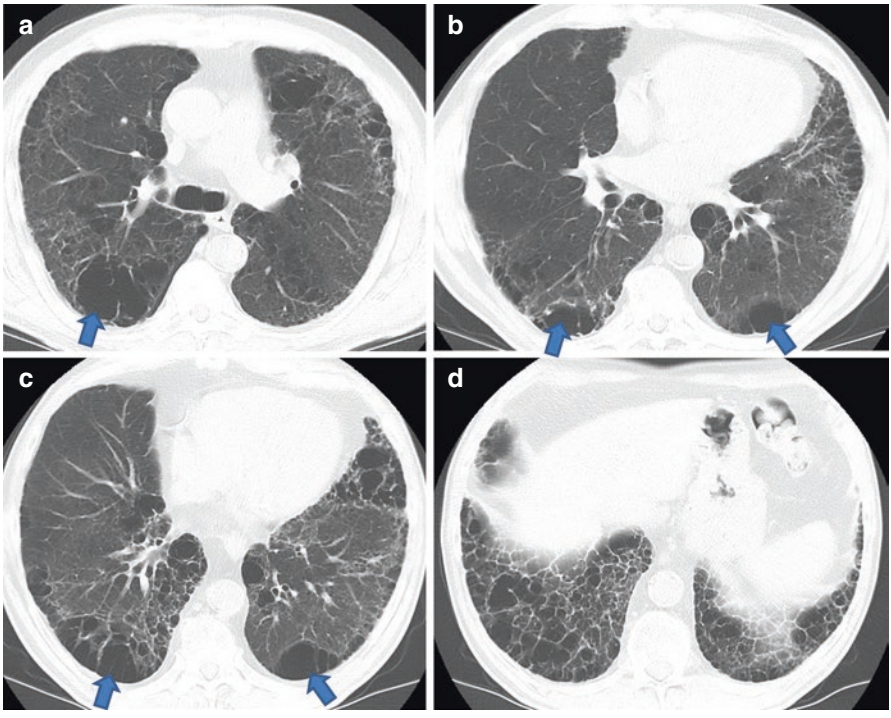

Fig. 11.2 A HRCT demonstrating typical combined pulmonary fibrosis and emphysema. The blue arrows highlight areas of paraseptal emphysema. Severe lower lung predominant fibrosis is seen in panel D

Genetic Abnormalities

A number of genetic mutations have been shown to predispose to IPF [39]. These may occur sporadically or in the context of familial disease. A single nucleotide polymorphism (SNP) in the promoter region of the MUC5B gene results in overproduction of a mucin involved in airway defense and has been found to be strongly associated with both familial and sporadic variants of IPF [40]. Interestingly, while this genetic aberration is associated with an increased incidence of IPF, it is paradoxically associated with a more favorable prognosis [41]. Telomere dysfunction and shortening has been linked with development of pulmonary fibrosis [42]. About 10% of familial pulmonary fibrosis and 1–3% of sporadic IPF patients have variations in one of two major enzymes responsible for telomere maintenance: telomerase reverse transcriptase (TERT) or telomerase RNA component (TERC) [43]. A number of abnormalities in genes related to inflammation or immunity including TOLLIP, ELMOD2, interleukins, major histocompatibility complex (MHC), tumor necrosis factor (TNF), and transforming growth factor β (TGF-β) have been associated with the development of pulmonary fibrosis. Finally, a predilection to IPF has been linked to genes coding for alveolar stabilizers including surfactant protein C (SFTPC), surfactant protein A2 (SFTPA), and ATP-binding cassette member A3 (ABCA3) [39, 43]. Given that no identifiable genetic susceptibility to IPF can be identified in the vast majority of patients who develop disease, it is clear that there is still a large void in our understanding of the genetic underpinnings of this complex condition.

Familial Idiopathic Pulmonary Fibrosis

There is wide variation in the percentage of IPF patients who are believed to have the familial variant. This is recognized when two or more individuals from the same family have pulmonary fibrosis consistent with an idiopathic interstitial pneumonia (IIP). The topic of familial IPF is covered in more detail in a dedicated chapter [39]. Whether this patient subgroup has a natural history of disease that is sufficiently different such that it can be regarded as a distinct phenotype is uncertain. However, what is known is that those with a familial predisposition can develop not only IPF but other forms of IIP, which suggests that genetic mutations predispose patients to a number of IIP pathologies other than UIP.

Patients with familial IPF, including those with telomerase mutations, tend to be diagnosed earlier and hence die at a younger age [44]. However, their clinical presentation and disease course appears similar to that of sporadic IPF patients with a mean life expectancy after diagnosis of 2.4–3 years [45, 46].

The Future of Phenotyping

While efforts to categorize IPF through clinical phenotyping have been valiant and well-intentioned, it is clear that there are major shortfalls to doing so. Epigenetics, specifically an individual's genetic makeup interacting with environmental exposures, are just a portion of the exceedingly complex pathophysiology of pulmonary fibrosis. Epigenetic mechanisms such as DNA methylation, histone modifications, and microRNA can influence gene expression in IPF [47]. Transcriptional (conversion of DNA to RNA) and posttranscriptional (conversion of primary RNA to mature RNA) factors can also influence the expression of disease. Important work is already being done on molecular endotyping. Two studies, COMET (Correlating Outcomes with biochemical Markers to Estimate Time to progression in Idiopathic Pulmonary Fibrosis) and PROFILE (PRospective Observation of Fibrosis In the Lung clinical Endpoints) are examining both clinical and molecular fingerprints in large cohorts of patients [48, 49].

It is hoped that further studies will provide greater insight into the complex pathophysiology of IPF and allow for molecular endotyping, which incorporates genetic, metabolic, transcriptional, and environmental factors, to eventually supplant clinical phenotyping [50]. If this goal is realized, in the future simple serum or bronchoalveolar lavage biomarkers might be utilized to provide individualized information regarding prognosis, diagnosis, and optimal treatment of patients not only with IPF but all forms of fibrotic lung diseases.

Comorbidities of Idiopathic Pulmonary Fibrosis

Patients with IPF have a significantly higher prevalence of several comorbid pulmonary and extrapulmonary conditions compared to the general population. These conditions often dictate the presentation and clinical course of IPF, and such conditions can significantly affect the morbidity and mortality attributable to IPF. As such, it seems logical to phenotype IPF based on those comorbidities (Figs. 11.3 and 11.4).

CPFE

Cigarette smoking is a common risk factor for both emphysema and pulmonary fibrosis, so it is not surprising that many patients have these two conditions in coexistence. Although many authors had previously described this association, Cottin and colleagues were the first to coin the term "combined pulmonary fibrosis and emphysema" in their description of 61 patients with a heavy smoking history,

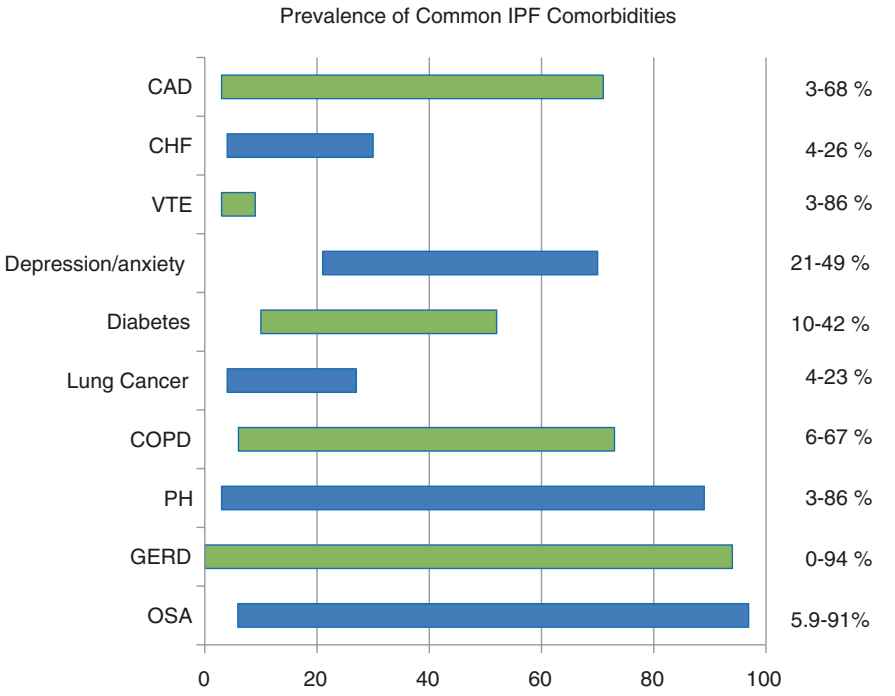

Fig. 11.3 Prevalence of common comorbidities of IPF. Abbreviations: CAD coronary artery disease, CHF congestive heart failure, COPD chronic obstructive pulmonary disease, GERD gastroesophageal reflux disease, OSA obstructive sleep apnea, PH pulmonary hypertension, VTE venous thromboembolism. (The data for this figure were derived from Ref. [52])

exercise hypoxemia, upper lobe emphysema and lower-lobe fibrosis, preserved lung volumes, and severely reduced DLCO [51]. About a third of patients with IPF have some evidence of emphysema, but the reported prevalence varies from 6% to 67% [52]. Patients with CPFE typically tend to be older males with a strong history of smoking and present with marked exertional hypoxemia. PFTs frequently demonstrate pseudonormalized lung volumes due to the net effect of hyperinflation from emphysema and restriction from pulmonary fibrosis, which is accompanied by a severely reduced DLCO. The diagnosis is made by CT scan showing the presence of emphysematous changes (usually in the upper lung fields) with subpleural, lower-lobe predominant fibrotic lung disease (Fig. 11.2).

It is possible that CPFE develops in individuals who have a genetic susceptibility to both COPD and IPF, since both have similar risk factors including cigarette smoking and environmental exposures. However, the exact pathophysiology is unclear, and it is possible that the development of one pathology predisposes to the development of the other in a patient with a unique genetic predisposition.

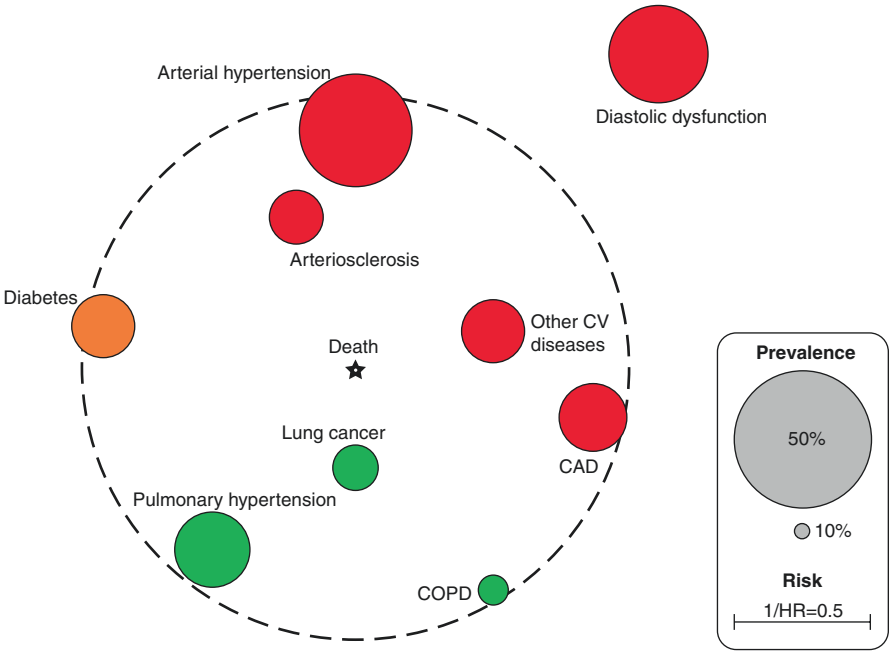

Fig. 11.4 Graphic depiction of comorbidities of IPF that have more than a 10% prevalence in the entire cohort. The area of the circles represents the prevalence of the comorbid disorder, and the proximity to the center of the circle represents the strength of association between the comorbidity and the risk of death. Comorbidities with a statistically significant increased risk of death will be fully inside the large circle. Bubble colors represent organ systems or disease clusters (cardiovascular = red, pulmonary = green, others = orange). (Reproduced from Kreuter et al. [130])

Patients with CPFE appear to have distinct clinical features [51, 53, 54]. Similar to IPF, CPFE has an unfavorable prognosis with a 5-year survival reported between 35% and 80% [55]. A recent study by Jacob et al. concluded that this is due to the additive effects of fibrosis and emphysema and that there was no prognostic impact of emphysema on fibrosis beyond the additive extents of both entities [56]. The incidence of PH as well as lung cancer in the CPFE phenotype are 50% higher than in patients with IPF alone. PH appears to be the major cause of mortality in these patients with a 1-year survival of only 60% in patients with CPFE who develop severe PH. Similarly, the prognosis of lung cancer associated with CPFE is poor with a median survival of 10.8 months [57]. Moreover, treatment options for lung cancer in CPFE are limited because of the increased risk of acute exacerbation with chemotherapy, radiation, and surgical resection [1].

Although CPFE is increasingly recognized as a distinct phenotype of IPF, there are no established treatment guidelines due to the lack of trials exclusively limited to patients with this phenotype. Smoking cessation is crucial for prevention of further progression, and other supportive measures including supplemental oxygen therapy, immunizations, and pulmonary rehabilitation would be helpful to most

patients. Bronchodilators should be considered if airway obstruction appears to be a major component. Data on the efficacy of antifibrotics (pirfenidone or nintedanib) is limited. A subgroup analysis of a sizable number of CPFE patients from the INPULSIS trials of nintedanib found that the drug was still efficacious in slowing disease progression [58]. A case series of 11 patients with CPFE treated with pirfenidone described a similar rate of disease progression as IPF sans emphysema [59]. As such, treating people with CPFE with these drugs should be considered. There is even less data on the management of PH complicating CPFE despite the severe impact of this complication. Although it is enticing to treat severe PH in any context, studies still need to be completed before the treatment of these patients with PH-specific medications can be endorsed.

IPF with PH

Pulmonary hypertension is commonly present in patients with IPF and impacts morbidity and mortality significantly. As such, IPF associated with PH should be considered a distinct phenotype. The prevalence of PH in IPF at the initial screening evaluation has been reported to be around 8–17% [60–62], but this number is as high as 30–50% later in the disease course, as derived from the data on patients with IPF who are entered into the lung transplant registry [36]. It was once believed that there was a subset of IPF patients who could be regarded as having "disproportionate" PH, but after the introduction of guidelines from the World Health Organization in 2013 defining severe PH as a mPAP greater than or equal to 35 mmHg or mPAP greater than or equal to 25 mmHg in the presence of a low CO (CI <2.5), the term "out-of-proportion PH" has largely been abandoned [63–65].

Patients with IPF and coexisting PH tend to have dyspnea out of proportion to their PFT abnormalities and a reduced distance on 6-min walk test (6MWT) that is typically accompanied by excessive desaturation and an impaired heart rate recovery. Other clues to the presence of PH include a severely reduced DLCO, an elevation of the brain natriuretic peptide, and an enlarged pulmonary artery segment on chest CT scan [1, 66] (Fig. 11.5). Right heart catheterization (RHC) is mandatory in the diagnosis of PH-IPF, since the accuracy of transthoracic echocardiography in advanced lung disease is suboptimal [67]. RHC also provides a comprehensive hemodynamic profile and allows differentiation of precapillary from postcapillary PH, which may be present in as many as 15–20% of IPF patients [62, 68].

The pathophysiology of PH in IPF appears to be complex but centers around distortion and destruction of the vascular bed from fibrosis and chronic hypoxemic vasoconstriction. Additional mechanisms thought to contribute to PH include uncorrected or partially corrected hypoxemia, aberrant angiogenesis, endothelial dysfunction, and cytokine duality with profibrogenic mediators that are also vasoactive including leukotrienes, tumor necrosis factor-α (TNF-α), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) [69]. Moreover, other known

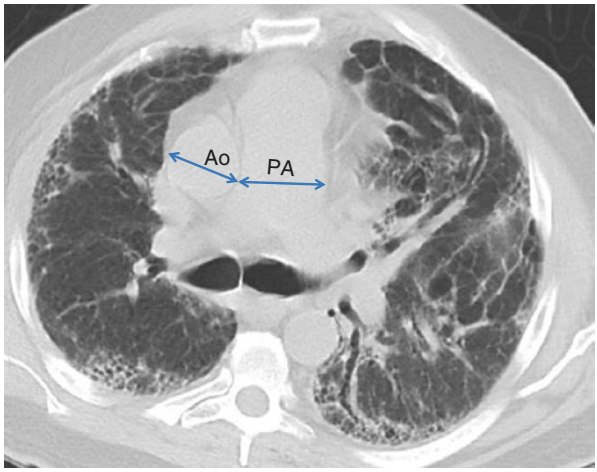

Fig. 11.5 Thoracic CT demonstrating an enlarged pulmonary artery to aorta ratio at the level of the carina

etiologies of PH such as obstructive sleep apnea (OSA), thromboembolic disease, or heart failure can coexist and contribute to PH in IPF.

The presence of PH in IPF appears to be a poor prognostic marker. In one study, patients with IPF and PH had a threefold increased risk of death that was independent of age, race, FVC % predicted, 6MWT distance, and other covariates [70]. Another study of patients who were undergoing assessment for lung transplant showed that the 1-year mortality was 28% in the group with PH as opposed to 5.5% in the group without PH; in the same study, PH was present in 52.4% of IPF non-survivors compared to only 24.1% of survivors [35]. Despite the associations noted, the optimal treatment for PH in IPF is not established. Studies assessing the role of pulmonary vasodilators in PH with IPF have shown mixed results at best, and the current consensus is that such therapy should only be initiated for patients with PH in IPF in the context of a clinical trial or at an expert center under close clinical observation [1]. The use of inhaled prostaglandins has shown promising results in group III PH, underscoring the need for further studies to evaluate these agents for PH related to IPF [71]. In addition, because of the strong association between PH and mortality, the guidelines on lung transplant candidate selection cite the development of PH in patients with IPF as a criterion to list for transplantation [72].

Gastroesophageal Reflux Disease (GERD)

Several studies dating back to the 1970s have shown an increased prevalence of GERD in patients with IPF compared to matched controls, although the reported prevalence varies [52, 73, 74]. In a study reported in 1998 evaluating the prevalence of GERD in IPF, over 90% of the patients with biopsy-proven IPF had significant GERD on esophageal PH monitoring compared to only 50% of the controls with non-IPF ILD. Interestingly, only 25% of the patients with increased acid exposure

had typical reflux symptoms, underscoring the fact that absence of symptoms does not preclude a diagnosis of GERD in this population [75]. However, despite the association between GERD and IPF, it is difficult to establish a cause-effect relationship. While it has long been observed that aspiration of gastric contents can cause pulmonary fibrosis in both animals and humans, it has been argued that GERD may be a result of mechanical effects of IPF including poor lung compliance, distortion of mediastinal anatomy, and weakening of the lower esophageal sphincter [76–78]. Moreover, a recent study of 45 IPF patients undergoing evaluation for lung transplant concluded that lung disease severity in those patients was more strongly associated with impedance measures of bolus reflux than pH parameters of acid reflux alone, supporting the notion that non-acid reflux may play a more significant role than previously understood [79].

Results of the studies looking at the efficacy of antiacid therapy in the treatment of IPF have been mixed at best. An analysis of data from three randomized controlled trials (STEP-IPF, ACE-IPF, and PANTHER-IPF) showed slower decline in FVC over time with antiacid therapy in patients with IPF. However, another post hoc analysis of data from three randomized trials of pirfenidone (CAPACITY 004, CAPACITY 006, and ASCEND) showed no association of antiacid therapy with survival in IPF but rather an increased risk of infection in those with more advanced disease [80, 81]. Current guidelines recommend that asymptomatic GERD should be medically treated in most patients with IPF with the caveat that this recommendation is based on very low-quality data [82]. Clearly more studies are needed to address this dilemma, including the role of therapies to prevent non-acid reflux.

Venous Thromboembolism (VTE)

IPF patients tend to have a higher incidence of venous thromboembolic disease compared to the general population [83, 84]. VTE is responsible for 0.4–3% of deaths in the IPF population [85, 86]. A population-based study from the United Kingdom found that the incidence of pulmonary embolism (PE) in IPF was 2.4% as opposed to 0.6% in controls [87]. A retrospective review of more than 9000 patients from a US insurance claims database reported a prevalence of 2.7% for PE compared with 0.4% in matched controls with a relative risk for PE of 6.97 (95% CI of 4.92–9.89) [88]. Patients with IPF were found to be more than four times as likely to be in a prothrombotic state in a study of over 200 patients with IPF when compared to controls that were matched for age and gender [89]. This could be related to decreased mobility of patients with IPF, but activation of the coagulation cascade in these patients could also possibly play a role [1]. The diagnosis of PE is frequently made by CT pulmonary angiography (CTPA); however, a recent study of 22 IPF patients with acute clinical deterioration showed that pulmonary embolism was detected more frequently by VQ-SPECT than by CTPA; however, this needs to be validated in a larger study [90].

The link between IPF and venous thrombosis offered a theoretical rationale for use of anticoagulants in IPF. However, a randomized controlled trial of dose-adjusted warfarin compared with placebo actually showed an increase in mortality in the group receiving warfarin, leading to early termination of the study [91]. In addition, a post hoc analysis of patients in the placebo arms of three major RCTs of antifibrotic therapy for IPF demonstrated an increased risk of IPF-related death in patients on anticoagulants. Therefore, the general consensus is not to place patients with IPF on anticoagulants in the absence of another indication [92].

Cardiovascular Disease

Cardiovascular diseases including arrhythmias, congestive heart failure (CHF), coronary artery disease (CAD), cerebrovascular accident (CVA), and systemic hypertension are significantly more common in patients with IPF compared to the general population [52, 83, 93]. In fact, cardiac diseases are the second most common cause of death in patients with IPF after respiratory failure and account for 10% of deaths [94]. IPF may be an independent risk factor for the development of CAD [95]. The prevalence of CAD in patients with IPF has been reported to be as variable as 3–68% based on definitions used, but severe CAD in IPF portends a poor prognosis with an unadjusted hazard ratio of 3.3 and a median survival from the time of LHC of just over 1.5 years [52, 96]. CHF, both systolic and diastolic, is common in IPF with a prevalence of 4–26%; similarly, arrhythmias complicate the course of 6–19% of patients with most of the arrhythmias being atrial fibrillation [52].

The increased prevalence of cardiac diseases might be related to common shared risk factors including history of cigarette smoking and advancing age, but local lung injury and repair with upregulation of protease inhibitors, coagulopathy, and promotion of atherosclerosis (as well as exacerbation of underlying CAD by the concomitant presence of hypoxemia with progressive IPF) may all contribute [97]. Regardless, cardiac comorbidities should always be considered in the differential diagnosis for any patient with IPF with clinical deterioration and investigated as indicated. While left heart catheterization remains the gold standard for the diagnosis of CAD, HRCT evidence of coronary calcification may provide clinicians with a readily available means of screening for CAD [1] (Fig. 11.6). Management of these comorbidities is beyond the scope of this chapter but should be in accordance with established guidelines.

Diabetes Mellitus (DM)

Diabetes mellitus (DM) is a significant comorbidity in IPF. The reported prevalence of DM in IPF patients ranges from 9.6% to 56% [98, 99]. A case control study of 920 patients with IPF and over 3500 matched controls in the United Kingdom found

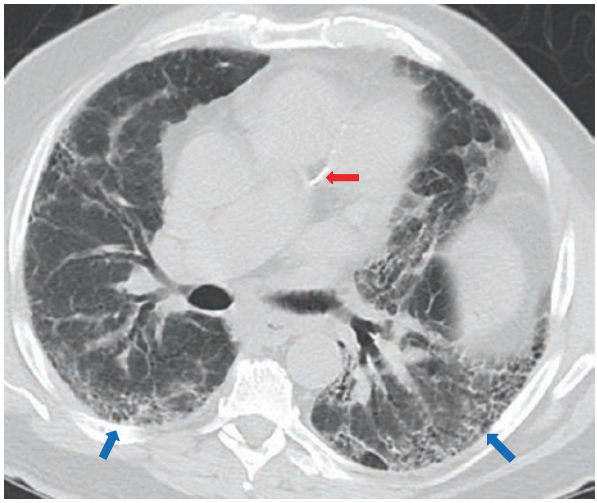

Fig. 11.6 Prominent coronary artery calcifications seen on a standard non-contrast CT of the chest in a patient with IPF

that IPF was significantly associated with exposures relating to the presence of DM, the strongest association being with the use of insulin. The results were similar even after excluding people on prednisolone, and the authors concluded that the results were consistent with a relationship in which DM might be a causal factor for IPF [98]. Also, another study showed that DM was a significant prognostic determinant of IPF in a population-based cohort [100]. Similarly, a meta-analysis of available studies concluded that the prevalence of DM in IPF was 13.9–25% in different continents and that DM increased the risk of IPF with an OR of 1.696 (95% CI, 1.34–2.14) [101].

The reasons for the relation between DM and IPF are not clear. It is possible that reactive oxygen species and advanced glycation end products that result from hyperglycemia may contribute to the development of fibrosis as hypothesized by some researchers [102]. A recent animal study showed that the oral hypoglycemic metformin attenuated lung fibrosis development in mouse models via NOX4 suppression, suggesting that it could be a promising antifibrotic agent for IPF [103]. Further research studies are needed to establish whether this holds true in humans and whether better overall control of DM improves outcomes in IPF. In the meantime, healthcare providers should be aware of the noted association and strive to manage DM aggressively in IPF patients according to standard guidelines.

Depression/Anxiety

Depression and anxiety are highly prevalent in IPF and are particularly associated with severe disease [1]. Moreover, those conditions seem to have a profound impact on health-related quality of life (HRQoL). A recent study of patients in the Australian IPF Registry showed that depression was a major symptomatic determinant of

HRQoL, aside from cough and dyspnea [104]. Similarly, another study from Japan demonstrated depression in 22.3% of IPF patients and seemed to be a significant determinant of HRQoL [105]. Patients with IPF and depression described dyspnea, feeling of social isolation, loss of independence, and inadequate sleep as major causes of psychological distress [106]. A vicious cycle between dyspnea and depression can occur with dyspnea contributing to depression and vice versa [107].

Due to the high prevalence and the profound psychological impacts of depression and anxiety, all patients with IPF should be screened for these disorders. Standard treatment would include cognitive behavioral therapy and antidepressant medications, although the effectiveness of these treatments has not been specifically validated in this population [1]. Pulmonary rehabilitation should be strongly considered, since studies have demonstrated a sustained improvement in depressive symptoms of anxiety and depression as well as functional improvement [108]. Patient support groups often provide good psychological support, while the psychosocial support may also be obtained through participation in pulmonary rehabilitation. Recent data suggests that the majority of patients with IPF die in a hospital setting with only a minority receiving formal palliative care referral [109]. These data underscore a deficiency in the current holistic management of IPF patients and serve notice for an earlier discussion of end-of-life care, including a palliative care referral that might help alleviate patients' burden of depression and anxiety.

Lung Cancer

Pulmonary fibrosis appears to be a risk factor for lung cancer independent of shared risk factors including cigarette smoking and advancing age [110]. The prevalence of lung cancer in populations with IPF was found to be 3–48% based on a review of 126 studies [52]. Squamous cell carcinomas appear to be the most common histologic type, but in contrast to the general population, these tend to be located in the lower lobes along the peripheral edges of fibrosis (Fig. 11.7) [111, 112]. The reason for an increased risk of lung cancer in pulmonary fibrosis is not clear. An aberrant expression of miRNAs regulating non-small cell lung cancer and IPF has been suggested together with a crucial role of tyrosine kinase inhibition directed against growth factors [111].

Symptoms of lung cancer like hemoptysis, unintentional weight loss, and constitutional symptoms can be minimal or nonspecific in patients with IPF, so a high index of suspicion is needed [113]. This is especially important since the survival in patients with IPF and lung cancer is significantly worse than that of patients with IPF alone. In an analysis of almost 200 patients, the median survival for patients with IPF but without lung cancer was 63.9 months vs 38.7 months for patients with lung cancer and IPF [114]. Moreover, treatment of lung cancers in patients with IPF is very challenging, as it is complicated by excess operative mortality and acute exacerbations of pulmonary fibrosis due to acute lung injury associated with

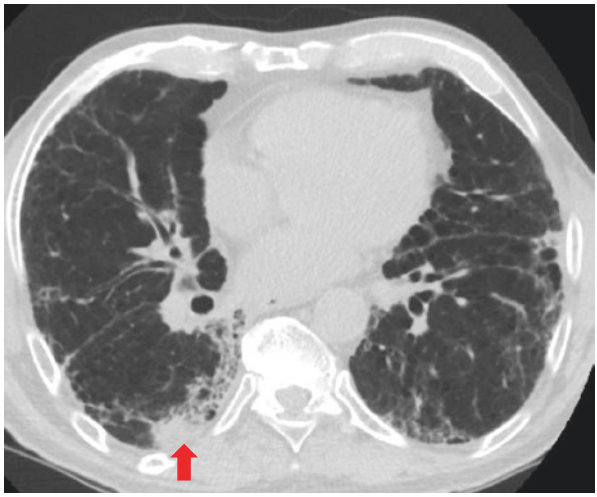

Fig. 11.7 Thoracic CT showing a non-small cell lung cancer in a patient with pulmonary fibrosis. The nodule is found in the lower lobe adjacent to the areas of fibrosis, which is typical for lung cancer complicating IPF

surgery, chemotherapy, and radiation therapy [115, 116]. The role of antifibrotics in the prevention and treatment of lung cancers in IPF patients is an area of current research because of possible shared pathways for both conditions. A retrospective review of 384 patients found a marked reduction in the incidence of lung cancer in those patients given pirfenidone [117]. Similarly, nintedanib in combination with docetaxel demonstrated significant overall survival benefits in adenocarcinoma patients in another study, which would be particularly relevant in IPF patients with lung cancer [118].

Sarcopenia/Deconditioning

Patients with IPF suffer from loss of muscle mass and strength as well as endurance, especially as their disease progresses. Deconditioning has been shown to be associated with decreased survival including patients with IPF waiting for a lung transplant [119, 120]. Deconditioning can be a result of exertional dyspnea that discourages activity as well as due to peripheral muscle dysfunction mediated by inactivity, hypoxemia, and adverse effects of medications; moreover, deconditioning can lead to worse dyspnea and further muscle dysfunction [121]. Thus, patients with IPF should be routinely counseled to stay active. Multiple trials of pulmonary rehabilitation have demonstrated improvements in several IPF outcome measures including deconditioning by providing a structured, supervised exercise training in addition to disease-specific education and psychosocial support [1]. The importance of managing deconditioning in IPF patients waiting for a lung transplant should be particularly emphasized, since pre-transplant wasting is an important prognostic marker for posttransplantation outcome [122].

Sleep-Disordered Breathing

Sleep disordered breathing has been recognized as an important comorbidity of IPF [52]. Patients with IPF have alterations in their sleep architecture, including decreased sleep efficiency, abnormal slow wave and rapid eye movement (REM) sleep, as well as increased sleep fragmentation [123]. Factors contributing to poor sleep include nocturnal cough, medications, nocturnal desaturations, and obstructive apneas. The prevalence of obstructive sleep apnea in IPF patients has been reported to be 59–90% [124–126]. The apnea-hypopnea index in IPF patients appears to correlate with TLC. Nocturnal desaturation, which can occur independent of obstructive apneas and is due to alveolar hypoventilation and worsened ventilation-perfusion mismatch, is also very common [127]. Sleep-disordered breathing, especially OSA, has been thought to cause subclinical lung injury through alveolar stretching, oxidative stress, and microaspiration [128]. Regardless, sleep-disordered breathing is associated with a reduction in quality of life and increased risk of mortality [123].

Sleep evaluation and polysomnographic study should be considered for all patients with IPF given the high prevalence of sleep-disordered breathing. Following the diagnosis of sleep-disordered breathing, these patients are best managed by referral to experienced centers capable of implementing therapy effectively while combating ancillary clinical problems such as cough, claustrophobia, and insomnia. Obstructive sleep apnea should be treated with CPAP, as this improves quality of life and decreases mortality [129]. Similarly, nocturnal hypoxemia is easily corrected with supplemental oxygen therapy; however, no studies have confirmed an effect on long-term survival or on the development of pulmonary hypertension [128].

Conclusion

IPF is a heterogenous disease in every respect, including possibly the role and response to individual therapies. The advent of antifibrotic therapies has highlighted the need for more accurate disease phenotyping in IPF and other fibrotic disorders. While there have been laudable efforts to phenotype based on clinical, radiographic, and other readily available modalities, it appears that molecular phenotyping might be the key to a future of precision medicine in IPF and other fibrotic lung diseases. Indeed, it is conceivable that some medications might only work in very select groups of patients, while available therapies might be more suited to one patient group versus another. A hidden downside to the advent of effective antifibrotic therapy is that this might become the focal point of care at the expense of a more holistic approach that includes attention to various comorbidities. In the zest and quest to improve both the quality and quantity of the lives of IPF patients, simple measures to proactively look for and address the common comorbidities discussed above should not be overlooked.

References

1. King CS, Nathan SD. Idiopathic pulmonary fibrosis: effects and optimal management of comorbidities. Lancet Respir Med. 2017;5(1):72–84.
2. Raghu G, Collard HR, Egan JJ, et al. 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.
3. 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.
4. King TE Jr, Tooze JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med. 2001;164(7):1171–81.
5. Erbes R, Schaberg T, Loddenkemper R. Lung function tests in patients with idiopathic pulmonary fibrosis. Are they helpful for predicting outcome? Chest. 1997;111(1):51–7.
6. Han MK, Murray S, Fell CD, et al. Sex differences in physiological progression of idiopathic pulmonary fibrosis. Eur Respir J. 2008;31(6):1183–8.
7. Ley B, Ryerson CJ, Vittinghoff E, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. Ann Intern Med. 2012;156(10):684–91.
8. Ley B, Bradford WZ, Weycker D, Vittinghoff E, du Bois RM, Collard HR. Unified baseline and longitudinal mortality prediction in idiopathic pulmonary fibrosis. Eur Respir J. 2015;45(5):1374–81.
9. Wells AU, Desai SR, Rubens MB, 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.
10. du Bois RM, Weycker D, Albera C, et al. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184(4):459–66.
11. Costabel U, Inoue Y, Richeldi L, et al. Efficacy of nintedanib in idiopathic pulmonary fibrosis across prespecified subgroups in INPULSIS. Am J Respir Crit Care Med. 2016;193(2):178–85.
12. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone for idiopathic pulmonary fibrosis: analysis of pooled data from three multinational phase 3 trials. Eur Respir J. 2016;47(1):243–53.
13. Selman M, Carrillo G, Estrada A, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. PLoS One. 2007;2(5):e482.
14. Trujillo G, Meneghin A, Flaherty KR, et al. TLR9 differentiates rapidly from slowly progressing forms of idiopathic pulmonary fibrosis. Sci Transl Med. 2010;2(57):57ra82.
15. Brown AW, Shlobin OA, Weir N, et al. Dynamic patient counseling: a novel concept in idiopathic pulmonary fibrosis. Chest. 2012;142(4):1005–10.
16. Boon K, Bailey NW, Yang J, et al. Molecular phenotypes distinguish patients with relatively stable from progressive idiopathic pulmonary fibrosis (IPF). PLoS One. 2009;4(4):e5134.
17. Hogaboam CM, Trujillo G, Martinez FJ. Aberrant innate immune sensing leads to the rapid progression of idiopathic pulmonary fibrosis. Fibrogenesis Tissue Repair. 2012;5(Suppl 1):S3.
18. Oak SR, Murray L, Herath A, et al. A micro RNA processing defect in rapidly progressing idiopathic pulmonary fibrosis. PLoS One. 2011;6(6):e21253.
19. Lee JS, Song JW, Wolters PJ, et al. Bronchoalveolar lavage pepsin in acute exacerbation of idiopathic pulmonary fibrosis. Eur Respir J. 2012;39(2):352–8.
20. Konishi K, Gibson KF, Lindell KO, et al. Gene expression profiles of acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;180(2):167–75.
21. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J. 2011;37(2):356–63.
22. Collard HR, Moore BB, Flaherty KR, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2007;176(7):636–43.
23. Ohshimo S, Ishikawa N, Horimasu Y, et al. Baseline KL-6 predicts increased risk for acute exacerbation of idiopathic pulmonary fibrosis. Respir Med. 2014;108(7):1031–9.

24. Azuma A, Nukiwa T, Tsuboi E, et al. Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2005;171(9):1040–7.
25. King CS, Nathan SD. Practical considerations in the pharmacologic treatment of idiopathic pulmonary fibrosis. Curr Opin Pulm Med. 2015;21(5):479–89.
26. Gay SE, Kazerooni EA, Toews GB, et al. Idiopathic pulmonary fibrosis: predicting response to therapy and survival. Am J Respir Crit Care Med. 1998;157(4 Pt 1):1063–72.
27. Sumikawa H, Johkoh T, Colby TV, et al. Computed tomography findings in pathological usual interstitial pneumonia: relationship to survival. Am J Respir Crit Care Med. 2008;177(4):433–9.
28. Flaherty KR, Thwaite EL, Kazerooni EA, et al. Radiological versus histological diagnosis in UIP and NSIP: survival implications. Thorax. 2003;58(2):143–8.
29. Ryerson CJ, Urbania TH, Richeldi L, et al. Prevalence and prognosis of unclassifiable interstitial lung disease. Eur Respir J. 2013;42(3):750–7.
30. Raghu G, Wells AU, Nicholson AG, et al. Effect of Nintedanib in subgroups of idiopathic pulmonary fibrosis by diagnostic criteria. Am J Respir Crit Care Med. 2017;195(1):78–85.
31. Solomon JJ, Ryu JH, Tazelaar HD, et al. Fibrosing interstitial pneumonia predicts survival in patients with rheumatoid arthritis-associated interstitial lung disease (RA-ILD). Respir Med. 2013;107(8):1247–52.
32. https://clinicaltrials.gov/ct2/show/NCT02597933. Accessed 18 Aug 2017.
33. https://clinicaltrials.gov/ct2/show/NCT02999178. Accessed Aug 18 2017.
34. Lin H, Jiang S. Combined pulmonary fibrosis and emphysema (CPFE): an entity different from emphysema or pulmonary fibrosis alone. J Thorac Dis. 2015;7(4):767–79.
35. Lettieri CJ, Nathan SD, Barnett SD, Ahmad S, Shorr AF. Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest. 2006;129(3):746–52.
36. Shorr AF, Wainright JL, Cors CS, Lettieri CJ, Nathan SD. Pulmonary hypertension in patients with pulmonary fibrosis awaiting lung transplant. Eur Respir J. 2007;30(4):715–21.
37. Nadrous HF, Pellikka PA, Krowka MJ, et al. Pulmonary hypertension in patients with idiopathic pulmonary fibrosis. Chest. 2005;128(4):2393–9.
38. Hayes D Jr, Black SM, Tobias JD, Kirkby S, Mansour HM, Whitson BA. Influence of pulmonary hypertension on patients with idiopathic pulmonary fibrosis awaiting lung transplantation. Ann Thorac Surg. 2016;101(1):246–52.
39. Lawson WE, Loyd JE, Degryse AL. Genetics in pulmonary fibrosis – familial cases provide clues to the pathogenesis of idiopathic pulmonary fibrosis. Am J Med Sci. 2011;341(6):439–43.
40. Seibold MA, Wise AL, Speer MC, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. N Engl J Med. 2011;364(16):1503–12.
41. Peljto AL, Zhang Y, Fingerlin TE, et al. Association between the MUC5B promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. JAMA. 2013;309(21):2232–9.
42. Cronkhite JT, Xing C, Raghu G, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. Am J Respir Crit Care Med. 2008;178(7):729–37.
43. Zhou W, Wang Y. Candidate genes of idiopathic pulmonary fibrosis: current evidence and research. Appl Clin Genet. 2016;9:5–13.
44. Ravaglia C, Tomassetti S, Gurioli C, et al. Features and outcome of familial idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis. 2014;31(1):28–36.
45. Lee HL, Ryu JH, Wittmer MH, et al. Familial idiopathic pulmonary fibrosis: clinical features and outcome. Chest. 2005;127(6):2034–41.
46. Diaz de Leon A, Cronkhite JT, Katzenstein AL, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. PLoS One. 2010;5(5):e10680.
47. Daccord C, Maher TM. Recent advances in understanding idiopathic pulmonary fibrosis. F1000 Res. 2016;5.
48. https://clinicaltrials.gov/ct2/show/NCT01134822. Accessed 12 Sept 2017.
49. https://clinicaltrials.gov/ct2/show/NCT01071707. Accessed 12 Sept 2017.

50. Goodwin AT, Jenkins G. Molecular endotyping of pulmonary fibrosis. Chest. 2016;149(1):228–37.
51. Cottin V, Nunes H, Brillet PY, et al. Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. Eur Respir J. 2005;26(4):586–93.
52. Raghu G, Amatto VC, Behr J, Stowasser S. Comorbidities in idiopathic pulmonary fibrosis patients: a systematic literature review. Eur Respir J. 2015;46(4):1113–30.
53. Ryerson CJ, Hartman T, Elicker BM, et al. Clinical features and outcomes in combined pulmonary fibrosis and emphysema in idiopathic pulmonary fibrosis. Chest. 2013;144(1):234–40.
54. Mejia M, Carrillo G, Rojas-Serrano J, et al. Idiopathic pulmonary fibrosis and emphysema: decreased survival associated with severe pulmonary arterial hypertension. Chest. 2009;136(1):10–5.
55. Fukui M, Suzuki K, Matsunaga T, Oh S, Takamochi K. Outcomes of lung cancer resection for patients with combined pulmonary fibrosis and emphysema. Surg Today. 2016;46(3):341–7.
56. Jacob J, Bartholmai BJ, Rajagopalan S, et al. Functional and prognostic effects when emphysema complicates idiopathic pulmonary fibrosis. Eur Respir J. 2017;50(1).
57. Cottin V, Le Pavec J, Prevot G, et al. Pulmonary hypertension in patients with combined pulmonary fibrosis and emphysema syndrome. Eur Respir J. 2010;35(1):105–11.
58. Pfeifer M, Cottin V, Taniguchi H, et al. Effect of baseline emphysema on reduction in FVC decline with nintedanib in the INPULSIS trials. Pneumologie. 2015;69:P254.
59. Oltmanns U, Kahn N, Palmowski K, et al. Pirfenidone in idiopathic pulmonary fibrosis: real-life experience from a German tertiary referral center for interstitial lung diseases. Respiration. 2014;88(3):199–207.
60. Kimura M, Taniguchi H, Kondoh Y, et al. Pulmonary hypertension as a prognostic indicator at the initial evaluation in idiopathic pulmonary fibrosis. Respiration. 2013;85(6):456–63.
61. Behr J, Kreuter M, Hoeper MM, et al. Management of patients with idiopathic pulmonary fibrosis in clinical practice: the INSIGHTS-IPF registry. Eur Respir J. 2015;46(1):186–96.
62. Raghu G, Nathan SD, Behr J, et al. Pulmonary hypertension in idiopathic pulmonary fibrosis with mild-to-moderate restriction. Eur Respir J. 2015;46(5):1370–7.
63. Corte TJ, Wort SJ, Wells AU. Pulmonary hypertension in idiopathic pulmonary fibrosis: a review. Sarcoidosis Vasc Diffuse Lung Dis. 2009;26(1):7–19.
64. Galie N, Humbert M, Vachiery JL, et al. 2015 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. Rev Esp Cardiol. 2016;69(2):177.
65. Seeger W, Adir Y, Barbera JA, et al. Pulmonary hypertension in chronic lung diseases. J Am Coll Cardiol. 2013;62(25 Suppl):D109–16.
66. Shin S, King CS, Puri N, et al. Pulmonary artery size as a predictor of outcomes in idiopathic pulmonary fibrosis. Eur Respir J. 2016;47(5):1445–51.
67. Arcasoy SM, Christie JD, Ferrari VA, et al. Echocardiographic assessment of pulmonary hypertension in patients with advanced lung disease. Am J Respir Crit Care Med. 2003;167(5):735–40.
68. Nathan SD, King CS. Treatment of pulmonary hypertension in idiopathic pulmonary fibrosis: shortfall in efficacy or trial design? Drug Des Devel Ther. 2014;8:875–85.
69. Sherner J, Collen J, King C, Nathan S. Pulmonary hypertension in idiopathic pulmonary fibrosis: epidemiology, diagnosis, and therapeutic implications. Curr Respir Care Rep. 2012;1:233–42.
70. Patel NM, Lederer DJ, Borczuk AC, Kawut SM. Pulmonary hypertension in idiopathic pulmonary fibrosis. Chest. 2007;132(3):998–1006.
71. https://clinicaltrials.gov/ct2/show/NCT02630316. Accessed 12 Sept 2017.
72. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014–an update from the pulmonary transplantation council of the international society for heart and lung transplantation. J Heart Lung Transplant. 2015;34(1):1–15.
73. Pearson JE, Wilson RS. Diffuse pulmonary fibrosis and hiatus hernia. Thorax. 1971;26(3):300–5.
74. Savarino E, Carbone R, Marabotto E, et al. Gastro-oesophageal reflux and gastric aspiration in idiopathic pulmonary fibrosis patients. Eur Respir J. 2013;42(5):1322–31.

75. Tobin RW, Pope CE 2nd, Pellegrini CA, Emond MJ, Sillery J, Raghu G. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998;158(6):1804–8.
76. Moran TJ. Experimental aspiration pneumonia. IV. Inflammatory and reparative changes produced by intratracheal injections of autologous gastric juice and hydrochloric acid. AMA Arch Pathol. 1955;60(2):122–9.
77. Sladen A, Zanca P, Hadnott WH. Aspiration pneumonitis – the sequelae. Chest. 1971;59(4):448–50.
78. Lee JS, Collard HR, Raghu G, et al. Does chronic microaspiration cause idiopathic pulmonary fibrosis? Am J Med. 2010;123(4):304–11.
79. Gavini S, Borges LF, Finn RT, et al. Lung disease severity in idiopathic pulmonary fibrosis is more strongly associated with impedance measures of bolus reflux than pH parameters of acid reflux alone. Neurogastroenterol Motil. 2017;29(5).
80. Lee JS, Collard HR, Anstrom KJ, et al. Anti-acid treatment and disease progression in idiopathic pulmonary fibrosis: an analysis of data from three randomised controlled trials. Lancet Respir Med. 2013;1(5):369–76.
81. Kreuter M, Wuyts W, Renzoni E, et al. Antacid therapy and disease outcomes in idiopathic pulmonary fibrosis: a pooled analysis. Lancet Respir Med. 2016;4(5):381–9.
82. Raghu G, Rochwerg B, Zhang Y, et al. An official ATS/ERS/JRS/ALAT clinical practice guideline: treatment of idiopathic pulmonary fibrosis. An update of the 2011 clinical practice guideline. Am J Respir Crit Care Med. 2015;192(2):e3–19.
83. Hubbard RB, Smith C, Le Jeune I, Gribbin J, Fogarty AW. The association between idiopathic pulmonary fibrosis and vascular disease: a population-based study. Am J Respir Crit Care Med. 2008;178(12):1257–61.
84. Sprunger DB, Olson AL, Huie TJ, et al. Pulmonary fibrosis is associated with an elevated risk of thromboembolic disease. Eur Respir J. 2012;39(1):125–32.
85. Panos RJ, Mortenson RL, Niccoli SA, King TE Jr. Clinical deterioration in patients with idiopathic pulmonary fibrosis: causes and assessment. Am J Med. 1990;88(4):396–404.
86. Olson AL, Swigris JJ, Lezotte DC, Norris JM, Wilson CG, Brown KK. Mortality from pulmonary fibrosis increased in the United States from 1992 to 2003. Am J Respir Crit Care Med. 2007;176(3):277–84.
87. Dalleywater W, Powell HA, Fogarty AW, Hubbard RB, Navaratnam V. Venous thromboembolism in people with idiopathic pulmonary fibrosis: a population-based study. Eur Respir J. 2014;44(6):1714–5.
88. Collard HR, Ward AJ, Lanes S, Cortney Hayflinger D, Rosenberg DM, Hunsche E. Burden of illness in idiopathic pulmonary fibrosis. J Med Econ. 2012;15(5):829–35.
89. Navaratnam V, Fogarty AW, McKeever T, et al. Presence of a prothrombotic state in people with idiopathic pulmonary fibrosis: a population-based case-control study. Thorax. 2014;69(3):207–15.
90. Leuschner G, Wenter V, Milger K, et al. Suspected pulmonary embolism in patients with pulmonary fibrosis: discordance between ventilation/perfusion SPECT and CT pulmonary angiography. Respirology. 2016;21(6):1081–7.
91. Noth I, Anstrom KJ, Calvert SB, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186(1):88–95.
92. Kreuter M, Wijsenbeek MS, Vasakova M, et al. Unfavourable effects of medically indicated oral anticoagulants on survival in idiopathic pulmonary fibrosis: methodological concerns. Eur Respir J. 2016;48(5):1524–6.
93. Dalleywater W, Powell HA, Hubbard RB, Navaratnam V. Risk factors for cardiovascular disease in people with idiopathic pulmonary fibrosis: a population-based study. Chest. 2015;147(1):150–6.
94. King TE Jr, Albera C, Bradford WZ, et al. All-cause mortality rate in patients with idiopathic pulmonary fibrosis. Implications for the design and execution of clinical trials. Am J Respir Crit Care Med. 2014;189(7):825–31.

95. Kim WY, Mok Y, Kim GW, et al. Association between idiopathic pulmonary fibrosis and coronary artery disease: a case-control study and cohort analysis. Sarcoidosis Vasc Diffuse Lung Dis. 2015;31(4):289–96.
96. Nathan SD, Basavaraj A, Reichner C, et al. Prevalence and impact of coronary artery disease in idiopathic pulmonary fibrosis. Respir Med. 2010;104(7):1035–41.
97. Zisman DA, Kawut SM. Idiopathic pulmonary fibrosis: a shot through the heart? Am J Respir Crit Care Med. 2008;178(12):1192–3.
98. Gribbin J, Hubbard R, Smith C. Role of diabetes mellitus and gastro-oesophageal reflux in the aetiology of idiopathic pulmonary fibrosis. Respir Med. 2009;103(6):927–31.
99. Sherbini N, Feteih MN, Wali SO, Alamoudi OS, Al-Faifi SM, Khalid I. Idiopathic pulmonary fibrosis in Saudi Arabia: demographic, clinical, and survival data from two tertiary care hospitals. Ann Thorac Med. 2014;9(3):168–72.
100. Hyldgaard C, Hilberg O, Bendstrup E. How does comorbidity influence survival in idiopathic pulmonary fibrosis? Respir Med. 2014;108(4):647–53.
101. He J, Huang Z, Yu X, et al. Diabetes mellitus in idiopathic pulmonary fibrosis: a systematic review and meta-analysis. Int J Clin Exp Med. 2016;9(9):17727–38.
102. Kim YJ, Park JW, Kyung SY, et al. Clinical characteristics of idiopathic pulmonary fibrosis patients with diabetes mellitus: the national survey in Korea from 2003 to 2007. J Korean Med Sci. 2012;27(7):756–60.
103. Sato N, Takasaka N, Yoshida M, et al. Metformin attenuates lung fibrosis development via NOX4 suppression. Respir Res. 2016;17(1):107.
104. Glaspole IN, Chapman SA, Cooper WA, et al. Health-related quality of life in idiopathic pulmonary fibrosis: data from the Australian IPF registry. Respirology. 2017;22(5):950–6.
105. Matsuda T, Taniguchi H, Ando M, et al. Depression is significantly associated with the health status in patients with idiopathic pulmonary fibrosis. Intern Med. 2017;56(13):1637–44.
106. Akhtar AA, Ali MA, Smith RP. Depression in patients with idiopathic pulmonary fibrosis. Chron Respir Dis. 2013;10(3):127–33.
107. Ryerson CJ, Arean PA, Berkeley J, et al. Depression is a common and chronic comorbidity in patients with interstitial lung disease. Respirology. 2012;17(3):525–32.
108. Ryerson CJ, Cayou C, Topp F, et al. Pulmonary rehabilitation improves long-term outcomes in interstitial lung disease: a prospective cohort study. Respir Med. 2014;108(1):203–10.
109. Lindell KO, Liang Z, Hoffman LA, et al. Palliative care and location of death in decedents with idiopathic pulmonary fibrosis. Chest. 2015;147(2):423–9.
110. Hubbard R, Venn A, Lewis S, Britton J. Lung cancer and cryptogenic fibrosing alveolitis. A population-based cohort study. Am J Respir Crit Care Med. 2000;161(1):5–8.
111. Antoniou KM, Tomassetti S, Tsitoura E, Vancheri C. Idiopathic pulmonary fibrosis and lung cancer: a clinical and pathogenesis update. Curr Opin Pulm Med. 2015;21(6):626–33.
112. Kishi K, Homma S, Kurosaki A, Motoi N, Yoshimura K. High-resolution computed tomography findings of lung cancer associated with idiopathic pulmonary fibrosis. J Comput Assist Tomogr. 2006;30(1):95–9.
113. Fulton BG, Ryerson CJ. Managing comorbidities in idiopathic pulmonary fibrosis. Int J Gen Med. 2015;8:309–18.
114. Tomassetti S, Gurioli C, Ryu JH, et al. The impact of lung cancer on survival of idiopathic pulmonary fibrosis. Chest. 2015;147(1):157–64.
115. Kumar P, Goldstraw P, Yamada K, et al. Pulmonary fibrosis and lung cancer: risk and benefit analysis of pulmonary resection. J Thorac Cardiovasc Surg. 2003;125(6):1321–7.
116. Kudoh S, Kato H, Nishiwaki Y, et al. Interstitial lung disease in Japanese patients with lung cancer: a cohort and nested case-control study. Am J Respir Crit Care Med. 2008;177(12):1348–57.
117. Miura Y, Saito T, Tsunoda Y, et al. Clinical effect of pirfenidone on incidence of lung carcinoma in chronic interstitial pneumonia. ERJ. 2014;44:P3497.
118. Gottfried M, Bennouna J, Bondarenko I, et al. Efficacy and safety of nintedanib plus docetaxel in patients with advanced lung adenocarcinoma: complementary and exploratory analyses of the phase III LUME-lung 1 study. Target Oncol. 2017;12(4):475–85.

119. Caminati A, Bianchi A, Cassandro R, Mirenda MR, Harari S. Walking distance on 6-MWT is a prognostic factor in idiopathic pulmonary fibrosis. Respir Med. 2009;103(1):117–23.
120. Lederer DJ, Arcasoy SM, Wilt JS, D'Ovidio F, Sonett JR, Kawut SM. Six-minute-walk distance predicts waiting list survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006;174(6):659–64.
121. Holland AE. Functional capacity in idiopathic pulmonary fibrosis: looking beyond the lungs. Respirology. 2015;20(6):857–8.
122. Kelm DJ, Bonnes SL, Jensen MD, et al. Pre-transplant wasting (as measured by muscle index) is a novel prognostic indicator in lung transplantation. Clin Transpl. 2016;30(3):247–55.
123. Milioli G, Bosi M, Poletti V, et al. Sleep and respiratory sleep disorders in idiopathic pulmonary fibrosis. Sleep Med Rev. 2016;26:57–63.
124. Pihtili A, Bingol Z, Kiyan E, Cuhadaroglu C, Issever H, Gulbaran Z. Obstructive sleep apnea is common in patients with interstitial lung disease. Sleep Breath = Schlaf Atmung. 2013;17(4):1281–8.
125. Mermigkis C, Stagaki E, Tryfon S, et al. How common is sleep-disordered breathing in patients with idiopathic pulmonary fibrosis? Sleep Breath = Schlaf Atmung. 2010;14(4):387–90.
126. Lancaster LH, Mason WR, Parnell JA, et al. Obstructive sleep apnea is common in idiopathic pulmonary fibrosis. Chest. 2009;136(3):772–8.
127. Troy LK, Corte TJ. Sleep disordered breathing in interstitial lung disease: a review. World J Clin Cases. 2014;2(12):828–34.
128. Schiza S, Mermigkis C, Margaritopoulos GA, et al. Idiopathic pulmonary fibrosis and sleep disorders: no longer strangers in the night. Eur Respir Rev. 2015;24(136):327–39.
129. Mermigkis C, Bouloukaki I, Antoniou K, et al. Obstructive sleep apnea should be treated in patients with idiopathic pulmonary fibrosis. Sleep Breath = Schlaf Atmung. 2015;19(1):385–91.
130. Kreuter M, Ehlers-Tenenbaum S, Palmowski K, Bruhwyler J, Oltmanns U, et al. Impact of comorbidities on mortality in patients with idiopathic pulmonary fibrosis. PLOS ONE. 2016;11(3):e0151425. https://doi.org/10.1371/journal.pone.0151425. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0151425.

Chapter 12
The Keys to Making a Confident Diagnosis of IPF

Jamie Sheth, Anish Wadhwa, and Kevin R. Flaherty

Introduction

Diffuse parenchymal lung diseases (DPLDs) are characterized by injury primarily to the interstitium of the lung but may involve alveolar spaces, airways and blood vessels [1]. Many DPLDs are idiopathic (referred to as idiopathic interstitial pneumonias, or IIPs), but DPLD can develop secondary to other factors including connective tissue disease (CTD), environmental exposures, drugs/toxins, etc [1]. The major IIPs include idiopathic pulmonary fibrosis (IPF), idiopathic nonspecific interstitial pneumonia (NSIP), respiratory bronchiolitis interstitial lung disease (RB-ILD), desquamative interstitial pneumonia (DIP), cryptogenic organizing pneumonia (COP), and acute interstitial pneumonia (AIP) [1, 2]. There is significant overlap in the clinical features of the IIPs including chronic dyspnea, interstitial changes on imaging studies, reduction in lung volumes, and impairment in diffusion capacity (DLCO) [1]. Distinct radiographic and histopathological features can distinguish among the clinical entities, and establishing an accurate diagnosis is critical to determining treatment and understanding prognosis [3, 4]. Of the over 150 recognized types of DPLDs, IPF is the most common and has the worst prognosis [3, 4]. IPF is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown etiology that occurs primarily in older adults, is limited to the lungs and is associated with a histopathologic and/or radiologic pattern of usual interstitial pneumonia (UIP) [5]. As outlined in the ATS/ERS 2011 consensus statement, the diagnosis requires the exclusion of known causes of DPLD and the

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presence of a UIP pattern on high-resolution computed tomography (HRCT) or surgical lung biopsy (SLB) [5]. The incidence and prevalence of IPF increase with age, and the diagnosis should be considered in older adult patients who present with nonproductive cough, dyspnea or bibasilar crackles [6–8]. This chapter reviews the key historical, physiologic, radiographic and histopathological features that are key to establishing a confident diagnosis of IPF.

Clinical Presentation, Disease Course, and Phenotypes

Signs and Symptoms

The clinical features of IPF are nonspecific. Most patients complain of a dry cough and dyspnea. These symptoms are sometimes attributed to comorbid conditions such as cardiac disease, infections, normal aging, or deconditioning, which can lead to a delay in diagnosis. A high index of suspicion is required to avoid missing a diagnosis of DPLD, including IPF. The most characteristic physical exam finding of IPF is bibasilar crackles on chest auscultation, and digital clubbing may be present but is nonspecific.

The duration of symptoms prior to presentation may offer insight in a patient without an obvious proximate cause for dyspnea. The classical chronic and insidious presentation of interstitial lung diseases (ILD) contrasts with the acute/subacute development and progression of particular diagnoses, including acute interstitial pneumonia (AIP), acute eosinophilic pneumonia, cryptogenic organizing pneumonia (COP), drug-induced lung diseases, and CTD-related ILD (CTD-ILD). Sarcoidosis and hypersensitivity pneumonitis (HP) may present in an acute, subacute, or chronic fashion. Chronic HP has overlapping radiologic features with IPF, and HP must always be considered if the high-resolution computed tomography (HRCT) is not definitive for the pathologic correlate of IPF, which is usual interstitial pneumonia (UIP). Evaluation for chronic HP is discussed later.

Age and gender may also help to distinguish among DPLDs,as certain diagnoses may be more common to particular age groups or have a male or female predominance. The prevalence of IPF has been estimated to range from 0.8 (age, 18–34) to 64.7 (age ≥75) per 100,000 and is generally higher among men than women [6]. The index of suspicion for a diagnosis of CTD-ILD should be higher in younger patients (less than age 50 years), especially in women. In contrast to other IIPs, most patients with IPF are older than age 50 years at the time of diagnosis [9]. The incidence and prevalence of IPF increase with each decade of life, and two-thirds of patients are over age 60 at the time of presentation [10]. Increasing age has been shown to be a powerful predictor of IPF, particularly in patients with mild radiographic disease, as shown in Table 12.1 [8]. Subsequent evaluation has shown that in the absence of honeycombing, patients age 60 years or older with reticular densi-

Table 12.1 Positive predictive value, specificity, sensitivity, and negative predictive value when classifying patients with IPF who are at least as old as the age specified

Age	PPV	Specificity	Sensitivity	NPV
30	72	0	100	N/A
40	74	11	98	67
50	78	34	92	62
60	87	89	61	43
70	95	97	21	32
80	100	100	1	28

Classification of patients with idiopathic pulmonary fibrosis based on age, positive predictive value (PPV), specificity, sensitivity, and negative predictive value (NPV). PPV and specificity increase in older patients. Data expressed as percentages

ties occupying at least one-third of lung volume, the probability of IPF exceeds 80%, with a specificity for IPF diagnosis of 96% [11].

Although IPF is by definition idiopathic, there are several potential risk factors that have been implicated. Tobacco use is strongly associated with the development of both sporadic and familial IPF, particularly for individuals with a smoking history of more than 20 pack-years [12–14], and smoking cessation may be the most modifiable risk factor [15]. Gastroesophageal reflux disease (GERD) is frequent in patients with IPF [16, 17] and may contribute to lung injury via microaspiration [18, 19]. It is also seen in pulmonary fibrosis associated with scleroderma [20]. Environmental exposure to stone, metal, wood, and inorganic dust, as well as occupations such as hairdressing and farm working, has also been associated with the development of IPF [21–23]. The clinical evaluation is critical to look for historical features or exam findings that may suggest an exposure or systemic illness leading to the development of a DPLD.

Genetic factors, particularly in familial and sporadic forms of IPF, may also be considered, especially when considering the likelihood of disease progression. Genetic analyses evaluating differential expression of genes have identified unique patterns that suggest there may be different phenotypes of IPF, and an evolving understanding of these holds promise in identifying subgroups of patients who are likely to have differing clinical courses. For example, data suggest that predominantly male smokers with less than 6 months of symptoms before their first presentation may be "rapid" progressors and show an upregulation of genes involved in cell motility, myofibroblast differentiation, coagulation, oxidative stress, and development [24]. These patients differ from those with greater than 24 months of symptoms prior to presentation and have been termed "slow" progressors [24]. While genetic studies in familial and sporadic pulmonary fibrosis have provided useful insights into the pathogenesis of IPF, these investigations require further validation. Therefore, genetic testing is not currently recommended as part of a clinical evaluation [5].

Clinical Course

The importance of a diagnosis of IPF as related to disease progression and poor prognosis is well established [3, 4]. Furthermore, therapy for IPF is different than for other IIPs, with recent data showing a positive impact with use of anti-fibrotic agents (pirfenidone and nintedanib), while anti-inflammatory therapy worsened outcomes [25–30]. IPF is characterized by a progressive decline in pulmonary function until death. Data suggest that 40–60% of patients will die from a respiratory cause with comorbid coronary artery disease or infections comprising the most common other proximate causes of mortality [31, 32]. The time and path of progression from asymptomatic to symptomatic IPF is variable. Patients may demonstrate a slow progression over years, a rapid progression in months, or episodes of sudden deterioration in their condition during a period of relative stability that are often called "exacerbations."

The potential clinical courses in IPF are depicted in Fig. 12.1 [33]. The rate of decline and progression to death may present in several clinical forms: subclinical IPF, where disease precedes symptoms; slowly progressive IPF, where there is a gradual physiologic decline and increasing exertional dyspnea; and rapidly progressive IPF, characterized by an acute decline from time of presentation with progression to death. This latter variant may also manifest with phases of stability alternating with periods of acute decline (so-called acute exacerbations of IPF (AE-IPF)), and such episodes often necessitate frequent hospitalizations for respiratory failure [33].

Accelerated worsening of IPF may occur at any time during the disease course and may be the initial manifestation of disease in some patients. The etiology of decompensation may be related to pulmonary embolism, infection, congestive heart failure, pneumothorax, or drugs. When a cause cannot be determined, the worsening is typically called an AE-IPF. Although Epstein-Barr virus has been identified with high prevalence in the lung tissue of patients with IPF and the general population [34], definitive conclusions about the contribution of acute infection in disease causation/progression cannot be made. Moreover, studies evaluating gene expression profiles in explanted tissue samples from patients with AE-IPF suggest that a marked inflammatory response, secondary to infection or otherwise, is less likely to contribute to the phenotype [35]. The true incidence of AE-IPF is not known. Clinical trials over the past decade have reported variable incidence rates, likely related to differences in study population (such as disease severity), definition of acute exacerbation used, follow-up time, and statistical methodology [36]. A recent meta-analysis of six clinical trials in patients with IPF revealed a weighted average of 4.1 acute exacerbations per 100 patient-years [37]. Microaspiration of gastric contents is a proposed risk factor for exacerbations as well as progression of IPF [38]. Subclinical IPF may be a risk factor for the development of an acute exacerbation, especially after surgery or invasive procedures [39]. Thoracic surgery, including lung resection or surgical lung biopsy (SLB), can precipitate an AE-IPF, often in the non-biopsied side and perhaps secondary to barotrauma incurred during the single-lung ventilation process [40, 41]. Patients with lower FVC have been shown to have more total and respira-

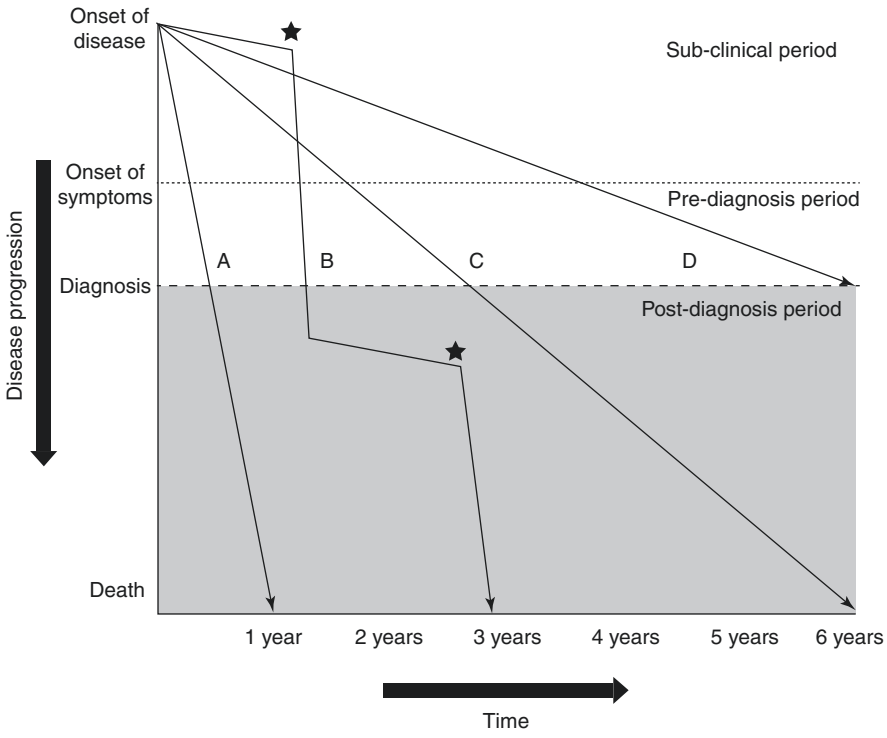

Fig. 12.1 Potential clinical courses of idiopathic pulmonary fibrosis (IPF). During the subclinical period, only radiographic findings of disease may be present. The rate of decline may be accelerated in some (line A), although the majority of patients experience a gradual progressive worsening of their disease (lines C and D). The rate of decline may have periods of relative stability interposed with periods of rapid progression of disease (line B, stars). (Reprinted with permission of the American Thoracic Society. Copyright © 2018 American Thoracic Society. Taken with permission from Ley et al. [33]. The *American Journal of Respiratory and Critical Care Medicine* is an official journal of the American Thoracic Society)

tory hospitalizations during subsequent follow-up [42, 43], while physiologic factors of advanced IPF, such as lower FVC, diffusion capacity, and 6-min walk distance, are associated with increased rates of AE-IPF [40, 44, 45]. Although the numbers of fibroblastic foci seen on biopsy are associated with poor survival [46–49], they cannot predict the development of acute exacerbations of IPF/UIP [48].

Combined Pulmonary Fibrosis and Emphysema (CPFE)

Smoking increases the risk of developing IPF [14]. Patients with combined emphysema and pulmonary fibrosis are recognized as a unique clinical phenotype of IPF, but not a distinct form of IIP [2, 50]. Patients with both emphysema and interstitial

disease often present with severe dyspnea, preserved lung volumes and marked reduction in DL_{CO}, and radiographic evidence of lower-lobe predominant pulmonary fibrosis and upper-lobe predominant emphysema [51, 52]. In a series of 110 patients with IPF, 28% of patients were found to have at least 10% of the lung affected with emphysema and thus were considered to have CPFE [52]. The risk of development of pulmonary hypertension (PH) is notably higher in patients with IPF and concomitant emphysema, as demonstrated by echocardiographic estimates of systolic pulmonary artery pressures (PAPs) [52]. Survival is worse for patients with CPFE versus those with IPF alone [52], and survival is even worse if these patients develop PH; 5-year survival may be as low as 25% for patients with PH on echocardiogram versus 75% for patients without PH [53].

Since lung volumes are relatively preserved in patients with CPFE [53], unlike in IPF patients without emphysema, serial measurement of lung volumes to monitor disease course may not be relevant in these patients. Instead, changes in FEV_1 or echocardiographic evidence for PH could be more appropriate surrogates for progression of disease and prediction of mortality [53, 54]. DL_{CO} may not reliably predict mortality [54]. Of note, clinical prediction tools like the clinical-radiographic-physiologic (CRP) score do not consider the presence and severity of emphysema and thus have limited utility in assessment of patients with CPFE [55]. A separate clinical tool, the composite physiologic index (CPI), predicts mortality more accurately than individual pulmonary function tests (PFTs) alone in patients with CPFE. However, it is not helpful in establishing a diagnosis and is less useful than the forced expiratory volume in 1 s (FEV_1) in predicting mortality when the disease progresses [54, 56].

Pulmonary Fibrosis with Pulmonary Hypertension

Patients with IPF may present with pulmonary hypertension (PH) that is disproportionate to the severity of underlying lung disease [57]. The prevalence of PH in a series of patients with DPLD may range from 14% to 41% [58], and most studies have used the NIH definition of pulmonary artery hypertension as a mean pulmonary artery pressure (PAP) of >25 mmHg at rest with normal pulmonary capillary wedge pressure [59, 60]. Estimates of severity and decisions regarding treatment of PH should not be based on echocardiography as this modality may overestimate the degree of PH when compared to right heart catheterization [61]. The incidence and severity of PH tends to correlate with the need for supplemental oxygen and decrements in DL_{CO} [60, 62], and PH is associated with increased risk of subsequent mortality [62–64]. Of note, a prospective analysis of IPF patients undergoing initial workup with RHC and PFTs identified a mean PAP of 17 mmHg as an appropriate threshold value to discriminate 5-year mortality [65].

The subset of patients with IPF who develop PH at earlier stages of disease may have disproportionate PH due to molecular mediators that are common to PH and IPF. There may be increases in 5-lipoxygenase (LO), transforming growth factor-β (TGF-β), and endothelin-1 (ET-1) but decreases in prostaglandin-E_2 (PGE_2) [66–68].

An altered balance between angiogenesis and angiostasis, as well as intermittent hypoxia (especially during sleep and exercise), may also contribute [57]. Pulmonary vascular remodeling associated with chronic alveolar hypoxia may be a consequence of "desensitization" to hypoxia (as seen in patients with nocturnal hypoventilation syndromes [69]) and thus make patients more vulnerable to daytime and exercise-induced hypoxia [57].

Physiologic Evaluation

Reduced lung volumes and impairment in DL_{CO} are common to all the IIPs, although normal PFTs cannot exclude a diagnosis of IPF [70], and discrimination of IPF versus other IIPs by use of PFTs alone is limited by a lack of specificity. Typical physiologic changes include an increase in elastic recoil and decrease in lung compliance that leads to reduction in vital capacity (VC) and total lung capacity (TLC) [71], while the functional residual capacity remains either normal or mildly reduced in comparison [72]. Preserved residual volume (RV) may be secondary to honeycombing or cystic air spaces that contribute to the TLC or may represent an increase in dead space ventilation [71, 72]. Both FEV_1 and forced vital capacity (FVC) may be reduced if lung volumes are reduced, but usually the FEV_1/FVC ratio is normal or elevated [71, 72]. As noted previously, concomitant emphysema and IPF can render measurements of lung volumes less reliable as hyperinflation and increased compliance can lead to pseudo-normalization of the VC and TLC [51, 53].

Hypoxemia is thought to be secondary to various mechanisms, including ventilation-perfusion mismatch, impaired diffusion secondary to abnormality of the alveolar-capillary membrane, and right-to-left shunting (from intracardiac/intrapulmonary shunting or elevated PAPs) [71]. Increased dead space ventilation likely accounts for the characteristic changes noted during exercise assessment, including increased minute ventilation (V_E) at rest and increased V_E as oxygen consumption (VO_2) increases [71]. A reduction in DL_{CO} may manifest with resting or exercise-induced hypoxemia, a reduced partial pressure of oxygen in arterial blood (PaO_2), or an elevated alveolar-arterial gradient ($P(A\text{-}a)O_2$) [72]. A decreased DL_{CO} below 40% predicts a subsequent risk of increased mortality [73]. Similarly, desaturation to less than 88% during a 6-min walk test [74, 75], low thresholds of maximal oxygen uptake during exercise [76], and declines in lung function over time also correlate with increased risk of mortality [7, 74, 75, 77–79].

The classical phenotype of IPF, where there is progressive decline in lung function and increasing dyspnea with death often within 5 years of diagnosis, demonstrates a mean rate of decline in FVC of 150–200 mL/year [33]. The variability in rate of progression of IPF is well established, and identification of baseline and short-term serial predictors of survival is critical to the accurate characterization of disease progression and consideration of appropriate interventions. In addition to providing evidence that a restrictive and probable interstitial pulmonary process is present, physiologic studies can aid in establishing baseline and longitudinal prognoses [73, 77, 80–82].

Radiographic Evaluation

Utilization of HRCT has become a key aspect in the evaluation of patients with a suspected IIP. Although typical radiographic changes are usually noted in established disease, normal radiology does not exclude the presence of IPF [83]. The recent ATS/ERS consensus statement clearly outlines the HRCT characteristics associated with a diagnosis of IPF: subpleural reticulation with a basal predominance and honeycombing without associated extensive ground-glass abnormality, micronodules, discrete cysts, mosaic attenuation/air trapping, or consolidation [5]. Honeycombing on HRCT, which is critical for establishing a definitive HRCT diagnosis of IPF, manifests as clustered cystic spaces varying between 3 and 25 mm in diameter, usually with well-defined walls [84]. Honeycomb lung may be preceded by the presence of patchy ground-glass opacities and reticulations within a secondary lobule [85]. Importantly, HRCT features of ground glass, fibrosis, and honeycombing correlate with measurements of FVC and DL_{CO} and pathologic fibrosis [86, 87]. Although HRCT can be used to make a definitive diagnosis by revealing a pattern of UIP, it cannot do the same for nonspecific interstitial pneumonia (NSIP). In current practice, the presence of honeycombing is considered specific for UIP. Thus per recent Fleischner Society recommendations, the term should be used with caution given the potential impact to care [84] (Fig. 12.2).

HRCT findings of UIP and other abnormalities (e.g., plaques, calcifications, and pleural effusions) should prompt consideration of alternative etiologies for the UIP pattern [5]. The list of alternative diagnoses to consider includes CTD-ILD, chronic HP, and certain pneumoconioses, particularly asbestosis [5]. With the exception of honeycomb changes, many of the characteristic features of UIP overlap with HRCT features of NSIP, as listed in Table 12.2 [88]. Examples of HRCT images showing

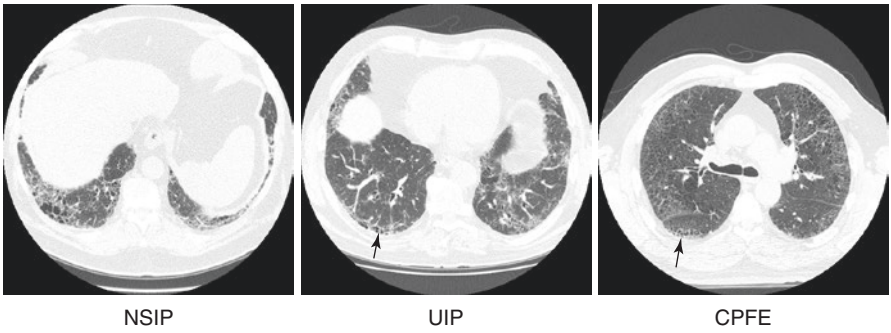

Fig. 12.2 HRCT images from three different patients with ILD. Areas of honeycombing are indicated by black arrows. (1) *Left*: Peripheral and lower-lung predominant interstitial disease without honeycombing in a patient with radiographic diagnosis of NSIP, although surgical lung biopsy was consistent with UIP. (2) *Middle*: There are areas of lower-lobe predominant septal thickening, traction bronchiectasis, and honeycombing that are consistent with UIP. (3) *Right*: Upper-lobe emphysema changes and lower-lobe interstitial changes compatible with UIP in a patient with CPFE. (Figures courtesy of Kevin R. Flaherty MD, MS. University of Michigan)

Table 12.2 Characteristic radiographic findings in NSIPoverlap with typical findings in UIP

Radiologic finding	Number (%)
Lower-lobe distribution	56 (92)
Diffuse (axial) distribution	29 (47)
Peripheral (axial) distribution	28 (46)
Reticulation	53 (87)
Traction bronchiectasis	50 (82)
Lobar volume loss	47 (77)
Ground-glass attenuation	27 (44)
Subpleural sparing	13 (21)
Substantial micronodules	2 (3)
Honeycombing	3 (5)

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Diffuse bilateral reticular opacities that are mostly lower-lobe predominant, associated traction bronchiectasis and lobar volume loss, and relative sparing of the subpleural space in approximately 20% of patients are common findings. Data expressed as percentages in a series of 61 cases

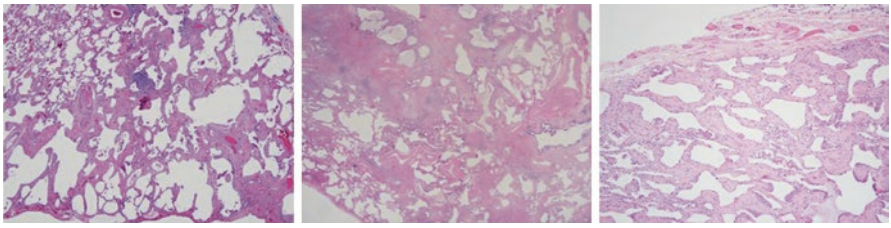

Fig. 12.3 Histologic images of SRIF, UIP, and NSIP. Original magnification 40×, hematoxylin and eosin staining. (1) *Left*: A typical SRIF has more emphysema changes with collagen deposition around airways and evidence of macrophages in airways (consistent with respiratory bronchiolitis). (2) *Middle*: A pathologic diagnosis of UIP requires identification of normal areas of lung interspersed with fibroblastic foci and honeycomb changes. (3) *Right*: A NSIP pattern has interstitial inflammation that is diffuse without evidence of honeycombing and scant evidence of fibroblastic foci. (Images courtesy of Lindsay Schmidt M.D. University of Michigan)

NSIP, UIP, and CPFE patterns are shown in Fig. 12.3. Patients with suspected NSIP by HRCT require a surgical lung biopsy (SLB) for confirmation; many of these patients will have a histopathological pattern of UIP [89].

Concurrence between radiologists regarding the presence of honeycomb lung may be inconsistent, as demonstrated in a study of 314 patients where inter-observer agreement for the presence of honeycomb lung ranged from 0.21 to 0.31 [87]. The presence of emphysema and cystic spaces can make the diagnostic process more challenging, especially in the presence of overlapping ground-glass opacities [90], possibly leading to misdiagnoses. Development of chronic interstitial pneumonia in

emphysematous lung or patterns of NSIP and DIP that demonstrate predominantly ground-glass opacities and have a honeycomb appearance (especially if involving areas of emphysematous lung) may also be misdiagnosed as UIP [85]. Emphysema and interstitial fibrosis can develop and progress simultaneously in the same lung area and lead to honeycomb changes, which may also contribute to misdiagnoses [91]. Paraseptal emphysema, which has definite walls and is often located subpleurally in clusters, may be accompanied by fibrosis in its walls, and when such changes occur in upper and middle lobes and are coexistent with typical honeycomb changes in the lower lobes, distinguishing the disease entities could conceivably be more difficult.

Bronchoscopy, Surgical Lung Biopsy, and Histopathology

Bronchoscopy

The 2000 ATS/ERS consensus statement regarding diagnosis and treatment of IPF included the use of transbronchial lung biopsy (TBLB) or bronchoalveolar lavage (BAL) to identifyany features that could support an alternative to IPF as a criterion for making an IPF diagnosis in patients who do not undergo SLB [92]. These criteria were not included in the 2011 statement, although bronchoscopy should still be considered when non-IPF diagnoses are in the differential [5]. Bronchoscopy can be useful for the diagnosis of sarcoidosis, infections, malignancy, and potentially HP; a BAL cell count of more than 30% lymphocytes has been suggested as predictive of HP [5, 93].

Conventional wisdom suggests that the amount of tissue obtained by TBLB is inadequate to make a diagnosis of UIP, although recent data suggest that characteristic histologic features of UIP can be identified on TBLB pathology more frequently than previously appreciated. In a series of 22 patients with UIP, 7 of 18 TBLBs were adequate specimens that contained features diagnostic of UIP (i.e., a patchwork pattern of involvement by fibrosis and temporal variability with fibroblastic foci, collagen, and honeycomb change), and an additional 2 cases were considered consistent with UIP [94]. A second study of 32 patients found changes consistent with UIP in only 9.4% of patients, although the authors did suggest that the approach could be helpful in patients unable to undergo SLB [95]. As the sensitivity and specificity of this approach for the diagnosis of UIP is unknown, the most recent ATS/ERS statement suggests that TBLB should not be used in the evaluation of IPF, but should be considered in the evaluation of selected conditions (e.g., granulomatous disorders such as sarcoidosis – for which there is a reasonable expectation of establishing a diagnosis) [5]. However, subsequent studies have shown that TBLB can detect a UIP pattern in 30% of cases with high specificity and positive predictive value but a low negative predictive value [96]. Furthermore, when used in a multidisciplinary setting, clinician confidence in an IPF diagnosis increased when a TBLB specimen contained the characteristic histologic features of UIP [97]. Transbronchial lung cryobiopsy is an alternative and less invasive method for

obtaining larger biopsies of lung parenchyma, and transbronchial lung cryobiopsy may represent advancement in IPF diagnostics with lower complication and mortality rates compared to SLB. However, it is not widely available and may have significant morbidity [98–102]. The exact role of transbronchial biopsy (either standard TBLB or cryobiopsy) in the diagnosis of UIP remains unclear.

Surgical Lung Biopsy

Accurate diagnosis of an IIP often requires obtaining a SLB, as histopathology may serve as the only distinguishing feature between similar clinical and radiographic presentations [103]. As many patients with advanced lung disease are of older age, have impaired lung function (including low DL_{CO}), require oxygen, have PH, and demonstrate impaired functional capacity at the time of evaluation, decision-making regarding whether to perform a SLB is complex [41, 104, 105]. Patients with comorbidities, the elderly and those with atypical clinical and HRCT features of UIP, have a higher mortality risk associated with SLB as well as increased risk of having an acute exacerbation post-procedure [41, 104–106]. In patients with nondiagnostic HRCT findings, SLB should be considered, although 30-day mortality following the procedure has been described to be as high as 17% [41]. The possibility of complications including bleeding, prolonged mechanical ventilation, or prolonged air leak must also be considered. Acute respiratory failure following surgery carries a high mortality [41, 42]. Risk factors for increased morbidity and mortality following SLB include prior treatment with immunosuppression, mechanical ventilation at the time of biopsy, PH, lower levels of lung function (specifically regarding lung volumes or DL_{CO} less than 40% predicted), and the need for supplemental oxygen [1, 104, 107–109]. Increased in-hospital mortality has also been linked to male sex, open SLB rather than thoracoscopic surgery, and a suspected diagnosis of IPF [110]. Although HRCT features of UIP in the presence of honeycombing have a diagnostic accuracy of greater than 90% [3, 89, 111, 112], other diseases with specific historical and radiographic findings may also be diagnosed without biopsy, such as asbestosis. A diagnosis of asbestosis should be considered in patients with extensive exposure history, pleural plaques, and classical HRCT findings.

The most recent ATS/ERS consensus statement reiterates that findings on TBLB and BAL fluid are not reliable for establishing a diagnosis [5]. With improvements in minimally invasive techniques, including video-assisted thoracoscopic surgery (VATS), complication rates have declined. Thirty day mortality is estimated at 4% but decreases to 1.5–3% when those already on mechanical ventilation, with an acute exacerbation, or on immunosuppression are excluded [104, 113]. Moreover, VATS lung biopsy has a diagnostic yield that is comparable to open SLB for both diffuse and focal pathology [114]. As previously mentioned, transbronchial lung cryobiopsy may represent an alternative method to obtain larger tissue samples with a reported diagnostic yield as high as 80% with lower complication and mortality rates compared to SLB [100], although the cryobiopsy technique is not widely

available and is likely operator-dependent. Biopsy is ideally performed early in the disease course, as histologic distinctions can be more difficult as disease progresses. Because single center analyses showed patterns of both UIP and NSIP in 12–26% of patients when biopsies were taken from multiple lobes, obtaining biopsies from multiple lobes is recommended [115, 116]. In addition, diagnostic yield is improved when diseased (but not end stage) areas are targeted, reducing the risk of finding nonspecific changes [117]. Biopsies from areas of severe fibrosis are likely to show end-stage lung and not the histopathologic patterns required to differentiate UIP/IPF from other IIPs (see section "Histopathology" below). HRCT may be helpful in guiding surgeons to areas that show intermediate or relatively preserved lung, as a pathologic identification of fibrotic lung next to normal lung aids in confirmation of a UIP pattern [115].

Histopathology

Prior to the 1960s, the term "honeycomb lung" had been used to describe the macroscopic appearance of lung diseases comprising various histopathologic processes and causes, but in 1965 the definition was limited to include chronic interstitial pneumonia (pulmonary fibrosis) regardless of etiology [91, 118]. The presence of honeycomb lung should not be considered specific as to cause, and other disease entities (e.g., IIPs, diffuse alveolar damage [DAD], asbestosis, interstitial granulomatous diseases, and eosinophilic granuloma) should be included in a list of differential diagnoses that includes acute interstitial pneumonia, scarred sarcoidosis, chronic HP, scarred Langerhans cell histiocytosis, and smoking-related interstitial fibrosis (SRIF) [119, 120]. Studies of honeycomb lung found in diseases other than IPF (scleroderma, dermatomyositis, Langerhans cell histiocytosis, tuberculosis, lipoid pneumonia, and sarcoidosis) suggest that the pathophysiologic changes may be independent of the original disease [121]. SRIF, which has also been referred to as RB-ILD with fibrosis, is a pathologic pattern of uniform thickening of alveolar septa by collagen deposition with minimal associated inflammation; fibroblastic foci are seen in combination with emphysema and respiratory bronchiolitis (a manifestation of cigarette smoking) without architectural distortion or honeycomb changes [120, 122].

The most important criteria for pathologic diagnosis of UIP are temporal and spatial heterogeneity in the distribution of normal lung, interstitial inflammation, fibroblastic foci, and honeycomb change. Scattered fibroblastic foci are usually found between areas of normal lung and older fibrosis, and the majority of changes are often in a lower-lobe predominant distribution. The histological changes correlate with findings on HRCT with the peripheral subpleural parenchyma most severely affected. A prospective cohort study of 87 patients with biopsy-proven UIP showed that the degree of granulation/connective tissue deposition, which is charac-

Table 12.3 Characteristic histologic findings in UIP, NSIP, and SRIF

	UIP	NSIP	SRIF
Distribution	Heterogeneous	Uniform	Uniform
Emphysema	Usually absent	Usually absent	Often severe
Respiratory bronchiolitis	Possible	Possible	Present
Honeycombing	Present	None/minimal	None/minimal
Fibroblastic foci	Present	None/rare	None/rare

Adapted from Katzenstein [165]
UIP is distinguished by the heterogeneous distribution of areas of active fibrosis with collagen deposition, parenchymal distortion, and honeycomb changes. In contrast, the fibrosis in SRIF and NSIP is more uniform and less patchy and lacks the characteristic honeycomb changes seen in UIP

teristic of fibroblastic foci, could predict lower survival rates [46]. The importance of the number of foci to the clinical phenotype was also demonstrated by a separate study of 108 patients with UIP where the 9 patients with CTD-ILD and a UIP pattern had fewer foci and improved survival [123]. At low magnification the pattern has a heterogeneous appearance, and identification of normal parenchyma interspersed with areas of fibrosis and honeycomb cysts can help to distinguish UIP from NSIP. In NSIP temporal and spatial uniformity are common, honeycomb changes are rare, interstitial inflammation is more likely, and fewer fibroblastic foci may be found [90]. A UIP pattern may be found in non-UIP diagnoses, although the possibility of other diagnoses does not necessarily confer a survival advantage, as in a series of 168 patients including various different IIPs (i.e., not just IPF) wherein the risk ratio of histological classification of UIP for mortality was 11.46 (95% confidence interval 4.13–31.83, $p < 0.0001$) [3]. A summary of contrasting histologic features of SRIF, UIP, and NSIP is listed in Table 12.3, and representative images for these diagnoses are shown in Fig. 12.3.

Inter-observer variation in the pathologic diagnosis of DPLDs parallels the variation that has been described with radiologic diagnoses. One study of 133 biopsy specimens identified a 100% confidence level for a single diagnosis in only 39% of biopsy specimens that were reviewed by 10 pulmonary pathologists ($\kappa = 0.38$) [124]. The level of agreement increased when multiple biopsy specimens were taken and when diagnostic confidence was higher ($\kappa = 0.43$ and $\kappa = 0.50$, respectively). Agreement improved only marginally for a diagnosis of UIP, even with multiple biopsy specimens and high diagnostic confidence ($\kappa = 0.42$, $\kappa = 0.49$ and $\kappa = 0.58$, respectively) [124]. Agreement was significantly improved for sarcoidosis ($\kappa = 0.76$, $\kappa = 0.82$ and $\kappa = 0.86$, respectively) [124]. Not surprisingly, significant variability was seen for a diagnosis of NSIP ($\kappa = 0.29$, $\kappa = 0.32$ and $\kappa = 0.31$, respectively), and distinction of NSIP from UIP was noted to be particularly problematic [124]. The degree of uncertainty in establishing a histologic diagnosis of particular DPLDs, particularly NSIP versus UIP, supports the use of a multidisciplinary approach to confirm a diagnosis. This paradigm (multidisciplinary discussion) is discussed later in this chapter.

Close Mimics of IPF

In a patient with a suspected IIP, a histological diagnosis of UIP confers a nearly 30-fold increased risk of mortality when compared to an alternative histological diagnosis, and the relative risk of mortality for a histological diagnosis of UIP is more than ten times higher than that associated with only the presence of honeycomb changes on HRCT [3]. Evaluation of patients with presentations similar to UIP should include consideration of differences in history, exposures, and HRCT patterns. Exclusion of other known causes of ILD is important given differences in clinical course, management, and outcomes.

Chronic HP and NSIP

A thorough reviewof history and physical examination (as related to comorbid conditions), medications, environmental exposures, and family history can be useful to distinguish among the IIPs and non-IIP ILDs, particularly when chronic HP is a potential diagnosis [5]. In HP type III hypersensitivity reactions related to precipitin-antibody deposition in alveolar walls may be considered pathologic, although 20–30% of patients may not have an inciting antigen identified by exposure history or serologic testing [125–127]. Histologic findings of lymphocytic interstitial infiltrates with granuloma formation and BAL findings of lymphocytosis are typical [125]. Patients with chronic HP and a fibrotic histopathology demonstrate a predominant T_H2 lymphocyte response in comparison to patients with organizing pneumonia (OP) or NSIP-like histopathology [128]. With chronic exposure the typical histopathologic findings in subacute HP (cellular NSIP and bronchiolitis, granulomatous inflammation, involvement of central regions of secondary lobules) can progress to fibrotic changes with honeycombing [129]. A review of 13 cases of chronic HP, all with presence of granulomas and/or giant cells, suggested patterns of fibrosis may be in a typical UIP distribution (peripheral, patchy, and with fibroblastic foci) that is similar to fibrotic NSIP (homogeneous linear fibrosis) or UIP-like (irregular, predominantly peribronchiolar) [130].

Specific HRCT findings prompting consideration of chronic HP include the distribution of abnormality in a patchy or geographic pattern, often with upper-lung predominance, ground-glass opacities, centrilobular nodules, mosaic attenuation, and air trapping [125, 131–133]. The HRCT distribution of changes in HP may be more prominent in upper lobes, but changes can occur in the lower lobes, although subpleural involvement is less likely [132]. A study evaluating the role of HRCT in distinguishing chronic HP from UIP and NSIP found the presence of lobular areas with decreased attenuation, centrilobular nodules, and a lack of lower-lung predominance of changes to be the most useful; the basal predominance of honeycombing and absence of subpleural sparing and centrilobular nodules is particularly useful to distinguish chronic HP versus UIP, although up to 64% of patients with chronic HP may have honeycomb changes as well [133].

Idiopathic NSIP is a distinct clinical entity with features that distinguish it from the other IIPs. Symptoms of breathlessness and cough are often present. Patients are usually non-smokers, tend to be women in the sixth decade of life, and often have serologic testing that is positive for CTD [88]. Key histopathologic features differ between predominantly cellular patterns (mild to moderate interstitial chronic inflammation and type II pneumocyte hyperplasia in areas of inflammation) and fibrosing patterns (dense areas of loose interstitial fibrosis with a uniform appearance, lung architecture frequently preserved, and mild to moderate interstitial chronic inflammation) [88]. Studies suggest that the distinction between cellular and fibrosing patterns is important, as a more favorable prognosis is seen with the cellular patterns [134]. Characteristic HRCT features include reticular opacities with lower-lung zone predominance, traction bronchiectasis with lobar volume loss, and a diffuse or subpleural distribution [88]. The most common finding may be symmetric ground-glass opacities [119, 135].

Connective Tissue Disease-Related ILD

Pulmonary disease may complicate several CTDs including rheumatoid arthritis (RA), systemic sclerosis/scleroderma (SSc), polymyositis (PM)/dermatomyositis (DM), Sjögren syndrome, and systemic lupus erythematosus (SLE). Patients often present with nonspecific complaints of cough, dyspnea, and fatigue. Approximately 15% of patients with UIP have an underlying CTD as well [5], and the incidence of an IPF diagnosis in younger women may often relate to misdiagnosis of these patients [136]. Within CTDs a pattern of UIP is most common in RA [137], and both disease course and prognosis for UIP related to RA are similar to IPF [138]. Although RA-associated ILD is often secondary to long-standing disease and progression of disease is usually slow, it may be an early manifestation of disease in up to 20% of patients and can occur prior to classical exam findings of synovitis [139, 140]. Risk factors for RA-associated ILD include older age, male sex, and history of tobacco use.

The most recent ATS/ERS consensus statement gives a weak recommendation (given low-quality evidence) for serologic testing for CTD in the evaluation for IPF, even in the absence of signs or symptoms of disease [5]. Rheumatoid factor, anti-citrullinated peptide, and antinuclear antibody titer and pattern should be considered first, as use of other serological tests may only be helpful in select cases. Regarding bronchoscopy, BAL neutrophilia correlates with poor lung function but has not been shown to consistently correlate with prognosis and/or response to therapy [141–144]. Still, there is utility in evaluating for possible drug reactions (for evidence of eosinophilia), diffuse alveolar hemorrhage (DAH), and opportunistic infection [145–147].

A typical pattern of bibasilar subpleural reticulations and honeycombing likely predicts a pathologic finding of UIP [137, 148], as is the case for idiopathic IPF, although ground-glass predominance may confer a better prognosis for CTD-ILD

[149]. In addition to ground-glass opacities, common HRCT features of CTD-ILD include reticulation, bronchiectasis, and micronodules [150]. Abnormalities are found predominantly at the periphery of the lung and are usually associated with architectural distortion, traction bronchiectasis, and honeycombing, and these features can make distinction of CTD-ILD versus IIPs difficult [151]. The correlation that exists between radiographic and pathologic manifestations of UIP is thought to exist in patients with CTD-ILD as well [148], whereas the correlations between radiographic and histologic patterns of NSIP are not reliable [152]. Over time, HRCT manifestations of CTD-ILD can include progressive reticular and honeycomb changes with temporal heterogeneity [149], and progressive fibrosis is associated with worse prognosis [151].

A number of histopathological patterns including NSIP and UIPmay exist simultaneously in a single-lung biopsy specimen in patients with CTD-ILD [153]. Overall the prognosis of patterns of NSIP and UIP in CTD-ILD is felt to be better than in idiopathic disease that is not linked to CTD [115, 136, 151, 154, 155]. This may relate in part to a higher profusion of fibroblastic foci noted on histopathology between idiopathic ILD and CTD-ILD [123]. It is unclear whether a different fibroblast phenotype exists in idiopathic UIP versus CTD-ILD or if there is an effect of age on fibroblast function, as studies of fibroblasts undergoing replicative senescence suggest that the senescent state mimics inflammatory wound repair processes [156].

Many patients with an IIP have clinical features that suggest an underlying autoimmune process but do not meet established criteria for CTD-ILD. The term "interstitial pneumonia with autoimmune features" (IPAF) has been proposed by an ERS/ATS task force along with classification criteria built around clinical, serologic, and morphologic domains to identify individuals with IIP and features suggestive of but not definitive for CTD [157]. These criteria require further validation and assessment of clinical implications before routine clinical use of IPAF as a diagnostic entity can be adopted.

The Elderly Patient

As stated previously, the incidence of IPF is increased in older patients, with two-thirds of patients over 60 years of age at the time of presentation [6, 10]. Many of the hallmarks of aging (e.g., genomic instability, telomere attrition, epigenetic alterations, mitochondrial dysfunction, and cellular senescence) have been proposed as essential mechanisms for the development of IPF [158, 159]. Mechanisms of disease pathogenesis suggest that the aging process itself may contribute to clinical progression through the effects of cellular and molecular factors (e.g., mutations in surfactant protein C, mutations in telomerase), environmental factors (e.g., tobacco use, viral infections), and comorbid conditions (e.g., GERD, PH) [160]. Improvements in radiographic studies have facilitated the diagnosis of some DPLDs without the need for surgical lung biopsy, although histopathologic evaluation is often required to establish diagnosis and determine appropriate prognosis and

treatment. As IPF is a progressive condition that inexorably leads to death from respiratory failure or complicating comorbid conditions [32] and therapeutic options are limited, the use of SLB must be carefully considered in the context of the patient's overall clinical condition, as age itself is a known risk factor for complications and mortality from SLB [41]. There may be a risk for an acute exacerbation even for patients undergoing BAL [161], and the role of transbronchial biopsy specimens is not yet established as a reliable way to make a diagnosis of IPF [5]. One retrospective series identified age as a reliable sole predictor for the diagnosis of IPF. Specifically, a cutoff of 75 years provided a 100% predictive value of confirming UIP/IPF by SLB, while a cutoff of 70 years is nearly as good. This information offers the clinician some confidence when considering an IPF diagnosis and the associated prognosis in elderly patients who are not likely to be surgical candidates. The predictive value increased when interstitial changes were also present on HRCT [8]. Subsequent studies have shown that in the absence of honeycombing, increasing age and extent of reticular densities on HRCT can be used to predict a diagnosis of IPF [11].

The Multidisciplinary Approach

Establishing the correct diagnosis in a patient with a suspected IIP can be challenging. The ATS/ERS consensus recommendation for creation of a collaborative process involving clinicians, radiologists, and pathologists working together to improve diagnostic confidence for patients with suspected IPF built upon the knowledge that the combination of HRCT and histologic features was more robust in predicting prognosis versus either modality alone [89]. Previous data suggested that a histological diagnosis of IPF as a standard by itself was limited by inter-observer variation, as the ability of experienced pathologists to discriminate between NSIP and UIP produced agreement only 50% of the time [124]. Radiologists' assessment of ILD was also found to be limited by nonspecific findings and inter-observer variations, and in this separate study, the frequency of disagreement was highest for the diagnosis of NSIP, particularly for the distinction between UIP and NSIP [162]. The creation of an interdisciplinary algorithm for clinicians, radiologists, and pathologists determined that SLB could be deferred if the clinical/radiographic impression was consistent with IPF [163]. The study concluded that a diagnosis of non-IPF IIPs would require biopsy, as consensus opinion would likely be affected by the histopathological diagnosis [163]. A second area of focus – comparison of academic-based clinicians and community-based physicians – found significant disagreement in the diagnosis of IIPs, identified a tendency for community-based physicians as more likely to make a diagnosis of IPF, and found that overall clinical experience likely had a profound effect on diagnostic confidence [164]. In sum, these studies suggest that pathologists should consider additional data (clinical, radiological) when making diagnoses and that patients should be referred to tertiary centers with expertise in DPLDs in order to better clarify diagnoses and provide suggestions regarding treatment options.

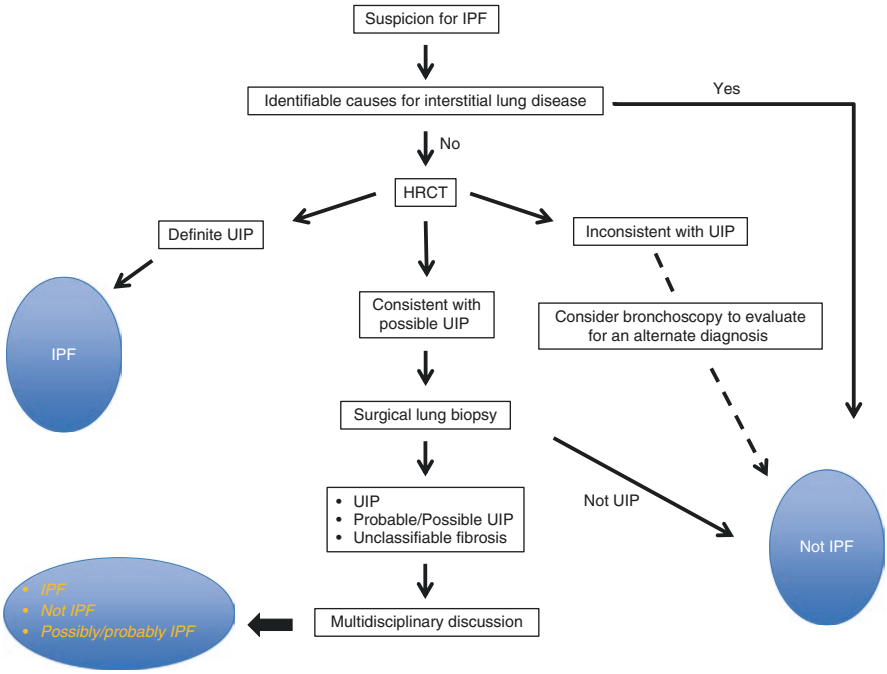

Fig. 12.4 Algorithm for evaluation of suspected IPF. HRCT may establish a diagnosis if there is a pattern consistent with UIP. Absent a pattern of UIP, a bronchoscopy should be considered to further evaluate for alternate diagnoses. A surgical lung biopsy should be considered if HRCT suggest possible UIP, but is not diagnostic for UIP. The accuracy of diagnosis of IPF increases with collaboration among a multidisciplinary team of specialists. (Reprinted with permission of the American Thoracic Society. Copyright © 2018 American Thoracic Society. Adapted from Raghu et al. [5]. The *American Journal of Respiratory and Critical Care Medicine* is an official journal of the American Thoracic Society)

Figure 12.4 demonstrates a diagnostic algorithm for evaluation of suspected IPF.

Future Directions

Prospective registries along with long-term follow-up data will continue to provide insights regarding the natural history of IPF. Identification of biomarkers that have prognostic significance and improvements in established multivariate predictive models will hopefully aid in the stratification of unique subpopulations with a goal of targeting therapies with greater precision. Specifically, the use of molecular and genetic techniques to establish diagnoses and distinguish among subtypes of IPF will be of paramount importance. Future investigation to elucidate other mechanisms as part of a broader collaboration between basic and clinical scientists will help in achieving goals of improved detection, prolonging survival, and improving quality of life.

References

1. 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.
2. Travis WD, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013;188(6):733–48.
3. Flaherty KR, et al. Clinical significance of histological classification of idiopathic interstitial pneumonia. Eur Respir J. 2002;19(2):275–83.
4. Bjoraker JA, et al. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998;157(1):199–203.
5. Raghu G, et al. 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.
6. Raghu G, et al. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006;174(7):810–6.
7. Douglas WW, Ryu JH, Schroeder DR. Idiopathic pulmonary fibrosis: impact of oxygen and colchicine, prednisone, or no therapy on survival. Am J Respir Crit Care Med. 2000;161(4 Pt 1):1172–8.
8. Fell CD, et al. Clinical predictors of a diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2010;181(8):832–7.
9. Wade JF 3rd, King TE Jr. Infiltrative and interstitial lung disease in the elderly patient. Clin Chest Med. 1993;14(3):501–21.
10. Raghu G, et al. Idiopathic pulmonary fibrosis in US Medicare beneficiaries aged 65 years and older: incidence, prevalence, and survival, 2001–11. Lancet Respir Med. 2014;2(7):566–72.
11. Salisbury ML, et al. Predictors of idiopathic pulmonary fibrosis in absence of radiologic honeycombing: a cross sectional analysis in ILD patients undergoing lung tissue sampling. Respir Med. 2016;118:88–95.
12. Steele MP, et al. Clinical and pathologic features of familial interstitial pneumonia. Am J Respir Crit Care Med. 2005;172(9):1146–52.
13. Schwartz DA, et al. The influence of cigarette smoking on lung function in patients with idiopathic pulmonary fibrosis. Am Rev Respir Dis. 1991;144(3 Pt 1):504–6.
14. Baumgartner KB, et al. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1997;155(1):242–8.
15. Schwartz DA, et al. Determinants of progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1994;149(2 Pt 1):444–9.
16. Lee JS, et al. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184(12):1390–4.
17. Raghu G, et al. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. Eur Respir J. 2006;27(1):136–42.
18. Lee JS. The role of gastroesophageal reflux and microaspiration in idiopathic pulmonary fibrosis. Clin Pulm Med. 2014;21(2):81–5.
19. Lee JS, et al. Does chronic microaspiration cause idiopathic pulmonary fibrosis? Am J Med. 2010;123(4):304–11.
20. D'Ovidio F, et al. Prevalence of gastroesophageal reflux in end-stage lung disease candidates for lung transplant. Ann Thorac Surg. 2005;80(4):1254–60.
21. Baumgartner KB, et al. Occupational and environmental risk factors for idiopathic pulmonary fibrosis: a multicenter case-control study. Collaborating centers. Am J Epidemiol. 2000;152(4):307–15.
22. Hubbard R, et al. Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. Lancet. 1996;347(8997):284–9.

23. Kitamura H, et al. Inhalation of inorganic particles as a risk factor for idiopathic pulmonary fibrosis – elemental microanalysis of pulmonary lymph nodes obtained at autopsy cases. Pathol Res Pract. 2007;203(8):575–85.
24. Selman M, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. PLoS One. 2007;2(5):e482.
25. Idiopathic Pulmonary Fibrosis Clinical Research, N, et al. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 2012;366(21):1968–77.
26. Idiopathic Pulmonary Fibrosis Clinical Research, N, et al. Randomized trial of acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2093–101.
27. Noble PW, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet. 2011;377(9779):1760–9.
28. King TE Jr, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2083–92.
29. Richeldi L, et al. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med. 2011;365(12):1079–87.
30. Richeldi L, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2071–82.
31. Daniels CE, Yi ES, Ryu JH. Autopsy findings in 42 consecutive patients with idiopathic pulmonary fibrosis. Eur Respir J. 2008;32(1):170–4.
32. Panos RJ, et al. Clinical deterioration in patients with idiopathic pulmonary fibrosis – causes and assessment. Am J Med. 1990;88(4):396–404.
33. 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.
34. Stewart JP, et al. The detection of Epstein-Barr virus DNA in lung tissue from patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1999;159(4 Pt 1):1336–41.
35. Konishi K, et al. Gene expression profiles of acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;180(2):167–75.
36. Ryerson CJ, et al. Acute exacerbation of idiopathic pulmonary fibrosis: shifting the paradigm. Eur Respir J. 2015;46(2):512–20.
37. Atkins CP, Loke YK, Wilson AM. Outcomes in idiopathic pulmonary fibrosis: a meta-analysis from placebo controlled trials. Respir Med. 2014;108(2):376–87.
38. Lee JS, et al. Anti-acid treatment and disease progression in idiopathic pulmonary fibrosis: an analysis of data from three randomised controlled trials. Lancet Respir Med. 2013;1(5):369–76.
39. Chida M, et al. Subclinical idiopathic pulmonary fibrosis is also a risk factor of postoperative acute respiratory distress syndrome following thoracic surgery. Eur J Cardiothorac Surg. 2008;34(4):878–81.
40. Kondoh Y, et al. Risk factors of acute exacerbation of idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis. 2010;27(2):103–10.
41. Utz JP, et al. High short-term mortality following lung biopsy for usual interstitial pneumonia. Eur Respir J. 2001;17(2):175–9.
42. Kim DS, et al. Acute exacerbation of idiopathic pulmonary fibrosis: frequency and clinical features. Eur Respir J. 2006;27(1):143–50.
43. Martinez FJ, et al. The clinical course of patients with idiopathic pulmonary fibrosis. Ann Intern Med. 2005;142(12 Pt 1):963–7.
44. Song JW, et al. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J. 2011;37(2):356–63.
45. Kondoh Y, et al. Risk factors for acute exacerbation of idiopathic pulmonary fibrosis – extended analysis of pirfenidone trial in Japan. Respir Investig. 2015;53(6):271–8.
46. King TE Jr, et al. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med. 2001;164(6):1025–32.
47. Nicholson AG, et al. The relationship between individual histologic features and disease progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2002;166(2):173–7.
48. Tiitto L, et al. Relationship between histopathological features and the course of idiopathic pulmonary fibrosis/usual interstitial pneumonia. Thorax. 2006;61(12):1091–5.

49. King TE Jr, et al. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med. 2001;164(7):1171–81.
50. Cottin V, Cordier JF. The syndrome of combined pulmonary fibrosis and emphysema. Chest. 2009;136(1):1–2.
51. Doherty MJ, et al. Cryptogenic fibrosing alveolitis with preserved lung volumes. Thorax. 1997;52(11):998–1002.
52. Mejia M, et al. Idiopathic pulmonary fibrosis and emphysema: decreased survival associated with severe pulmonary arterial hypertension. Chest. 2009;136(1):10–5.
53. Cottin V, et al. Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. Eur Respir J. 2005;26(4):586–93.
54. Schmidt SL, et al. Pulmonary function measures predict mortality differently in IPF versus combined pulmonary fibrosis and emphysema. Eur Respir J. 2011;38(1):176–83.
55. Watters LC, et al. A clinical, radiographic, and physiologic scoring system for the longitudinal assessment of patients with idiopathic pulmonary fibrosis. Am Rev Respir Dis. 1986;133(1):97–103.
56. Wells AU, 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.
57. Corte TJ, Wort SJ, Wells AU. Pulmonary hypertension in idiopathic pulmonary fibrosis: a review. Sarcoidosis Vasc Diffuse Lung Dis. 2009;26(1):7–19.
58. Andersen CU, et al. Pulmonary hypertension in interstitial lung disease: prevalence, prognosis and 6 min walk test. Respir Med. 2012;106(6):875–82.
59. D'Alonzo GE, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. Ann Intern Med. 1991;115(5):343–9.
60. Patel NM, et al. Pulmonary hypertension in idiopathic pulmonary fibrosis. Chest. 2007;132(3):998–1006.
61. Arcasoy SM, et al. Echocardiographic assessment of pulmonary hypertension in patients with advanced lung disease. Am J Respir Crit Care Med. 2003;167(5):735–40.
62. Nathan SD, et al. Serial development of pulmonary hypertension in patients with idiopathic pulmonary fibrosis. Respiration. 2008;76(3):288–94.
63. Lettieri CJ, et al. Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest. 2006;129(3):746–52.
64. Nathan SD, Noble PW, Tuder RM. Idiopathic pulmonary fibrosis and pulmonary hypertension: connecting the dots. Am J Respir Crit Care Med. 2007;175(9):875–80.
65. Hamada K, et al. Significance of pulmonary arterial pressure and diffusion capacity of the lung as prognosticator in patients with idiopathic pulmonary fibrosis. Chest. 2007;131(3):650–6.
66. Charbeneau RP, Peters-Golden M. Eicosanoids: mediators and therapeutic targets in fibrotic lung disease. Clin Sci (Lond). 2005;108(6):479–91.
67. Richter A, et al. Impaired transforming growth factor-beta signaling in idiopathic pulmonary arterial hypertension. Am J Respir Crit Care Med. 2004;170(12):1340–8.
68. Wright L, et al. 5-lipoxygenase and 5-lipoxygenase activating protein (FLAP) immunoreactivity in lungs from patients with primary pulmonary hypertension. Am J Respir Crit Care Med. 1998;157(1):219–29.
69. Weitzenblum E, Chaouat A. Sleep and chronic obstructive pulmonary disease. Sleep Med Rev. 2004;8(4):281–94.
70. Risk C, Epler GR, Gaensler EA. Exercise alveolar-arterial oxygen pressure difference in interstitial lung disease. Chest. 1984;85(1):69–74.
71. O'Donnell D. Physiology of interstitial lung disease. In: Schwarz MI, King TE, editors. Interstitial lung disease. Hamilton: Marcel Dekker; 1998. p. 51–70.
72. Gottlieb D, Snider G. Lung function in pulmonary fibrosis. In: Phan S, Thrall R, editors. Lung biology in health and disease: pulmonary fibrosis. New York: Marcel Dekker; 1995. p. 85–135.
73. Collard HR, et al. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2003;168(5):538–42.
74. Flaherty KR, et al. Idiopathic pulmonary fibrosis: prognostic value of changes in physiology and six-minute-walk test. Am J Respir Crit Care Med. 2006;174(7):803–9.

75. Lama VN, et al. Prognostic value of desaturation during a 6-minute walk test in idiopathic interstitial pneumonia. Am J Respir Crit Care Med. 2003;168(9):1084–90.
76. Fell CD, et al. The prognostic value of cardiopulmonary exercise testing in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;179(5):402–7.
77. Latsi PI, et al. Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. Am J Respir Crit Care Med. 2003;168(5):531–7.
78. du Bois RM, et al. Six-minute-walk test in idiopathic pulmonary fibrosis: test validation and minimal clinically important difference. Am J Respir Crit Care Med. 2011;183(9):1231–7.
79. du Bois RM, et al. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184(4):459–66.
80. Flaherty KR, et al. Prognostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. Am J Respir Crit Care Med. 2003;168(5):543–8.
81. Jegal Y, et al. Physiology is a stronger predictor of survival than pathology in fibrotic interstitial pneumonia. Am J Respir Crit Care Med. 2005;171(6):639–44.
82. Ley B, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. Ann Intern Med. 2012;156(10):684–91.
83. Orens JB, et al. The sensitivity of high-resolution CT in detecting idiopathic pulmonary fibrosis proved by open lung biopsy. A prospective study. Chest. 1995;108(1):109–15.
84. Hansell DM, et al. Fleischner society: glossary of terms for thoracic imaging. Radiology. 2008;246(3):697–722.
85. Akira M, Sakatani M, Ueda E. Idiopathic pulmonary fibrosis: progression of honeycombing at thin-section CT. Radiology. 1993;189(3):687–91.
86. Kazerooni EA, et al. Thin-section CT obtained at 10-mm increments versus limited three-level thin-section CT for idiopathic pulmonary fibrosis: correlation with pathologic scoring. AJR Am J Roentgenol. 1997;169(4):977–83.
87. Lynch DA, et al. High-resolution computed tomography in idiopathic pulmonary fibrosis: diagnosis and prognosis. Am J Respir Crit Care Med. 2005;172(4):488–93.
88. Travis WD, et al. Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. Am J Respir Crit Care Med. 2008;177(12):1338–47.
89. Flaherty KR, et al. Radiological versus histological diagnosis in UIP and NSIP: survival implications. Thorax. 2003;58(2):143–8.
90. du Bois R, King TE Jr. Challenges in pulmonary fibrosis x 5: the NSIP/UIP debate. Thorax. 2007;62(11):1008–12.
91. Arakawa H, Honma K. Honeycomb lung: history and current concepts. AJR Am J Roentgenol. 2011;196(4):773–82.
92. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med. 2000;161(2 Pt 1):646–64.
93. Ohshimo S, et al. Significance of bronchoalveolar lavage for the diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;179(11):1043–7.
94. Berbescu EA, et al. Transbronchial biopsy in usual interstitial pneumonia. Chest. 2006;129(5):1126–31.
95. Shim HS, Park MS, Park IK. Histopathologic findings of transbronchial biopsy in usual interstitial pneumonia. Pathol Int. 2010;60(5):373–7.
96. Tomassetti S, et al. Transbronchial biopsy is useful in predicting UIP pattern. Respir Res. 2012;13:96.
97. Sheth JS, et al. Utility of transbronchial vs surgical lung biopsy in the diagnosis of suspected fibrotic interstitial lung disease. Chest. 2017;151(2):389–99.
98. Casoni GL, et al. Transbronchial lung cryobiopsy in the diagnosis of fibrotic interstitial lung diseases. PLoS One. 2014;9(2):e86716.
99. Poletti V, Hetzel J. Transbronchial cryobiopsy in diffuse parenchymal lung disease: need for procedural standardization. Respiration. 2015;90(4):275–8.
100. Ravaglia C, et al. Safety and diagnostic yield of transbronchial lung cryobiopsy in diffuse parenchymal lung diseases: a comparative study versus video-assisted thoracoscopic lung biopsy and a systematic review of the literature. Respiration. 2016;91(3):215–27.

101. Gasparini S, Bonifazi M. Cryobiopsy for interstitial lung diseases. J Bronchol Interv Pulmonol. 2016;23(1):4–6.
102. Tomassetti S, et al. Bronchoscopic lung cryobiopsy increases diagnostic confidence in the multidisciplinary diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2016;193(7):745–52.
103. Hunninghake GW, et al. Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2001;164(2):193–6.
104. Lettieri CJ, et al. Outcomes and safety of surgical lung biopsy for interstitial lung disease. Chest. 2005;127(5):1600–5.
105. Kreider ME, et al. Complications of video-assisted thoracoscopic lung biopsy in patients with interstitial lung disease. Ann Thorac Surg. 2007;83(3):1140–4.
106. Tiitto L, et al. Thoracoscopic lung biopsy is a safe procedure in diagnosing usual interstitial pneumonia. Chest. 2005;128(4):2375–80.
107. Canver CC, Mentzer RM Jr. The role of open lung biopsy in early and late survival of ventilator-dependent patients with diffuse idiopathic lung disease. J Cardiovasc Surg. 1994;35(2):151–5.
108. Hazelrigg SR, Nunchuck SK, LoCicero J 3rd. Video assisted thoracic surgery study group data. Ann Thorac Surg. 1993;56(5):1039–43. discussion 1043–4
109. Nicod P, Moser KM. Primary pulmonary hypertension. The risk and benefit of lung biopsy. Circulation. 1989;80(5):1486–8.
110. Hutchinson JP, et al. In-hospital mortality after surgical lung biopsy for interstitial lung disease in the United States. 2000 to 2011. Am J Respir Crit Care Med. 2016;193(10):1161–7.
111. Hunninghake GW, et al. Radiologic findings are strongly associated with a pathologic diagnosis of usual interstitial pneumonia. Chest. 2003;124(4):1215–23.
112. Raghu G, et al. The accuracy of the clinical diagnosis of new-onset idiopathic pulmonary fibrosis and other interstitial lung disease: a prospective study. Chest. 1999;116(5):1168–74.
113. Park JH, et al. Mortality and risk factors for surgical lung biopsy in patients with idiopathic interstitial pneumonia. Eur J Cardiothorac Surg. 2007;31(6):1115–9.
114. Kadokura M, et al. Pathologic comparison of video-assisted thoracic surgical lung biopsy with traditional open lung biopsy. J Thorac Cardiovasc Surg. 1995;109(3):494–8.
115. Park JH, et al. Prognosis of fibrotic interstitial pneumonia: idiopathic versus collagen vascular disease-related subtypes. Am J Respir Crit Care Med. 2007;175(7):705–11.
116. Monaghan H, et al. Prognostic implications of histologic patterns in multiple surgical lung biopsies from patients with idiopathic interstitial pneumonias. Chest. 2004;125(2):522–6.
117. Qureshi RA, et al. Does lung biopsy help patients with interstitial lung disease? Eur J Cardiothorac Surg. 2002;21(4):621–6. discussion 626
118. Meyer EC, Liebow AA. Relationship of interstitial pneumonia honeycombing and atypical epithelial proliferation to cancer of the lung. Cancer. 1965;18:322–51.
119. Johkoh T, et al. Nonspecific interstitial pneumonia: correlation between thin-section CT findings and pathologic subgroups in 55 patients. Radiology. 2002;225(1):199–204.
120. Katzenstein AL, et al. Clinically occult interstitial fibrosis in smokers: classification and significance of a surprisingly common finding in lobectomy specimens. Hum Pathol. 2010;41(3):316–25.
121. Pimentel JC. Tridimensional photographic reconstruction in a study of the pathogenesis of honeycomb lung. Thorax. 1967;22(5):444–52.
122. Katzenstein AL. Smoking-related interstitial fibrosis (SRIF): pathologic findings and distinction from other chronic fibrosing lung diseases. J Clin Pathol. 2013;66(10):882–7.
123. Flaherty KR, et al. Fibroblastic foci in usual interstitial pneumonia: idiopathic versus collagen vascular disease. Am J Respir Crit Care Med. 2003;167(10):1410–5.
124. Nicholson AG, et al. Inter-observer variation between pathologists in diffuse parenchymal lung disease. Thorax. 2004;59(6):500–5.
125. Costabel U, Bonella F, Guzman J. Chronic hypersensitivity pneumonitis. Clin Chest Med. 2012;33(1):151–63.
126. Hanak V, et al. High-resolution CT findings of parenchymal fibrosis correlate with prognosis in hypersensitivity pneumonitis. Chest. 2008;134(1):133–8.

127. Sahin H, et al. Chronic hypersensitivity pneumonitis: CT features comparison with pathologic evidence of fibrosis and survival. Radiology. 2007;244(2):591–8.
128. Kishi M, et al. Pathogenesis of cBFL in common with IPF? Correlation of IP-10/TARC ratio with histological patterns. Thorax. 2008;63(9):810–6.
129. Coleman A, Colby TV. Histologic diagnosis of extrinsic allergic alveolitis. Am J Surg Pathol. 1988;12(7):514–8.
130. Churg A, et al. Chronic hypersensitivity pneumonitis. Am J Surg Pathol. 2006;30(2):201–8.
131. Johannson KA, et al. A diagnostic model for chronic hypersensitivity pneumonitis. Thorax. 2016;71(10):951–4.
132. Lynch DA, et al. Can CT distinguish hypersensitivity pneumonitis from idiopathic pulmonary fibrosis? AJR Am J Roentgenol. 1995;165(4):807–11.
133. Silva CI, et al. Chronic hypersensitivity pneumonitis: differentiation from idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia by using thin-section CT. Radiology. 2008;246(1):288–97.
134. Travis WD, et al. Idiopathic nonspecific interstitial pneumonia: prognostic significance of cellular and fibrosing patterns: survival comparison with usual interstitial pneumonia and desquamative interstitial pneumonia. Am J Surg Pathol. 2000;24(1):19–33.
135. Park JS, et al. Nonspecific interstitial pneumonia with fibrosis: radiographic and CT findings in seven patients. Radiology. 1995;195(3):645–8.
136. Kim DS, et al. The major histopathologic pattern of pulmonary fibrosis in scleroderma is nonspecific interstitial pneumonia. Sarcoidosis Vasc Diffuse Lung Dis. 2002;19(2):121–7.
137. Lee HK, et al. Histopathologic pattern and clinical features of rheumatoid arthritis-associated interstitial lung disease. Chest. 2005;127(6):2019–27.
138. Kim EJ, et al. Usual interstitial pneumonia in rheumatoid arthritis-associated interstitial lung disease. Eur Respir J. 2010;35(6):1322–8.
139. Gochuico BR, et al. Progressive preclinical interstitial lung disease in rheumatoid arthritis. Arch Intern Med. 2008;168(2):159–66.
140. Mori S, et al. A simultaneous onset of organizing pneumonia and rheumatoid arthritis, along with a review of the literature. Mod Rheumatol. 2008;18(1):60–6.
141. Biederer J, et al. Correlation between HRCT findings, pulmonary function tests and bronchoalveolar lavage cytology in interstitial lung disease associated with rheumatoid arthritis. Eur Radiol. 2004;14(2):272–80.
142. Garcia JG, et al. Lower respiratory tract abnormalities in rheumatoid interstitial lung disease. Potential role of neutrophils in lung injury. Am Rev Respir Dis. 1987;136(4):811–7.
143. Komocsi A, et al. Alveolitis may persist during treatment that sufficiently controls muscle inflammation in myositis. Rheumatol Int. 2001;20(3):113–8.
144. Nagasawa Y, et al. Inflammatory cells in lung disease associated with rheumatoid arthritis. Intern Med. 2009;48(14):1209–17.
145. Costabel U, et al. Bronchoalveolar lavage in other interstitial lung diseases. Semin Respir Crit Care Med. 2007;28(5):514–24.
146. Ramirez P, Valencia M, Torres A. Bronchoalveolar lavage to diagnose respiratory infections. Semin Respir Crit Care Med. 2007;28(5):525–33.
147. Schnabel A, et al. Bronchoalveolar lavage cell profile in methotrexate induced pneumonitis. Thorax. 1997;52(4):377–9.
148. Kim EJ, Collard HR, King TE Jr. Rheumatoid arthritis-associated interstitial lung disease: the relevance of histopathologic and radiographic pattern. Chest. 2009;136(5):1397–405.
149. Dawson JK, et al. Predictors of progression of HRCT diagnosed fibrosing alveolitis in patients with rheumatoid arthritis. Ann Rheum Dis. 2002;61(6):517–21.
150. Stack BH, Grant IW. Rheumatoid interstitial lung disease. Br J Dis Chest. 1965;59(4):202–11.
151. Kocheril SV, et al. Comparison of disease progression and mortality of connective tissue disease-related interstitial lung disease and idiopathic interstitial pneumonia. Arthritis Rheum. 2005;53(4):549–57.
152. Kligerman SJ, et al. Nonspecific interstitial pneumonia: radiologic, clinical, and pathologic considerations. Radiographics. 2009;29(1):73–87.

153. Leslie KO, Trahan S, Gruden J. Pulmonary pathology of the rheumatic diseases. Semin Respir Crit Care Med. 2007;28(4):369–78.
154. Douglas WW, et al. Polymyositis-dermatomyositis-associated interstitial lung disease. Am J Respir Crit Care Med. 2001;164(7):1182–5.
155. Tansey D, et al. Variations in histological patterns of interstitial pneumonia between connective tissue disorders and their relationship to prognosis. Histopathology. 2004;44(6):585–96.
156. Shelton DN, et al. Microarray analysis of replicative senescence. Curr Biol. 1999;9(17):939–45.
157. Fischer A, et al. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. Eur Respir J. 2015;46(4):976–87.
158. Thannickal VJ, Loyd JE. Idiopathic pulmonary fibrosis: a disorder of lung regeneration? Am J Respir Crit Care Med. 2008;178(7):663–5.
159. Selman M, Pardo A. Revealing the pathogenic and aging-related mechanisms of the enigmatic idiopathic pulmonary fibrosis. An integral model. Am J Respir Crit Care Med. 2014;189(10):1161–72.
160. Castriotta RJ, et al. Workshop on idiopathic pulmonary fibrosis in older adults. Chest. 2010;138(3):693–703.
161. Sakamoto K, et al. Acute exacerbation of IPF following diagnostic bronchoalveolar lavage procedures. Respir Med. 2012;106(3):436–42.
162. Aziz ZA, et al. HRCT diagnosis of diffuse parenchymal lung disease: inter-observer variation. Thorax. 2004;59(6):506–11.
163. Flaherty KR, et al. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? Am J Respir Crit Care Med. 2004;170(8):904–10.
164. Flaherty KR, et al. Idiopathic interstitial pneumonia: do community and academic physicians agree on diagnosis? Am J Respir Crit Care Med. 2007;175(10):1054–60.
165. Katzenstein AL. Smoking-related interstitial fibrosis (SRIF), pathogenesis and treatment of usual interstitial pneumonia (UIP), and transbronchial biopsy in UIP. Mod Pathol. 2012;25(Suppl 1):S68–78.

Chapter 13
Pharmacologic Treatment of IPF

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General Introduction

The pathogenetic mechanisms leading to lung fibrosis in idiopathic pulmonary fibrosis (IPF) are mostly unknown, but significant results have been achieved since 2000 when the first guidelines for the diagnosis and treatment of IPF were published. The previous paradigm of inflammation leading to fibrosis has been largely abandoned and replaced by the current hypothesis of a dysregulated wound healing response that involves many profibrotic pathways and cell types with an excessive production of extracellular matrix (ECM) [1]. The paradigm shift has also influenced the therapeutic approach to the disease. The older treatment approach was directed to halting inflammation with anti-inflammatory, immunosuppressive, or cytotoxic drugs (now proven to be ineffective and potentially harmful), and it has now been replaced by therapies targeting fibrosis and its pathways. The IPF treatment guidelines of 2011 (updated in 2015) highlight these changes and provide the rationale for recommendations in favor or against treatment interventions [1, 2].

The statements provided in these guidelines were formulated by a panel of non-conflicted experts who reviewed results from randomized clinical trials, systematic reviews, and meta-analyses. They rated the evidence according to the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach. The committee graded each therapeutic intervention according to the quality of evidence available (high, moderate, low, very low) and provided a recommendation in favor of or against its use, and the strength of these recommendations was also specified as either weak or conditional.

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According to the 2015 clinical practice guideline from the American Thoracic Society (ATS)/European Respiratory Society (ERS)/Japanese Respiratory Society (JRS)/Latin American Thoracic Society (ALAT), only two drugs are approved and found to be capable of slowing disease progression [2]. The aim of this chapter is to outline the evolution of IPF treatment (both stable and acute disease) during the last two decades, including an evaluation of past treatments and the reasons why they were discontinued, as well as review present management of the disease and ongoing studies with investigational drugs targeting possible pathogenetic mechanisms of IPF.

Past Therapies in IPF

Introduction

The IPF guidelines of 2000 [3] and 2011 [1] represent two fundamental watersheds in knowledge and management of this pulmonary disorder. Until 2000, different names were used in order to refer to IPF including terms such as fibrosing alveolitis, chronic idiopathic pneumonia, and fibrosing pneumonitis. IPF was also linked with many different histological patterns. Clinical trials and published studies were difficult to interpret because of the lack of standardized criteria to define this disease.

The international Consensus Statement on IPF diagnosis and treatment of 2000 established a standardized definition and diagnostic criteria, which provided an important foundation for the implementation of successive clinical trials. The international panel of experts reviewed studies published prior to 2000 and acknowledged that no pharmacological therapy existed that was effective in treating IPF. However, the committee suggested that a treatment regimen with prednisone, azathioprine, or cyclophosphamide could be used, but the caveat that this therapy might not be appropriate for all patients with IPF was provided. They also suggested that it should be started as early as possible in the natural course of the disease. However, this treatment approach was focused on the old paradigm of IPF as an inflammatory disease of lung parenchyma leading to fibrosis [4].

The guidelines of 2011 acknowledged that the use of aggressive immunosuppressive and cytotoxic treatment regimens largely failed to reduce the death rate in patients with IPF [3, 5, 6]. They further recognized the disease as a fibrosing epithelial/mesenchymal disorder rather than an inflammatory process. The second edition of the guidelines did not find sufficient evidence to support the use of any specific pharmacological therapy for patients affected by IPF. However, based on results from many clinical trials, they provided recommendations not to use many drugs that were previously thought to be of possible benefit. In this section, an overview of past pharmacological approaches to IPF will be discussed with an emphasis on failed treatment trials.

Corticosteroids

Corticosteroid use evolved into accepted practice during the last 50 years despite the fact that no prospective, placebo-controlled randomized trial has ever been performed. A transient clinical response with no survival benefit has been described in a small minority of patients in some studies [5, 7]. However, a systematic review found no high-quality prospective trials on which to base any recommendation [8]. In older studies the definition of IPF was less specific, and it is likely that any physiologic or radiographic improvement reported occurred in a subgroup of responders who did not have IPF but had other diseases (such as respiratory bronchiolitis interstitial lung disease [RB-ILD], nonspecific interstitial pneumonia [NSIP], or desquamative interstitial pneumonia [DIP]) [9].

Moreover, when high-dose corticosteroids have been used in trials, significant toxicity has been reported (weight gain, hyperglycemia, osteoporosis, avascular necrosis, and adverse gastrointestinal [GI] effects) [10–12]. Current evidence suggests that corticosteroid monotherapy is not indicated in the treatment of IPF [13, 14].

Cyclophosphamide

Cyclophosphamide is an alkylating agent of the nitrogen mustard group, and its metabolites suppress lymphocyte function. Several small nonrandomized trials and case reports in idiopathic interstitial pneumonias (IIPs) have suggested a variable benefit with cyclophosphamide [11, 15–17].

The best prospective data on cyclophosphamide are from Johnson and coworkers, who randomized 43 patients with IIP to high-dose prednisolone versus low-dose prednisolone plus cyclophosphamide [15]. However, the distinction of IPF from other IIPs was unknown at that time, and probably more than 20% of cases were associated with connective tissue disease (CTD). Among this heterogeneous group, no difference in clinical markers or mortality was seen. A retrospective review compared 82 patients with IPF treated with combination cyclophosphamide and prednisone with 82 untreated patients matched for age and forced vital capacity (FVC) [18]. All subjects had IPF as defined by the current American Thoracic Society criteria. There was no significant mortality difference (including in cases of presumed early disease). In addition, cyclophosphamide is associated with an increase in risk of infection, myelosuppression, hepatotoxicity, hemorrhagic cystitis, and several cancers [19]. In a small prospective trial of cyclophosphamide in patients with IPF, 68% experienced adverse effects, and discontinuation was necessary in 47% [16]. No evidence exists to justify the routine use of cyclophosphamide alone in the management of IPF or its use as a steroid-sparing agent, and significant potential side effects are associated with its use. However, other forms of interstitial lung disease (ILD) may show a better response to this agent (as has also been reported for azathioprine).

Colchicine

Colchicine inhibits collagen formation by fibroblasts and may increase collagen degradation. It also suppresses the release of alveolar-macrophage-derived growth factor and fibronectin by alveolar macrophages from patients with pulmonary fibrosis. In vitro and animal models have suggested that colchicine may reduce fibrotic processes [20, 21]. However, several clinical studies have failed to show a significant effect on lung function decline or improved survival in patients treated with colchicine [10, 22–25].

In a retrospective analysis, Douglas and coworkers found no evidence to support the use of colchicine in the treatment of IPF/UIP [10]. A prospective controlled clinical trial [22] showed that treatment with colchicine was no more effective than prednisone and had no significant effect on outcomes (e.g., survival, pulmonary function), although colchicine seems to be safer and better tolerated. Side effects that may be encountered include nausea, vomiting, abdominal pain, and diarrhea. In summary, no evidence exists to justify the routine use of colchicine in the management of IPF.

Azathioprine + N-Acetylcysteine + Prednisone

The treatment guidelines published in 2000 suggested that corticosteroids combined with an immunosuppressant (azathioprine or cyclophosphamide) could be useful for the treatment of IPF patients. The multicenter randomized trial (IFIGENIA) tested N-acetylcysteine (NAC) combined with azathioprine and high-dose corticosteroids versus azathioprine and high-dose corticosteroids alone in a population of IPF patients [26]. N-Acetylcysteine was given at the dose of 1800 mg daily, and although both groups showed lower FVC and diffusion capacity for carbon monoxide (DLCO) after 12 months of treatment, the rate of decline was significantly lower in the group that received NAC. Because the IFIGENIA trial did not have a "no treatment" group, it was impossible to establish whether NAC in combination with prednisone and azathioprine was better than no treatment (placebo). Indeed, NAC might only serve to mitigate the potential deleterious effects of azathioprine. Despite the limitations of this study, the combination of azathioprine, NAC, and prednisone ("triple therapy") was widely used by pneumologists.

In 2012 a randomized, placebo-controlled, double-blind study (PANTHER-IPF) was published in which patients with IPF (mild to moderate lung function impairment) were assigned to one of three groups (triple therapy, NAC alone, or placebo). The triple therapy arm was stopped because of an increased rate of death and hospitalization in this group as compared with the placebo arm [27]. These findings provided evidence against the use of this combination in IPF patients.

N-Acetylcysteine

N-Acetylcysteine is a sulfhydryl substance with antioxidant and cellular detoxifying properties. The antioxidant properties of NAC made it an attractive option for the treatment IPF patients, since the disease process has been associated with excessive oxidative stress [28]. The first small study on NAC was conducted by Behr et al. in 1997. They reported that NAC, a precursor of glutathione, restored depleted pulmonary glutathione levels and improved lung function in IPF patients [29].

As previously discussed, both IFIGENIA trial and PANTHER-IPF have shown how NAC has failed to reduce IPF progression and mortality [26, 27, 30]. Another study found that inhaled NAC monotherapy was not associated with beneficial effects, although a post hoc analysis suggested that NAC therapy could have some beneficial effects in early stage IPF [31]. Review of these studies led to the conditional recommendation against the use of NAC in IPF [2].

Recent genetic studies could modify the recommendation against the use of NAC. Polymorphisms of TOLLIP and MUC5B genes have been linked with IPF susceptibility and survival. These genes are involved in lung host defense, namely, an immunologic response sensitive to oxidative signaling and the presence of antioxidants [32]. Based on this evidence, Oldham and coworkers conducted a post hoc analysis of PANTHER-IPF patients. They demonstrated that NAC may be an efficacious treatment strategy in IPF patients who have an rs3750920 (TOLLIP) TT genotype; however NAC therapy was associated with a trend toward harm in those with a CC genotype [33]. A genotype-stratified prospective clinical trial is needed to assess the potential efficacy of NAC in this population.

Interferon Gamma

Interferon-γ (IFN-γ) is an endogenously produced cytokine that is characterized by antifibrotic, antiproliferative, anti-infective, and immunomodulatory effects. In vitro and in vivo studies have demonstrated that IFN-γ modulates expression of transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and other profibrotic interleukins, and IFN-γ can suppress lung fibroblast proliferation and collagen synthesis [34]. A study of various forms of pulmonary fibrosis including IPF has indicated that there may be a general impairment of the production of IFN-γ in patients with pulmonary fibrosis [35]. These studies led to the evaluation of IFN-γ in clinical trials in patients with IPF.

The first study to evaluate the clinical effect of IFN-γ-1b was published in 1999 by Ziesche and colleagues [36]. They studied a small population of 18 patients affected by IPF who had no apparent responses to treatment with glucocorticoids or

other immunosuppressive agents and randomized nine patients to oral prednisolone alone and nine patients to a combination of IFN-γ-1b and prednisolone. Patients assigned to combination therapy showed substantial improvement in total lung capacity and partial pressure of arterial oxygen compared to patients treated with prednisolone alone after 12 months of treatment.

Two phase 3 clinical trials of subcutaneous IFN-γ-1b were subsequently implemented sequentially to assess the efficacy of this agent in IPF patients. In 2004 Raghu and coworkers [37] randomized 330 patients that were unresponsive to corticosteroid therapy to receive IFN-γ or placebo for 48 weeks. There was a suggestion of an effect on progression-free survival in patients with a FVC value >55% predicted, although the primary endpoint was not reached for the entire IFN-γ-1b-treated arm versus the placebo group. However, the subsequent INSPIRE study [38] of 826 patients randomized to receive IFN-γ-1b or placebo for 90–96 weeks was stopped early because of the absence of any treatment benefit. Both of these prospective trials failed to show any survival benefit with subcutaneous IFN-γ-1b treatment compared to placebo. This lack of benefit of IFN-γ-1b in improving the overall survival in IPF patients was subsequently confirmed in a meta-analysis [39]. These cumulative data led to the strong recommendation in 2011 against the use of IFN-γ-1b in patients with IPF [1].

Warfarin

Recent epidemiological studies have revealed a link between IPF and thrombotic vascular events, such as deep vein thrombosis, pulmonary embolism, and acute coronary syndromes. This observation is supported by in vitro and in vivo evidence of a prothrombotic state in fibrotic lungs, which is related to overexpression of tissue factor and coagulation factors X and VII in the alveolar compartment [40]. Additionally, data from a population-based study demonstrated that IPF patients were more likely to have a procoagulant state compared to the general population, and patients with a prothrombotic state were more likely to have severe disease and a worse prognosis [41]. Based on these observations, it was thought that anticoagulation therapy may be of benefit in patients with IPF.

The use of anticoagulant therapy with warfarin was first investigated in 2005 in a small (56 patients), randomized, multicenter clinical trial in Japan [42]. IPF patients were randomized to prednisolone or prednisolone plus oral warfarin in an outpatient setting (or prednisolone plus low-molecular weight heparin if admitted to the hospital). A significant survival benefit was demonstrated in the anticoagulated study group. This was thought to be linked to a decreased mortality for acute exacerbations of IPF, although the two groups didn't differ in terms of hospitalization-free periods. Based on this evidence, anticoagulant therapy received a weak recommendation against its use in IPF in the 2011 guidelines [1].

Results from the ACE-IPF (AntiCoagulant Effectiveness in Idiopathic Pulmonary Fibrosis) study of 2012 [43] led to a strong recommendation against the use of warfarin in IPF patients in the updated treatment guideline of 2015 [2]. In this double-blind, randomized, placebo-controlled trial, 145 participants were randomized to receive either warfarin or placebo for a total of 48 weeks. However, the study was terminated prematurely due to increased mortality related to disease progression or acute exacerbations and low evidence of benefit in the anticoagulant group.

Warfarin should be used only in IPF patients who have a known alternative indication other than IPF for its use, such as venous thromboembolic disease or atrial fibrillation, despite a post hoc analysis of ASCEND and CAPACITY trials suggesting that anticoagulants used for non-IPF indications may be associated with unfavorable outcomes [44]. A potential beneficial effect might still be possible with direct thrombin inhibitors [45, 46]; however, clinical trials are needed to further address this (see NCT02885961).

Sildenafil

Sildenafil is a phosphodiesterase type 5 (PDE-5) inhibitor that is characterized by vasoactive properties and approved for the treatment of pulmonary arterial hypertension (PH). The first trial evaluating the effect of sildenafil in lung fibrosis complicated by PH was conducted by Ghofrani et al. in 2002; sildenafil appeared to induce vasodilatation preferentially in well-ventilated lung areas, thereby improving ventilation-perfusion matching and gas exchange [47]. Results from another study suggested major anti-contractile and anti-remodeling effects of sildenafil in the arteries of IPF patients with PH, and the authors suggested that this could explain the possible beneficial effects of sildenafil in IPF patients with associated PH [48].

Current treatment guidelines provide a conditional recommendation against the use of sildenafil in IPF patients that is based on results from two randomized clinical trials [2]. The first one was the Sildenafil Trial of Exercise Performance in Idiopathic Pulmonary Fibrosis (STEP-IPF) [49], a double-blind, randomized, placebo-controlled trial (RCT) in which 180 patients were randomized to receive oral sildenafil or placebo for 12 weeks, which was then followed by a second phase of open-label phase of sildenafil for an additional of 12 weeks. There was no significant beneficial effect of sildenafil on the primary outcome, which was an improvement in 6-min walking distance (6MWD) of 20%. However, the presence of some positive secondary outcomes (improvement of DLCO, dyspnea, and quality of life) suggested a possible beneficial effect. A subsequent subgroup analysis was performed in patients with an available echocardiogram. The authors demonstrated a significant improvement in outcomes in patients with right ventricular hypertrophy or right ventricular systolic dysfunction [50]. The second, smaller RCT randomized 29 patients to receive oral sildenafil or placebo for 24 weeks; no proven benefit and an increase in adverse events were demonstrated in the treatment arm [51].

Based on results obtained by the subgroup analysis of Han et al. [50] and by a recent network meta-analysis suggesting sildenafil as being one of the three treatments with the highest probability of reducing mortality in IPF [52], two clinical trials are currently recruiting IPF patients with advanced disease to receive sildenafil added to base therapy: a phase IIb trial of pirfenidone plus sildenafil vs. pirfenidone plus placebo (NCT02951429) with a duration of 52 weeks and a phase III trial (INSTAGE) of nintedanib plus sildenafil vs. nintedanib plus placebo with a duration of 24 weeks (NCT02802345). Data from these trials will elucidate the role of sildenafil as an add-on therapy in patients with advanced lung disease.

Endothelin Receptor Antagonists

There is evidence from animal models of pulmonary fibrosis and from human studies that suggests a potential role for endothelin-1 (ET-1) in the development and progression of lung disease through a profibrotic effect [53]. Specifically, there is an increased level of ET-1 receptors, both endothelin type A (ET-A) and endothelin type B1 (ET-B1) receptors, in IPF-affected fibrotic lung [54]. Based upon this biological rationale, clinically available endothelin receptor antagonist (ERA) drugs have been tested in a number of RCTs including ambrisentan (a selective ET-A receptor antagonist), bosentan, and macitentan (dual antagonists targeting both ET-A and ET-B1 receptors).

The first clinical trial of an ERA was of bosentan, through the BUILD-1 (Bosentan Use in Interstitial Lung Disease) trial, the results of which were published in 2008 [55]. In this study 158 IPF patients were randomly assigned to receive bosentan or placebo for 12 months. However, the primary endpoint of an increased exercise capacity (as measured by 6-min walk distance) was not achieved, although a trend in delayed time to death or disease progression was observed. The follow-up BUILD-3 RCT was designed to demonstrate whether bosentan could delay IPF worsening or death. However, bosentan again failed to meet its primary endpoint [56]. Similarly, macitentan showed no significant differences compared to placebo in the MUSIC RCT (the primary outcome was improvement in the FVC) [57]. Current guidelines [2], according to results of these RCTs, provide a conditional recommendation against the use of dual ET-A and ET-B receptor antagonists for the treatment of IPF. Additionally, a strong recommendation against the use of ambrisentan is provided [2] that is based on the results of the ARTEMIS-IPF trial, which was halted early due to lack of efficacy as well as a high likelihood of disease progression and respiratory hospitalization [58].

Imatinib

Imatinib is a tyrosine kinase inhibitor (TKI) that can inhibit lung fibroblast-myofibroblast differentiation, proliferation, and extracellular matrix production

through inhibition of PDGF and TGF-β signaling [59]. On the basis of a RCT of imatinib versus placebo that showed no difference in mortality between the two groups, no differences in disease progression (study primary outcome), and an increased risk of adverse events in the imatinib group [60], current treatment guidelines strongly recommend that clinicians do not use imatinib to treat patients with IPF [2]. Table 13.1 shows a summary of past treatments for IPF.

Table 13.1 Past treatments

Treatment	Mechanism of action	Clinical trial/ retrospective series/ Cochrane review	2011 guidelines and quality of evidence	2015 guidelines and quality of evidence
Corticosteroid	Immunosuppressive and anti-inflammatory activities	Richeldi et al. [8] Flaherty et al. [12]	Strong against (VLQ)	Not addressed
Cyclophosphamide	Immunosuppressive	Collard et al. [18]	Strong against (LQ)	Not addressed
Colchicine	Anti-inflammatory and antifibrotic	Douglas et al. [22]	Strong against (VLQ)	Not addressed
Azathioprine + NAC + prednisone	Immunosuppressive, anti-inflammatory, and antioxidant activities	IFIGENIA [26] PANTHER-IPF [27]	Weak against (LQ)	Strong against (LC)
NAC	Antioxidant	IFIGENIA [26] PANTHER-IPF [27] Homma et al. [31]	Weak against (LQ)	Conditional against (LC)
Interferon-γ 1b	Antifibrotic and immunomodulatory properties	Raghu et al. [37] INSPIRE [38]	Strong against (HQ)	Not addressed
Warfarin	Anticoagulant	Kubo et al. [42] ACE-IPF [43]	Weak against (VLQ)	Strong against (LC)
Sildenafil	Phosphodiesterase-5 inhibitor	STEP-IPF [49] Jackson et al. [51]	Not addressed	Conditional against (MC)
Ambrisentan	Endothelin receptor-A antagonist	ARTEMIS-IPF [58]	Not addressed	Strong against (LC)
Bosentan	Dual endothelin receptor antagonist (ET-A, ET-B1)	BUILD-1 [55] BUILD-3 [56]	Strong against (MQ)	Conditional against (LC)
Macitentan	Dual endothelin receptor antagonist (ET-A, ET-B1)	MUSIC [57]	Strong against (MQ)	Conditional against (LC)
Imatinib	Tyrosine kinase inhibitor	Imatinib-IPF [60]	Not addressed	Strong against (MC)

NAC N-acetylcysteine, *VLQ* very low quality, *LQ* low quality, *MQ* moderate quality, *HQ* high quality, *LC* low confidence, *MC* moderate confidence

Current Therapies in IPF

Introduction

The 2015 American Thoracic Society (ATS)/European Respiratory Society (ERS)/Japanese Respiratory Society (JRS)/Latin American Thoracic Society (ALAT) clinical practice guideline [2] is a landmark document on the management of IPF. Its purpose was to update the prior guidelines published in 2011 in accordance with the more currently available evidence. Recommendations were formulated and graded by a panel of non-conflicted experts and adjudicated as either "strong" or "conditional." In recent years a plethora of compounds has been investigated as potential treatments for IPF; unfortunately, nearly all have proven to be ineffective. Negative results emanating from these trials and advances in the understanding of disease pathogenesis led to a shift to agents with antifibrotic and antiproliferative effects. Specifically, the new pathogenetic model recognizes the pivotal role of aberrant reparative mechanisms in the fibrotic process. The failure of the inflammation hypothesis and of studies testing anti-inflammatory and immunomodulatory drugs led experts to formulate a strong recommendation against the use of the combination of prednisone, azathioprine, and N-acetylcysteine. In 2014 two drugs, pirfenidone and nintedanib, were approved by the US Food and Drug Administration (FDA) for the treatment of IPF based on the positive results of large RCTs. They were found to be effective in slowing disease progression in mild to moderate disease, offering a new hope to IPF patients and raising optimism about the future of pharmacological treatment for IPF. Based on this new evidence, the 2015 updated treatment guidelines formulated a conditional recommendation for the use of either pirfenidone or nintedanib to treat IPF. Because for the first time IPF-specific therapies were recommended and new standards for therapy were established, the approval of these drugs by the FDA and the recommendation for their use to treat IPF can be considered historic events. The approval of these two drugs has enabled clinicians to embark on a new era in IPF care. However, it is commonly recognized that there is still a long and arduous road to travel to attain a definitive cure and to resolve many unanswered questions about this devastating disease.

Pirfenidone

Pirfenidone is an orally available pyridine recently approved for the treatment of IPF. It is readily absorbed by the gastrointestinal tract, which reaches a peak blood level after 1–2 h, and it is eliminated through the urine within 6 h [61]. This small synthetic compound undoubtedly has multiple different mechanisms of action that are largely unknown. It has antifibrotic, anti-inflammatory, and antioxidant properties as suggested by data from in vitro studies and animal models of pulmonary fibrosis [62, 63]. It regulates the activity of profibrotic growth factors such as

TGF-β, inhibits fibroblast proliferation and collagen synthesis, inhibits cytokines and inflammatory cells, and scavenges hydroxyl radicals [64].

The first clinical study of pirfenidone in patients with IPF was a phase II open-label trial in 1999. Fifty-four patients were enrolled and evaluated for mortality, change in lung function, and adverse effects; however, there was no control group [65]. This early study was followed by a phase II multicenter RCT that enrolled 107 Japanese patients with IPF. These patients were randomly assigned in a 2:1 ratio to receive 600 mg of pirfenidone or placebo for 12 months (72 patients were treated with pirfenidone and 35 patients with placebo). The study was stopped at 9 months before reaching the primary endpoint because of a greater incidence of acute exacerbations in the placebo-treated group and because of a significant reduction in decline in FVC in the pirfenidone-treated group [66]. The encouraging results of this study led to four randomized, double-blind, placebo-controlled, phase 3 RCTs. The first was conducted in Japan, and 275 patients were randomly assigned in a 2:1:2 ratio to one of three cohorts that received high-dose (1800 mg/day) pirfenidone, low-dose (1200 mg/day) pirfenidone, or placebo for 52 weeks. The primary endpoint was the change in vital capacity (VC) from baseline to week 52, and a significantly lower decline was described in the pirfenidone arm. Significant differences between the two groups were also observed with regard to progression-free survival (PFS) time (the secondary endpoint); indeed, high-dose pirfenidone was associated with a longer PFS. The drug was relatively well tolerated with photosensitivity being the major adverse event [67]. Pirfenidone was subsequently approved in 2008 for the treatment of IPF in Japan. This study was followed by the CAPACITY program, which sought to confirm the efficacy of pirfenidone in reducing the decline in lung function in patients with IPF with mild to moderate functional impairment. CAPACITY consisted of two nearly identical phase 3, multinational RCTs (PIPF 004 and PIPF 006). Patients aged 40–80 years diagnosed with IPF within the previous 48 months and who lacked evidence of significant improvement over the preceding year were eligible to participate. In study PIPF 004, 435 patients were assigned in a 2:1:2 ratio to high-dose (2403 mg/day) pirfenidone, low-dose (1197 mg/day) pirfenidone, or placebo for 72 weeks. In study PIPF 006, 344 patients were assigned in a 1:1 ratio to pirfenidone 2403 mg/day or placebo. Inclusion criteria mandated a FVC of 50–90% predicted, DLCO of 35–90% predicted, and a 6MWT distance of at least 150 m. The primary endpoint was change in percent predicted FVC from baseline to week 72, but this endpoint was only met in the PIPF 004 RCT. Treatment efficacy was noted by week 24 and persisted until week 72. In the PIPF 006 RCT, no significant difference was found between pirfenidone and placebo on the primary outcome of percentage predicted FVC change at 72 weeks. Pirfenidone was generally well tolerated with a high compliance rate [68]. Combined findings from these two phase 3 trials confirmed the efficacy of pirfenidone in IPF and showed a favorable benefit-risk profile that leads to its approval by the European Medicines Agency (EMA) in 2011, and pirfenidone was the first licensed, evidence-based treatment in Europe for IPF. However, the US FDA requested an additional phase 3 trial to support the efficacy of pirfenidone.

The Assessment of Pirfenidone to Confirm Efficacy and Safety in Idiopathic Pulmonary Fibrosis (ASCEND) studywas a multinational, double-blind, placebo-controlled RCT. A total of 555 IPF patients were randomly assigned in a 1:1 ratio to receive pirfenidone 2403 mg/day or placebo for 52 weeks. Eligible patients were aged 40–80 years and had a diagnosis of IPF based on consensus guidelines, a FVC of 50–90%, a DLCO of 30–90%, a 6MWT distance of at least 150 m, and no significant obstructive airway disease (FEV_1/FVC ratio was required to be ≥80%). The primary endpoint was the change from baseline to week 52 in the % predicted FVC, and a significant difference was observed between the two groups in favor of pirfenidone. Pirfenidone lessened the decline in the 6MWT distance and reduced the relative risk of death or disease progression, showing an improvement in PFS. No significant difference was noted in all-cause mortality. In the pirfenidone-treated group, the most common adverse events reported were GI (nausea, diarrhea, dyspepsia, anorexia, and vomiting) and skin-related events (rash/photosensitivity reactions). These adverse events were generally mild to moderate in severity, and they were not associated with any clinically significant sequelae. Elevations in aminotransferase levels occurred more frequently in the pirfenidone group, but all elevations were reversible and did not have clinically significant consequences [69]. The safety profile of the drug and the confirmed efficacy in preventing FVC decline led the FDA to license pirfenidone in 2014.

Analysis of pooled data from the three multinational RCTs (ASCEND and CAPACITY) supported the clinically significant benefit of pirfenidone on multiple efficacy outcomes that reflect a reduction of disease progression [70]. After a treatment period of 1 year, pirfenidone reduced the proportion of patients with a ≥10% decline in % predicted FVC or death and the proportion of patients with a ≥50 m decline in 6MWT distance. Beneficial effects were also reported also for dyspnea [70]. Additional support for the use of pirfenidone in IPF has come from a recent analysis and meta-analysis of pooled data from the previously mentioned three clinical trials (ASCEND and CAPACITY) that showed a reduced relative risk of mortality (treatment-emergent all-cause mortality, IPF-related mortality, and treatment-emergent IPF-related mortality) compared with placebo over a period of 120 weeks [71].

Long-term safety and tolerability of pirfenidone was confirmed in RECAP, an open-label extension study [72], and in PASSPORT (Pirfenidone Post-Authorisation Safety Registry), a multinational prospective safety study. Dose modification (either reduction or temporary discontinuation) was the most efficient strategy for managing adverse events and enabling patients to continue on treatment [73]. A total of 1299 patients were included in a recent integrated analysis of safety data from five clinical trials (CAPACITY studies 004 and 006, ASCEND, RECAP studies 002 and 012); long-term treatment with pirfenidone was demonstrated to be safe and generally well-tolerated [74]. Animal studies observed that food, when co-administered with pirfenidone, reduced the peak concentration of the drug and the risk of GI side effects. To increase compliance with treatment, patients are therefore instructed to take pirfenidone with food, to avoid sun exposure, and to use high ultraviolet A and B protection during treatment. Liver chemistry tests are required before starting

pirfenidone treatment and are recommended at monthly intervals for the first 6-month and at 3-month intervals thereafter. Patients are advised to follow the dose modification guidelines incorporated into the summary of product characteristics if GI or skin-related adverse events occur.

Nintedanib

Nintedanib is a multiple tyrosine kinase inhibitor that targets fibroblast growth factor receptor (FGFR), PDGF receptor (PDGFR), and vascular endothelial growth factor (VEGF) receptor (VEGFR) [75]. It competitively binds to the adenosine triphosphate-binding pocket of these receptors and interferes with fibroblast proliferation, migration, differentiation, and collagen secretion. Nintedanib also showed antifibrotic and anti-inflammatory activity in mouse models of bleomycin- and silica-induced lung fibrosis. It is rapidly absorbed by the GI tract with peak blood concentration achieved after 2–4 h and steady-state plasma levels within 7 days, and it is excreted via the fecal route. The TOMORROW trial was a phase 2 RCT that investigated the effects of nintedanib in IPF patients. A total of 432 patients were randomly assigned to receive one of four different doses of nintedanib (50 mg daily, 50 mg twice a day, 100 mg twice a day, 150 mg twice a day) or placebo for 52 weeks. The primary endpoint was the annual rate of decline in FVC, but this was not achieved. However, a reduction in FVC decline and in the incidence of acute exacerbations as well as a better quality of life was observed in the high-dose treatment group compared to placebo [76]. Two multinational, 52-week, phase 3 RCTs (INPULSIS-1 and INPULSIS-2) were subsequently conducted. Patients were randomly assigned in a 3:2 ratio to receive nintedanib 150 mg twice a day or placebo. Patients aged ≥40 years with a diagnosis of IPF made within the previous 5 years were eligible. In contrast to previous trials, patients with "possible UIP" (with traction bronchiectasis but lacking honeycomb change) on HRCT were included without having to undergo a surgical lung biopsy, and there was no upper threshold for FVC. Additional inclusion criteria were a FVC of 50% or more of the predicted value and a DLCO of 30–79% predicted. The primary endpoint was the annual rate of decline in FVC, and this outcome was achieved in both trials. In INPULSIS-2 (but not in INPULSIS-1), a significant increase in the time to the first acute exacerbation was also observed in the nintedanib-treated arm. The most common adverse event was diarrhea [77]. The encouraging results of these trials led the FDA to approve nintedanib for the treatment of IPF in 2014, and pooled data from the replicate INPULSIS trials demonstrated a manageable safety and tolerability profile of nintedanib [78].

The TOMORROW and INPULSIS trials were not powered to show a difference in mortality between nintedanib and placebo [79]. However, a meta-analysis and an analysis of pooled data from these three international phase 2/3 trials were performed on the primary and key secondary endpoints in the INPULSIS trials. A beneficial effect that favored nintedanib was observed for reduction of FVC decline, time to first acute exacerbation, and time to all-cause and on-treatment mortality [80].

Pre-specified subgroup analysis of pooled data from the two INPULSIS trials investigated the effect of nintedanib in IPF patients with baseline FVC >90% versus ≤90% predicted. No significant difference was observed between these subgroups in the treatment effect of nintedanib in terms of annual rate of decline in FVC, time to first acute exacerbation, and the St George's Respiratory Questionnaire (SGRQ) total score [81]. Additionally, a previous study demonstrated a consistent effect of nintedanib across a range of IPF patients with different demographic and clinical variables [82]. All these findings support the concept of early treatment of IPF patients, even if they still have preserved lung function. An interim analysis of data from the INPULSIS-ON open-label extension trial showed that IPF patients with a severe functional impairment (FVC <50% predicted) received the same beneficial effects on FVC decline as patients with less severe impairment and that the treatment benefits are maintained beyond 52 weeks. These results support the hypothesis that treatment with nintedanib can also be offered to patients with advanced disease, but these data have to be considered with caution because of the small number of patients with FVC ≤50% predicted [83].

Comparison of the Two Drugs

Nintedanib and pirfenidone both showed a beneficial effect in slowing disease progression, and both drugs represent a valid choice for treatment of IPF patients. However, neither drug was able to reverse or halt disease progression in all patients or demonstrate a definitive decrease in mortality. A direct comparison of these two drugs has not been investigated; therefore, no clear evidence is available as yet about which agent performs better than the other. Consequently, therapeutic decisions are based on a comprehensive evaluation of potential side effects, comorbidities, concomitant therapies, and patient preferences. A network meta-analysis provided an indirect comparison of the two approved treatments that showed a superior benefit of nintedanib in slowing the FVC decline, but this finding should be interpreted cautiously due to the limitations related to indirect comparisons [84]. A recent network meta-analysis found no superiority of one drug over the other on mortality outcomes or on decline in pulmonary function [85].

Nonetheless, despite the encouraging results of the recent clinical trials, many practical questions remain concerning the further development of available therapies for a broader group of patients and the standardization of strategies to manage treatment failure. At present some subgroups of patients are excluded from therapeutic options because of severe functional impairment, the presence of comorbidities, diagnostic inaccuracy, or advanced age. It is also necessary to better define the features of treatment failure and to investigate alternative approaches such as combination therapies. As concerns potential combination therapies, evidence of efficacy and safety are still lacking, but combination therapy could be a promising therapeutic option.

Antiacid Therapy

The 2015 ATS/ERS/JRS/ALAT clinical practice guidelines confirmed the conditional recommendation for the use of antiacid therapy reported in the previous guideline document published in 2011. This therapeutic choice is supported by the higher prevalence of abnormal acid gastroesophageal reflux (GER) in patients affected by IPF compared with control subjects and by a likely pathogenic role of GER in IPF. However, this potential causal relationship remains unclear. Therefore, further investigations are needed. Several studies have highlighted the potential beneficial effect of antiacid treatment. Laparoscopic fundoplication has been reported to stabilize oxygen requirements in patients with end-stage IPF awaiting lung transplantation [86], and it has also been found to improve nearly all measurements of lung function in patients with end-stage IPF before and after lung transplantation [87]. In a retrospective single-center study, no significant differences were reported in rates of FVC decline in IPF patients pre- and post-laparoscopic anti-reflux surgery over 1 year, suggesting a possible trend toward functional stabilization [88]. In a two-center retrospective cohort study, Lee et al. observed a lower radiologic fibrosis score in patients with IPF with the use of GER medications, and anti-reflux therapy was identified as an independent predictor of longer survival time [89]. The relationship between the routine use of antiacid therapy and change in FVC was further investigated in IPF patients treated with placebo in three IPFnet randomized controlled trials. A beneficial effect on FVC decline was demonstrated, and fewer acute exacerbations were reported in patients receiving antiacid treatment [90]. In addition an increased longevity was recently described in IPF patients treated with proton pump inhibitors (PPIs) [91], and PPI use for at least 4 months may have a protective effect in lowering the IPF-related mortality rate [92]. Chronic use of anti-reflux therapy was found to be an independent predictor of longer survival time in IPF patients, especially when receiving a combination of antiacid and gastrointestinal motility drugs [93]. However, a post-hoc analysis of antiacid use from CAPACITY/ASCEND pooled data, showed no significant difference at 52 weeks in disease progression between antiacid and no-antiacid users. Results showed that antiacid therapy may be potentially associated with an increased risk of infection in those with more-advanced disease [94].

Despite some studies seem to support the use of PPIs, further investigations are needed to define the optimal antiacid pharmacotherapy and the subsets of patients with IPF who could largely benefit from this treatment. The causal relationship between GER and IPF and the pathogenetic mechanisms are still unclear; consequently, the most appropriate anti-reflux treatment and management has yet to be established. Most of these studies were retrospective, and results could be influenced by the design and the small sample sizes. A comprehensive analysis of antiacid therapy, including the exact dose and risk of side effects, is needed to investigate the efficacy and safety of PPIseither alone or in combination with antifibrotic treatment. Possible interactions between antiacid therapy and antifibrotics also need to be further evaluated. There are several potential adverse effects associated with

PPIs, such as alteration of the gut microbiome, diarrhea, increased risk of community-acquired pneumonia, *Clostridium difficile* infection, risk of adverse neurologic effects, cardiovascular events, and osteoporosis. Moreover, a direct comparison between acid suppression therapy and laparoscopic anti-reflux surgery is still lacking. In light of the recent findings and of the unresolved questions regarding the safety and the efficacy of antiacid treatment, two ongoing clinical trials are investigating the effects of omeprazole (NCT02085018) and of laparoscopic anti-reflux surgery (NCT01982968) in IPF [95].

Future Therapies in IPF

Introduction

Pirfenidone and nintedanib, which are the only approved drugs for pharmacologic treatment of IPF, can slow disease progression but do not block or reverse the disease. Additionally, many drugs evaluated in several clinical trials in the past decades have not provided any significant ameliorating effects on the disease. This is probably related to the pathogenetic heterogeneity of this disease. Many molecular pathways have been identified and could be potential targets for novel agents as well as useful diagnostic, prognostic, and theragnostic biomarkers [96].

The challenge of the next decade will be to develop targeted therapies for use in combination with current treatments. The aim is to stop fibrosis progression and to preserve quality of life for patients with idiopathic pulmonary fibrosis [97].

Combination of Approved Drugs

A recent network meta-analysis suggested similar effects of nintedanib and pirfenidone on FVC decline and respiratory and all-cause mortality [85]. An obvious subsequent hypothesis is that combination of the two drugs may represent a more effective therapeutic approach, and the recently updated guidelines have provided a conditional recommendation for the use of either drug [2]. The possibility of combination therapy with pirfenidone and nintedanib together is based on recent preliminary studies. For example, an in vitro study showed a greater reduction of fibroblast and myofibroblast proliferation with both drugs compared with the use of only one of the drugs [98]. Additionally, several clinical trials are evaluating the possibility of combining nintedanib and pirfenidone.

NCT02579603, a phase 4, open-label, randomized, parallel group study, assessed a combination therapy to determine the number of patients with significant GI side effects. NCT01136174, a phase 2, double-blind, randomized, dose-escalation trial, and NCT01417156, a phase 2 open-label, follow-up study of nintedanib, are being conducted in Japanese IPF patients to assess the safety, tolerability, and pharmacokinetics of nintedanib either alone or combination with pirfenidone. This study

demonstrated lower plasma level of nintedanib during co-administration with pirfenidone [99]. NCT02606877, a phase 4, open-label, multiple-dose, two-group study, is evaluating the effect of steady-state pirfenidone on the pharmacokinetics of nintedanib and vice versa. Finally, NCT02598193, a phase 4, open-label, single-arm study, is assessing the tolerability and safety of nintedanib administered in patients who have already been taking pirfenidone for at least 16 weeks and are on a stable dose.

Combination of Approved and Experimental Drugs

The combination of other experimental drugs, acting on specific pathways involved in IPF, with either nintedanib or pirfenidone could be a potential treatment strategy. Vismodegib has been tested in combination with pirfenidone in a single-arm, multicenter, open-label, phase 1b study that aims to assess safety and tolerability (NCT02648048). Vismodegib is a selective inhibitor of hedgehog pathways (Hh). IPF patients show significantly greater expression of Hh-related genes versus controls, and Hh signaling contributes to profibrotic processes. As the upregulation of chemokine CXCL14 is the main effect of Hh-related genes expression, it has been proposed that CXCL14 could be a useful biomarker to monitor vismodegib treatment in IPF patients [100].

Sildenafil, a selective inhibitor of phosphodiesterase type 5 (PDE5) that is already known for its use in PH and erectile dysfunction, is currently under evaluation for IPF patients, although guidelines do not currently recommend treatment with this drug [2]. Sildenafil will be tested in combination with nintedanib versus nintedanib alone in patients with advanced IPF in a 24-week, double-blind, randomized, parallel-group study. The change from baseline in SGRQ total score at week 12 will be considered as the primary outcome measure (NCT02802345). Another study, a phase 2b, multicenter, double-blind, placebo-controlled RCT, will evaluate the efficacy, safety, and tolerability of sildenafil combined with pirfenidone in IPF patients with PH. The primary outcome measure is disease progression as determined by relevant decline in 6MWD, respiratory-related non-elective hospitalization, or death from any cause (NCT02951429).

Emerging Therapies Targeting Specific Molecular Pathways

Extracellular Matrix (ECM) Deposition

Anti-connective tissue growth factor antibodies Connective tissue growth factor (CTGF) is a matricellular protein that connects ECM and cells, and it has a role as an effective inducer of fibroblast proliferation, migration, and ECM deposition. Also known as CCN2 and belonging to the CCN family, CTGF is involved in regulation of many cellular processes that lead to wound healing. TGF-β, CCL2, and

IL-13 represent the main cytokines stimulating the production of CTGF by epithelial, endothelial, and mesenchymal cells [101, 102].

In murine lung models, it has been noted that CTGF has a crucial role in inducing an intense, but transient, profibrotic response [103]. Moreover, co-administration of TGF-β and CTGF increases the bleomycin-induced fibrotic response [104]. However, Smad3-null mice are resistant to TGF-β-induced fibrosis because of their inability to express the CTGF gene [105].

IPF patients manifest higher levels of CTGF on BAL fluid analysis than healthy subjects [106]. Therefore, CTGF may represent a valuable target for the treatment of IPF, and anti-CTGF antibodies have been shown to inhibit collagen deposition in murine models of fibrosis [104]. In IPF patients, the humanized antibody, FG-3019, administered intravenously showed safety and tolerability in preliminary results of an open-label phase 2 study (NCT01262001). The efficacy of FG-3019 is being evaluated in an ongoing placebo-controlled, double-blind phase 2 RCT (NCT01890265) with the primary outcome change of FVC from baseline at 48 weeks.

PBI-4050 is a novel, first-in-class drug that binds the G-protein-coupled fatty acid receptors, GPR40 and GPR84, and has shown efficacy in murine models of bleomycin-induced fibrosis, reducing CTGF production and collagen I mRNA expression [107]. The safety and tolerability of orally administered PBI-4050 will be assessed in an ongoing phase 2, open-label, single-arm trial (NCT02538536).

Therapies targeting Integrins Integrins are a large family of receptors that are used by cells to bind to ECM. Moreover, integrins are able to activate TGF-β by unbinding it from its ligand, latency-activated peptide (LAP). There are three isoforms of TGF-β. Among these isoforms, TGF-β1 has shown the most important profibrotic effects. It promotes the recruitment of fibroblasts, myofibroblast differentiation and survival, and ECM deposition. Integrin αvβ6 specifically mediates TGF-β1 activation [108]. Therefore, it has been proposed as a biomarker of fibrosing ILDs [109].

It has been reported that partial inhibition of the αvβ6 integrin is able to reduce fibrosis in animal models of bleomycin-induced fibrosis [110].

The humanized monoclonal antibody, BG00011 (STX100), is currently being tested in a phase 2 study to determine its safety and tolerability when administered subcutaneously (NCT01371305).

A randomized, double-blind phase 1 study (NCT02612051), of a nebulized solution of the integrin αvβ6-antagonist, GSK3008348, has yet to be published.

Inhibitors of PI3 kinase pathway Similarities between IPF and cancer have been proposed in recent years [111]. Phosphoinositide 3-kinases (PI3K) are a family of enzymes that mediate an array of intracellular transducing signals and are activated by tyrosine kinase receptors and Ras and G-protein-coupled receptors. There are eight PI3K isoforms that are divided into three classes. Class I PI3K signaling has been found to be involved in cell cycle progression, growth, and proliferation and is one of the most frequently deregulated pathways in cancer. It has been observed that

class I PI3K isoforms are overexpressed in IPF tissues [112] and could be involved in fibroblast proliferation, collagen deposition by activation of the TGF-β1 pathway, and myofibroblast differentiation [113].

Inhibition of class I PI3K showed antiproliferative effects for fibroblasts in vitro and antifibrotic effects in vivo (TNF-α-induced fibrosis). Therefore, P13K has been proposed as a potential therapeutic target for IPF. The inhibitor of PI3K/mammalian target of rapamycin (mTOR), known as GSK2126458, showed activity in reducing functional responses in IPF-derived lung fibroblasts [114]. A dose-finding, double-blind, placebo-controlled study (NCT01725139) testing GSK2126458 in subjects with IPF has recently been completed, but results have yet to be published.

The mammalian target of rapamycin (mTOR) is expressed in every cell type, and it is involved in cell motility, proliferation, and survival. As a consequence, rapamycin itself (known as sirolimus), an antibiotic and immunosuppressive drug, can be used as an antagonist of PI3K/mTOR in clinical studies. It has been demonstrated that sirolimus can have antifibrotic proprieties in bleomycin-induced fibrosis in mice [115]. An ongoing double-blind, placebo-controlled trial is evaluating the ability of sirolimus to reduce the number of circulating fibrocytes (NCT01462006).

Inhibition of Rho-associated protein kinases 2 (ROCK2) Rho-associated protein kinases (ROCKs) are a family of kinases involved in cellular proliferation, contraction, and migration [116]. In fibrotic rodent lungs, ROCK inhibition has been shown to reduce fibroblast migration and differentiation in myofibroblasts [117]. A selective inhibitor of ROCK2, KD025 (formerly Slx-2119), has been found to be well tolerated in a phase 1 study when administered orally [118]. An ongoing phase 2 trial is testing the safety and efficacy of oral KD025 while assessing change in FVC from baseline to 24 weeks (NCT02688647).

Inhibition of Autotaxin Autotaxin, also known as ENPP2, is an enzyme that converts lysophophatidylcholine into lysophosphatidic acid (LPA). LPA works as a lipidic signal that induces fibroblast recruitment and survival and epithelial apoptosis [119]. Furthermore, it can also induce endothelial damage and vascular dysfunction with subsequent vascular leak, coagulation, and fibrosis [120].

High levels of LPA have been found in BAL fluid from patients with IPF [121]. In addition, autotaxin levels have been found to be higher in lungs of IPF patients compared with normal subjects [122].

For these reasons, reducing LPA production by inhibiting autotaxin may provide a good target for therapy in IPF. GLPG1690 is a selective inhibitor of autotaxin and has demonstrated efficacy in animal models as well as tolerability and safety in healthy humans in a phase I trial (NCT02179502). An ongoing phase 2, placebo-controlled trial (NCT02738801) will evaluate the safety, tolerability, and pharmacological properties in IPF patients.

LPA receptor antagonists LPA effects can be reduced by inhibiting LPA receptors. Five types of receptors are known (LPA 1–5) and belong to the subfamily of G-protein-coupled receptors (GPCRs). LPA binds its receptors and activates the

RHO kinase pathway [123]. The effects of the activation of two receptors in particular (LPA-1 and LPA-2) have been studied. LPA-1 is involved in chemotactic activity of fibroblasts, while LPA-2 is expressed in epithelial cells and mediates TGF-β activation by αvβ6 integrin through the RHO kinase pathway.

Complexes of LPA-LPA1 have been found to be overexpressed in BAL fluid from mice with bleomycin-induced pulmonary fibrosis [121], and antagonists of the LPA-1 receptor have been studied to evaluate their ability to reduce vascular damage, fibroblast recruitment, and collagen deposition. The receptor antagonist, AM966, has been shown to reduce fibrosis in a murine model of bleomycin-induced fibrosis [124]. BMS-986020 is the name of an orally administered LPA-1 antagonist that has been compared to placebo in IPF patients treated for 26 weeks (NCT01766817). However, results of this RCT have not yet become available.

Inhibition of the JNK pathway The JNK kinases (c-Jun N-terminal kinases) belong to the superfamily of MAP kinases (mitogen-activated protein kinase), and JNK1 is also known as MAPK8. The JNK pathway is activated by different stress stimuli and is involved in cell proliferation, differentiation, and apoptosis. High activated levels of JNK have been found in IPF patients and in models of bleomycin-induced fibrosis. The JNK pathway is also involved in the downregulation of VEGF-D expression by TGF-β, thereby promoting fibrosing processes [125, 126]. An ongoing clinical trial (NCT02510937) enrolling IPF patients is evaluating the JNK inhibitor, CC-90001, to determine safety, tolerability, and pharmacologic features.

Inhibition of Galectin-3 The galactoside-binding lectin, galectin-3 (Gal-3), is strictly related to the TGF-β pathway and induces pulmonary fibrosis through the activation and differentiation of myofibroblasts and the production of collagen [127]. In Gal-3-deficient murine models, fibrotic mechanisms were significantly reduced both in the case of TGF-β and bleomycin-induced lung fibrosis. Moreover, BAL fluid analysis from IPF patients showed increased levels of Gal-3 [128].

An antagonist of Gal-3 (TD139) administered via dry powder inhaler (DPI) has been tested with promising results in terms of safety, tolerability, and pharmacological properties in healthy subjects and IPF patients in a phase 1/2 study (NCT02257177).

Antibodies to anti-lysyl oxidase (LOX) Lysyl oxidasesare a group of enzymes involved in collagen type I cross-linking. Through this mechanism, they contribute to the stiffness of the ECM. Indeed, mechanical properties of extracellular matrix (ECM) are highly relevant in fibrosing processes. The stiffness of fibrotic ECM and the contraction of myofibroblasts amplify TGF-β pathway activation. The resulting collagen deposition by fibroblasts builds the framework in which fibroblasts and myofibroblasts themselves may develop and differentiate in a self-sustained cycle [129].

Lysyl oxidase-like 2 (LOXL2) is the most studied enzyme of the LOX family. LOXL2 was found to be increased in fibrotic lung biopsies, and it has been suggested that LOXL2 might be involved in IPF progression [46]. It has therefore been proposed that inhibiting this enzyme could stop the self-maintaining process of ECM

deposition [130]. The humanized monoclonal antibody, GS-6624 (simtuzumab), that binds LOXL2 has been studied in a phase 2 double-blind, placebo-controlled RCT (NCT01769196). However, no significant differences in study endpoints were found for simtuzumab-treated patients with IPF versus placebo [131].

Immunity and Autoimmunity

Therapies targeting Leukotrienes Leukotrienes (LTs)are a group of pro-inflammatory and pro-fibrogenic mediators derived from the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism that are involved in inflammation, hypersecretion, contraction of smooth muscle cells, and increased vascular permeability. LTs (LTB4 in particular) have been identified as playing a role in sustaining pulmonary fibrosis [132]. Greatly increased levels of leukotriene B4 (LTB4) have been found in lung homogenates IPF patients [133].

The oral LTB4-antagonist (ONO-4057) has shown effects in reducing degranulation of neutrophils; decreasing levels of TGF-β, IL-6, and IL-13; and inhibiting bleomycin-related pulmonary fibrosis [134].

A phase 2, placebo-controlled, double-blind RCT (NCT02503657) is currently evaluating the efficacy, safety, and tolerability of oral tipelukast (also known as MN-001). This drug has been already tested for mild to moderate asthma, and it has been shown to inhibit PDE 3–4, antagonize the LTB4 receptor, and inhibit LOXL2.

Antibodies to Interleukins (IL-13 and IL-4) IL-13 and IL-4 are mainly secreted by T-helper type 2 (Th2) lymphocytes and activated macrophages. These cytokines are involved in a wide variety of disorders including IPF [135]. IL-13 is a key mediator of tissue fibrosis and promotes collagen deposition [136]. It has been found in both BAL fluid and lung tissues from patients with IPF and IL-13 levels correlating inversely with FVC [137]. IL-4 has been shown to increase alpha-smooth muscle actin and collagen III synthesis by human lung fibroblasts and may play a relevant role in the abnormal fibroblast proliferation in fibrotic lungs and in the differentiation of myofibroblasts [138].

Lebrikizumab is a humanized anti-IL-13 antibody that is being evaluated in a phase 2, double-blind, placebo-controlled RCT to assess the safety and efficacy of this agent in IPF patients with or without a background of pirfenidone treatment. The primary endpoint of the study is the absolute change from baseline in percent predicted FVC at week 52 (NCT01872689). Another anti-IL-13 antibody, tralokinumab, was tested in a phase 2 RCT, but the trial was stopped for lack of efficacy (NCT01629667). A third anti-IL-13 antibody (QAX576) has been evaluated for the treatment of IPF (NCT00532233); although the study has been completed, results have not yet been reported. Finally, a monoclonal antibody that targets both IL-13 and IL-4 (SAR156597) is being tested in a phase 2, double-blind, placebo-controlled, 52-week, dose-ranging RCT (NCT02345070). The primary efficacy endpoint for this subcutaneously administered antibody is the absolute change in FVC% predicted from baseline.

Human recombinant Pentraxin-2 (PTX-2) Pentraxins (PTXs)are a family of proteins synthetized by the liver. PTX-1, generally known as C-reactive protein, and PTX-3 are acute phase molecules. PTX-2 (also known as serum amyloid P) appears to play a regulatory role in wound healing and scar resolution [139]. IPF patients have low circulating levels of PTX-2, and its supplementation in animal models of fibrosis has shown promising results [140]. Therefore, it has been hypothesized that administration of the recombinant form of human PTX-2 (PRM-151) could be a potential therapeutic strategy for treating IPF [141].

Intravenous administration of the drug has been found to be tolerable and safe, and treatment with PRM-151 was associated with a reduction in circulating fibrocytes [142]. A phase 1 double-blind, placebo-controlled RCT (NCT01254409) reported increased pulmonary function measures in PRM-151-treated patients, although this result was not statistically significant. A phase 2, double-blind, placebo-controlled RCT (NCT02550873) is currently evaluating PRM-151 with a primary study outcome of change in FVC% predicted from baseline.

Type V collagen-induced immunotolerance Type V collagen is a form of fibrillary collagen that associates with type I collagen fibrils and regulates collagen fibrillogenesis. Lung injury can result in the release of collagen V fragments that may be recognized as "foreign antigens" by the immune system leading to a T-cell-dependent immune response with abnormal lung remodeling collagen accumulation. Peripheral blood mononuclear cells from patients with IPF or lung transplant recipients can be sensitized to collagen V [143], and levels of both anti-collagen V antibodies in the peripheral circulation and expression of the alpha-1 chain of collagen V in lung tissues were found to be increased in patients with IPF [144]. Because inducing immune tolerance to collagen V represents a potential therapeutic strategy in IPF, the immunomodulator, IW001 (an orally administered collagen V solution given to induce tolerance to collagen V), has been tested in an open-label, phase 1 study (NCT01199887) that demonstrated adequate safety and tolerability, showing a trend toward dose-dependent efficacy (stabilization of FVC) [145].

Rituximab The abnormal reactivity of B lymphocytes against self-matrix components and the presence of circulating autoantibodies in IPF patients support the rationale for using rituximab, a monoclonal antibody directed against CD20 [146]. A phase 2 RCT testing two different doses of rituximab against placebo is currently recruiting IPF patients (NCT01969409). Another trial is evaluating the efficacy of combination therapy including rituximab in acute exacerbations of IPF (NCT01266317).

Dasatinib Lck and Fyn are two members of the Src family kinases. These tyrosine kinase proteins are key components of the T-cell antigen receptor (TCR) signaling complex. Activated Src influences the production and differentiation of myofibroblasts, proliferation of mesenchymal cells, and collagen deposition. Dasatinib is an inhibitor of the Src family of kinases and other tyrosine kinase proteins such as platelet-derived growth factor receptor (PDGFR). Dasatinib has been shown to reduce bleomycin-induced lung fibrosis in animal models [147, 148]. Dasatinib together with quercetin, a flavonol that reduces free radicals and modulates oxida-

tive processes, will be tested in a phase 1, open-label study evaluating the expression of pro-inflammatory cells in skin biopsies from patients with IPF (NCT02874989). Table 13.2 shows a summary of emerging therapies targetng specific molecular pathways.

Antibiotics and Antiviral Drugs

One of the pathogenetic mechanisms that leads to development and progression of IPF is repetitive alveolar damage and aberrant wound healing in genetically

Table 13.2 Emerging therapies targeting specific molecular pathways

Mechanism of action	Investigational drug	Clinical trial number
Extracellular matrix (ECM) deposition		
Antibodies anti-connective tissue growth factor (CTGF)	FG-3019	NCT01262001 NCT01890265 Phase II
	PBI-4050	NCT02538536 Phase II
Integrin αvβ6-antagonist	BG00011 (STX100)	NCT01371305 Phase II
	GSK3008348	NCT02612051 Phase I
Antagonist of PI3K/mTOR	GSK2126458	Mercer et al. [114] NCT01725139
	Sirolimus	Jin et al. [115] NCT01462006
Inhibitor of ROCK2	KD025	Zanin-Zhorov et al. [118] NCT02688647 Phase II
Inhibitor of autotaxin	GLPG1690	NCT02179502 NCT02738801 Phase II
LPA1 receptor antagonists	AM966	Swaney etal. [124]
	BMS-986020	NCT01766817 Phase II
Inhibition of JNK pathway	CC-90001	NCT02510937 Phase I/II
Inhibition of galactoside-binding lectin galectin-3	TD139	NCT02257177 Phase I/II
Antibodies anti-lysyl oxidase	GS-6624 (simtuzumab)	Raghu et al. [128]
Immunity and autoimmunity		
Inhibition of PDE 3-4, LTB4-antagonist, inhibition of LOXL2	Tipelukast	NCT02503657 Phase II
Antibodies anti-interleukins-13	Lebrikizumab	NCT01872689 Phase II
	Tralokinumab	NCT01629667
	QAX576	NCT00532233 Phase II
Antibodies anti-interleukins-13 and IL-4	SAR156597	NCT02345070 Phase II
Recombinant form of human PTX-2 involved in wound healing	PRM-151	NCT01254409 NCT02550873 Phase II
Type V collagen immunomodulation	IW001	Wilkes et al. [145]
B-lymphocyte immunomodulation	Rituximab	NCT01969409 Phase II NCT01266317
Inhibition of Src family kinases and reduction of oxidative processes	Dasatinib + quercetin	NCT02874989 Phase I

predisposed patients. Viruses and bacteria could be involved in these processes, but their role is still open to debate [149].

The herpesvirus family may play a relevant role in this regard. It has been demonstrated that anti-cytomegalovirus (CMV) IgG antibodies were present in 80% of IPF patients versus 30% of controls [150], and anti-Epstein-Barr virus (EBV) IgA antibodies have been reported in 60% of IPF patients versus 22% of controls [151]. These observations suggest that antiviral therapy with valacyclovir and ganciclovir might represent a potential strategy that may modify the course of IPF. A small open-label study (14 patients) testing a 2-week course of ganciclovir in severe IPF patients with positive EBV-IgG serology reported that nine patients showed a significant clinical response [152].

The role of bacteria in IPF pathogenesis is even less clear, but there is evidence that suggests an association between IPF and the lung microbiome [149, 153]. It has been reported that IPF patients have an increased bacterial burden in their BAL fluid compared with controls, and these investigators showed that microbial as well as host transcriptome signatures in peripheral blood that reflect a host response to the presence of an altered or more abundant microbiome remained elevated over time in patients with disease progression versus those with stable disease, suggesting that bacteria in the distal airways may represent persistent stimuli that cause repetitive alveolar injury [153]. Additionally, another study showed an association between disease progression and the presence of specific members within the *Staphylococcus* and *Streptococcus* genera [154]. These findings suggest that antibiotic therapies might be useful in modifying IPF progression.

Co-trimoxazole has been tested against placebo in IPF patients in conjunction with approved antifibrotic therapy; no effect on lung function was reported, but co-trimoxazole was shown to be associated with improvement in quality of life and reduction in all-cause mortality [155]. An unblinded, phase 3, multicenter RCT comparing the effect of standard care versus standard-of-care plus antimicrobial therapy (co-trimoxazole or doxycycline) is currently recruiting patients (NCT02759120). The hypothesis being tested in this RCT is that reducing the microbial burden with antimicrobial therapy will reduce the risk of nonelective, respiratory hospitalization or death in IPF patients. In addition, a prospective, randomized, double-blind, two-period crossover study is currently evaluating the possible role of azithromycin versus placebo (NCT02173145). Additionally, immunomodulatory properties of macrolides could improve lung function and cough.

Pharmacologic Treatment of Acute Exacerbation of IPF

Introduction

The new definition of acute exacerbation of IPF (AE-IPF) includes any acute, clinically significant respiratory deterioration during a brief period of time, which is typically less than 1 month. This condition is characterized by the evidence of

new alveolar abnormalities that are not fully explained by cardiac failure or fluid overload [156].

AE-IPF is notoriously difficult to manage, especially since there are no validated or effective therapies available. To date, preventing the development of AE-IPF episodes could represent a very effective therapeutic strategy. Results from clinical trials of both pirfenidone and nintedanib suggest that these IPF therapies may help prevent the development of AE-IPF [156], and antiacid therapy with PPIs or histamine-2 blockers might also have a preventive effect on AE-IPF [90].

Corticosteroids

High-dose systemic corticosteroids are usually administered in clinical practice for treatment of AE-IPF. This medical intervention has a weak positive recommendation by the ATS/ERS/JRS/ALAT guidelines published in 2011 and is based on the potential benefit of corticosteroids for organizing pneumonia and a possible effect on acute lung injury [1]. The dosage and duration of corticosteroid therapy are not specified in the literature, but a daily dose of 0.5–1.0 g of methylprednisolone is usually administered for 3–5 days in patients with respiratory failure and followed by a dosage taper [157]. Doubts and uncertainties about this recommendation persist as corticosteroids as a monotherapy have no beneficial role in the management of stable IPF [158], and their reported benefits have only emanated from observational and anecdotal studies [8].

In the first reports of corticosteroid use for AE-IPF, improvements in chest X-ray findings, pulmonary function, and blood gas values were reported [159]. However, consecutive retrospective reviews reported a very high mortality rate among patients treated with steroids [160, 161]. Moreover, treatment with corticosteroids does not seem to prevent AE-IPF. Indeed, in studies where administration of corticosteroids was allowed, the incidence of AE-IPF and lower respiratory tract infections was reported to be higher [162].

Antibiotics

As stated in the revised definition and diagnostic criteria for acute exacerbation of IPF, exclusion of infection or other potential triggers is no longer required for the diagnosis of acute exacerbation [156]. Many patients receive empiric broad-spectrum antibiotic therapy even if there is no definite evidence of infection. The long-standing issue of antibiotic resistance is of concern as are the associated high costs and antibiotic-related adverse events. A single-center experience shows that a procalcitonin-guided strategy could be useful in AE-IPF as a means of reducing exposure to antibiotics, and this reduced antibiotic treatment duration has not been shown to be associated with worse outcomes [163]. Evidence from some studies

suggest that therapy with co-trimoxazole and macrolides may reduce the incidence and mortality of AE-IPF, most likely due to their anti- inflammatory effects and antimicrobial activity [164–166].

Other Therapies

Because AE-IPF is associated with acute clinical declines and a poor prognosis, other strategies have been evaluated but have not been proven to be efficacious. Small and uncontrolled trials including corticosteroid monotherapy, cyclophosphamide, cyclosporine, polymyxin B-immobilized fiber column hemoperfusion, rituximab with plasma exchange, and intravenous immunoglobulin, tacrolimus, and thrombomodulin have all been performed. All these therapies should, however, be considered for testing in RCTs to better elucidate their potential role in the management of AE-IPF [156].

Cyclosporine A (CsA)

Cyclosporine A is a calcineurin inhibitor that inhibits activation of T lymphocytes by blocking the transcription of IL-2 and related cytokines that are regulated by the nuclear factor of activated T-cell (NF-AT) transcription factor. Moreover, CsA has a direct effect on macrophages by inhibiting inflammatory cytokine production. It inhibits tumor growth factor (TGF)-β-induced signaling in vitro and collagen deposition in human lung fibroblasts [167]. The potential role of CsA in the management of AE-IPF is predicated by these specific characteristics, and a few retrospective analyses of AE-IPF cases have suggested a better survival rate for patients treated with CsA plus steroids in comparison with untreated subjects [168–170].

Tacrolimus

Tacrolimus (Tac) is also a calcineurin inhibitor and a specific inhibitor of T-lymphocyte function that has similar characteristics to that of CsA [171] but with 100-fold greater immunosuppressant potency [172]. A retrospective Japanese study in a small group of patients experiencing AE-IPF suggested that Tac plus methylprednisolone was more effective than steroids alone in the acute phase of AE-IPF [173].

Cyclophosphamide

Cyclophosphamide (CYC) is a cytotoxic immunosuppressive agent that suppresses lymphokine production and modulates lymphocyte function by alkylating various cellular constituents and suppressing the inflammatory response. It has been used in scleroderma lung disease with a marginal effect on lung function [174]. However,

treatment of AE-IPF with CYC has not been shown to have significant efficacy, and its routine use is not supported [166, 175–177].

Rituximab with Plasma Exchange

Autoantibodies have been shown to be involved in IPF progression, but autoantibody-related aspects of IPF pathogenesis do not appear to respond to corticosteroid therapy. A pilot study (clinical trial NCT01266317) has demonstrated that rituximab plus plasma exchange plus intravenous immunoglobulin for treatment of AE-IPF improved 1-year survival as compared to a group of historical controls [178].

Oral Anticoagulant and Thrombomodulin

Historically, IPF was considered to be associated with a procoagulant condition, suggesting a link between fibrosis and thrombosis. One study by Kubo et al. [42] comparing steroid treatment with steroid plus warfarin suggested that anticoagulant therapy reduced the mortality associated with AE-IPF. However, this study had significant deficiencies in the study protocol [42]. A subsequent RCT of warfarin as a therapy for IPF was halted early because of a lack of benefit and increased mortality in the anticoagulated group [43].

Thrombomodulin is an integral membrane protein that is expressed on the surfaces of endothelial cells which plays an important role in regulating coagulation, and it has both potent anticoagulant and anti-inflammatory effects. Recombinant thrombomodulin (rhTM) is used in clinical conditions such as disseminated intravascular coagulation (in Japan). In a prospective study, Tsushima et al. demonstrated that administering rhTM at a dose of 0.06 mg/kg/day reduced mortality in patients with AE-IPF on mechanical ventilation [179]. Other studies have also examined the use of rhTM as a treatment for AE-IPF in association with other modalities of care [180, 181]. Although these studies supported a better survival in AE-IPF patients treated with rhTM compared to the groups without rhTM, one study was retrospective and the other used a historical control group.

Polymyxin B-Immobilized Fiber Column Hemoperfusion

Polymyxin B-immobilized fiber column hemoperfusion is an instrument for extracorporeal hemoperfusion consisting of PMX-linked polystyrene chloroacetamide-methyl fibers chemically immobilized through covalent bonds. It was first used in sepsis to reduce circulating pro-inflammatory, profibrotic, and pro-angiogenic cytokines as well as reactive oxygen species (ROS) [182, 183]. Its use in sepsis provided the rationale for polymyxin B-immobilized fiber column hemoperfusion as a possible therapy for AE-IPF. Although a positive effect on mortality has been suggested [184–186], additional studies are needed to confirm a therapeutic role for AE-IPF.

Supportive Care

An important aspect of AE-IPF management is to provide supportive care, and all available measures should be used to increase patient comfort. Patients that undergo hospitalization with respiratory failure should be treated with oxygen to correct hypoxemia and improve dyspnea, and high-flow oxygen delivery mechanisms may be required in many cases [187]. International evidence-based guidelines make a weak recommendation against the use of noninvasive ventilation (NIV) in patients with respiratory failure due to IPF [1]. With this approach, the option of NIV should be considered with due consideration of the patient's wishes and their presumptive prognosis. Pharmacologic modulation of dyspnea with benzodiazepines and/or opiates is an acceptable strategy and should be used with close monitoring of oxyhemoglobin saturation to avoid overt respiratory depression.

Ongoing Trials

As far as future therapeutic options are concerned, the identification of effective therapies for treating AE-IPF should be a major field of interest in the next decade. Currently there are two ongoing double-blind, placebo-controlled RCTs for patients with AE-IPF. A phase 3 RCT is evaluating the effect of combined cyclophosphamide and prednisolone in comparison with prednisolone alone (NCT02460588). A second phase 3 RCT is comparing recombinant thrombomodulin (ART-123; 380 U/kg/day) via intravenous drip infusion)) given in addition to corticosteroids versus corticosteroid alone to evaluate the effect of recombinant human thrombomodulin alpha versus placebo on a 90-day survival (primary endpoint) in patients with AE-IPF (NCT02739165).

References

1. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med [Internet]. 2011;183(6):788–824. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21471066.
2. Raghu G, Rochwerg B, Zhang Y, et al. An official ATS/ERS/JRS/ALAT clinical practice guideline: treatment of idiopathic pulmonary fibrosis: an update of the 2011 clinical practice guideline. Am J Respir Crit Care Med [Internet]. 2015 [cited 2017 May 29];192(2):e3–e19. https://doi.org/10.1164/rccm.201506-1063ST.
3. Idiopathic pulmonary fibrosis: diagnosis and treatment. Am J Respir Crit Care Med. [Internet] 2000 [cited 2017 May 28];161(2):646–664. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10673212.
4. McAnulty RJ, Laurent GJ. Pathogenesis of lung fibrosis and potential new therapeutic strategies. Exp Nephrol [Internet]. [cited 2017 May 28]3(2):96–107. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7773645

5. Mapel DW, Samet JM, Coultas DB. Corticosteroids and the treatment of idiopathic pulmonary fibrosis. Past, present, and future. Chest [Internet]. 1996 [cited 2017 May 28];110(4):1058–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8874268.
6. B.T. SOCIETY and S.O. COMMITTEE. The diagnosis, assessment and treatment of diffuse parenchymal lung disease in adults. Introduction. Thorax [Internet] 1999 [cited 2017 May 28];54 Suppl 1:S1–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11006787.
7. Hanson D, Winterbauer RH, Kirtland SH, Wu R. Changes in pulmonary function test results after 1 year of therapy as predictors of survival in patients with idiopathic pulmonary fibrosis. Chest [Internet]. 1995 [cited 2017 May 28];108(2):305–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7634857.
8. Richeldi L, Davies HRHR, Ferrara G, Franco F. Corticosteroids for idiopathic pulmonary fibrosis. Cochrane Database Syst Rev. [Internet] 2003 [cited 2017 May 28];(3):CD002880. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12917934.
9. Turner-Warwick M, Burrows B, Johnson A. Cryptogenic fibrosing alveolitis: clinical features and their influence on survival. Thorax [Internet]. 1980 [cited 2017 May 28];35(3):171–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7385089.
10. Douglas WW, Ryu JH, Schroeder DR. Idiopathic pulmonary fibrosis: impact of oxygen and colchicine, prednisone, or no therapy on survival. Am J Respir Crit Care Med [Internet]. 2000 [cited 2017 Jun 12];161(4 I):1172–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10764308.
11. Raghu G, Depaso WJ, Cain K, et al. Azathioprine combined with prednisone in the treatment of idiopathic pulmonary fibrosis: a prospective double-blind, randomized, placebo-controlled clinical trial. Am Rev Respir Dis [Internet]. 1991 [cited 2017 May 29];144(2):291–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1859050.
12. Flaherty KR, Toews GB, Lynch JP, et al. Steroids in idiopathic pulmonary fibrosis: a prospective assessment of adverse reactions, response to therapy, and survival. Am J Med [Internet]. 2001 [cited 2017 May 29];110(4):278–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11239846.
13. Turner-Warwick M, Burrows B, Johnson A. Cryptogenic fibrosing alveolitis: response to corticosteroid treatment and its effect on survival. Thorax [Internet]. 1980 [cited 2017 May 29];35(8):593–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7444826.
14. Shah NR, Noble P, Jackson RM, et al. A critical assessment of treatment options for idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffus lung Dis Off J WASOG [Internet]. 2005 [cited 2017 May 29];22(3):167–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16315778.
15. Johnson MA, Kwan S, Snell NJ, Nunn AJ, Darbyshire JH, Turner-Warwick M. Randomised controlled trial comparing prednisolone alone with cyclophosphamide and low dose prednisolone in combination in cryptogenic fibrosing alveolitis. Thorax [Internet]. 1989 [cited 2017 May 29];44(4):280–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2669218.
16. Zisman DA, Lynch JP 3rd, Toews GB, Kazerooni EA, Flint A, Martinez FJ. Cyclophosphamide in the treatment of idiopathic pulmonary fibrosis: a prospective study in patients who failed to respond to corticosteroids. Chest [Internet]. 2000 [cited 2017 May 29];117(6):1619–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10858393.
17. Baughman RP, Lower EE. Use of intermittent, intravenous cyclophosphamide for idiopathic pulmonary fibrosis. Chest [Internet]. 1992 [cited 2017 May 29];102(4):1090–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1395749.
18. Collard HR, Ryu JH, Douglas WW, et al. Combined corticosteroid and cyclophosphamide therapy does not alter survival in idiopathic pulmonary fibrosis. Chest [Internet]. 2004 [cited 2017 May 29];125(6):2169–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15189938.
19. Lynch JP, McCune WJ. Immunosuppressive and cytotoxic pharmacotherapy for pulmonary disorders. Am J Respir Crit Care Med [Internet]. 1997 [cited 2017 May 28];155(2):395–420. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9032171.

20. Zhang L, Zhu Y, Luo W, Xi P, Yan Y. The protective effect of colchicine on bleomycin-induced pulmonary fibrosis in rats. Chin Med Sci J Chung-kuo i hsueh k'o hsueh tsa chih [Internet]. 1992 [cited 2017 May 29];7(1):58–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1384784.
21. Rennard SI, Bitterman PB, Ozaki T, Rom WN, Crystal RG. Colchicine suppresses the release of fibroblast growth factors from alveolar macrophages in vitro. The basis of a possible therapeutic approach of the fibrotic disorders. Am Rev Respir Dis [Internet]. 1988 [cited 2017 May 29];137(1):181–5. https://doi.org/10.1164/ajrccm/137.1.181.
22. Douglas WW, Ryu JH, Swensen SJ, et al. Colchicine versus prednisone in the treatment of idiopathic pulmonary fibrosis. A randomized prospective study. Members of the Lung Study Group. Am J Respir Crit Care Med [Internet]. 1998 [cited 2017 May 29];158(1):220–5. https://doi.org/10.1164/ajrccm.158.1.9709089.
23. Selman M, Carrillo G, Salas J, et al. Colchicine, D-penicillamine, and prednisone in the treatment of idiopathic pulmonary fibrosis: a controlled clinical trial. Chest [Internet]. 1998 [cited 2017 May 29];114(2):507–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9726738.
24. Peters SG, McDougall JC, Douglas WW, Coles DT, DeRemee RA. Colchicine in the treatment of pulmonary fibrosis. Chest [Internet]. 1993 [cited 2017 May 29];103(1):101–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8417861.
25. Addrizzo-Harris DJ, Harkin TJ, Tchou-Wong KM, et al. Mechanisms of colchicine effect in the treatment of asbestosis and idiopathic pulmonary fibrosis. Lung [Internet]. 2002 [cited 2017 May 29];180(2):61–72. https://doi.org/10.1007/s004080000083.
26. Demedts M, Behr J, Buhl R, et al. High-dose acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med [Internet]. 2005 [cited 2017 May 29];353(21):2229–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16306520
27. Idiopathic Pulmonary Fibrosis Clinical Research Network. Prednisone, azathioprine, and N -acetylcysteine for pulmonary fibrosis. N Engl J Med [Internet]. 2012 [cited 2017 Jun 12];366(21):1968–77. https://doi.org/10.1056/NEJMoa1113354.
28. Montuschi P, Ciabattoni G, Paredi P, et al. 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. Am J Respir Crit Care Med [Internet]. 1998 [cited 2017 Jun 13];158(5 PART I):1524–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9817703.
29. Behr J, Maier K, Degenkolb B, Krombach F, Vogelmeier C. Antioxidative and clinical effects of high-dose N-acetylcysteine in fibrosing alveolitis. Adjunctive therapy to maintenance immunosuppression. Am J Respir Crit Care Med [Internet]. 1997 [cited 2017 May 29];156(6):1897–901. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9412572.
30. The Idiopathic Pulmonary Fibrosis Clinical Research Network. Randomized trial of acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med [Internet]. 2014 [cited 2017 Jun 13];370(22):2093–101. https://doi.org/10.1056/NEJMoa1401739.
31. Homma S, Azuma A, Taniguchi H, et al. Efficacy of inhaled N-acetylcysteine monotherapy in patients with early stage idiopathic pulmonary fibrosis. Respirology [Internet]. 2012 [cited 2017 Jun 13];17(3):467–77. https://doi.org/10.1111/j.1440-1843.2012.02132.x.
32. Noth I, Zhang Y, Ma S-F, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. Lancet Respir Med [Internet]. 2013 [cited 2017 Jun 12];1(4):309–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24429156.
33. Oldham JM, Ma S-F, Martinez FJ, et al. TOLLIP, MUC5B, and the response to N-acetylcysteine among individuals with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Internet]. 2015 [cited 2017 Jun 12];192(12):1475–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26331942.
34. Narayanan AS, Whithey J, Souza A, Raghu G. Effect of gamma-interferon on collagen synthesis by normal and fibrotic human lung fibroblasts. Chest [Internet]. 1992 [cited 2017 May 18];101(5):1326–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1582292.
35. Prior C, Haslam PL. In vivo levels and in vitro production of interferon-gamma in fibrosing interstitial lung diseases. Clin Exp Immunol [Internet]. 1992 [cited 2017 May 17];88(2):280–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1572093.

36. Ziesche R, Hofbauer E, Wittmann K, Petkov V, Block L-H. A preliminary study of long-term treatment with interferon gamma-1b and low-dose prednisolone in patients with idiopathic pulmonary fibrosis. N Engl J Med [Internet]. 1999 [cited 2017 May 17];341(17):1264–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10528036.
37. Raghu G, Brown KK, Bradford WZ, et al. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. N Engl J Med [Internet]. 2004 [cited 2017 May 17];350(2):125–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14711911.
38. King TE, Albera C, Bradford WZ, et al. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. Lancet [Internet]. 2009 [cited 2017 May 18];374(9685):222–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19570573.
39. Spagnolo P, Giovane C Del, Luppi F, et al. Non-steroid agents for idiopathic pulmonary fibrosis. Cochrane Database Syst Rev. [Internet] 2010 [cited 2017 May 18];(9):CD003134. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20824834.
40. Crooks MG, Hart SP. Coagulation and anticoagulation in idiopathic pulmonary fibrosis. Eur Respir Rev [Internet]. 2015;24(137):392–9. Available from: http://err.ersjournals.com/content/24/137/392.
41. Navaratnam V, Fogarty AW, McKeever T, et al. Presence of a prothrombotic state in people with idiopathic pulmonary fibrosis: a population-based case–control study. Thorax [Internet]. 2014 [cited 2017 May 18];69(3):207–15. https://doi.org/10.1136/thoraxjnl-2013-203740.
42. Kubo H, Nakayama K, Yanai M, et al. Anticoagulant therapy for idiopathic pulmonary fibrosis. Chest [Internet]. 2005 [cited 2017 May 19];128(3):1475–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16162746.
43. Noth I, Anstrom KJ, Calvert SB, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Internet]. 2012 [cited 2017 May 19];186(1):88–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22561965.
44. Kreuter M, Wijsenbeek MS, Vasakova M, et al. Unfavourable effects of medically indicated oral anticoagulants on survival in idiopathic pulmonary fibrosis. Eur Respir J [Internet]. 2016 [cited 2017 May 19];47(6):1776–84. https://doi.org/10.1183/13993003.01482-2016.
45. Shea BS, Probst CK, Brazee PL, et al. Uncoupling of the profibrotic and hemostatic effects of thrombin in lung fibrosis. JCI Insight. [Internet] 2017 [cited 2017 May 19];2(9). Available from: http://www.ncbi.nlm.nih.gov/pubmed/28469072.
46. Schuliga M, Jaffar J, Berhan A, et al. Annexin A2 contributes to lung injury and fibrosis by augmenting factor Xa fibrogenic activity. Am J Phys Lung Cell Mol Phys [Internet]. 2017 [cited 2017 May 19];312(5):L772–82. https://doi.org/10.1152/ajplung.00553.2016.
47. Ghofrani HA, Wiedemann R, Rose F, et al. Sildenafil for treatment of lung fibrosis and pulmonary hypertension: a randomised controlled trial. Lancet [Internet]. 2002 [cited 2017 May 19];360(9337):895–900. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12354470.
48. Milara J, Escrivá J, Ortiz JL, et al. Vascular effects of sildenafil in patients with pulmonary fibrosis and pulmonary hypertension: an ex vivo/in vitro study. Eur Respir J [Internet]. 2016 [cited 2017 May 20];47(6):1737–49. https://doi.org/10.1183/13993003.01259-2015.
49. Idiopathic Pulmonary Fibrosis Clinical Research Network, Zisman DA, Schwarz M, et al. A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. N Engl J Med [Internet]. 2010 [cited 2017 May 19];363(7):620–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20484178.
50. Han MK, Bach DS, Hagan PG, et al. Sildenafil preserves exercise capacity in patients with idiopathic pulmonary fibrosis and right-sided ventricular dysfunction. Chest [Internet]. 2013 [cited 2017 May 20];143(6):1699–708. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0012369213604018.
51. Jackson RM, Glassberg MK, Ramos CF, Bejarano PA, Butrous G, Gómez-Marín O. Sildenafil therapy and exercise tolerance in idiopathic pulmonary fibrosis. Lung [Internet]. 2010 [cited 2017 May 19];188(2):115–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20012639.

52. Rochwerg B, Neupane B, Zhang Y, et al. Treatment of idiopathic pulmonary fibrosis: a network meta-analysis. 2016 [cited 2017 May 18];14(1):18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26843176
53. Fonseca C, Abraham D, Renzoni EA. Endothelin in pulmonary fibrosis. Am J Respir Cell Mol Biol [Internet]. 2011 [cited 2017 May 21];44(1):1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20448055.
54. Park SH, Saleh D, Giaid A, Michel RP. Increased endothelin-1 in bleomycin-induced pulmonary fibrosis and the effect of an endothelin receptor antagonist. Am J Respir Crit Care Med [Internet]. 1997 [cited 2017 May 21];156(2 I):600–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9279246.
55. King TE, Behr J, Brown KK, et al. BUILD-1: a randomized placebo-controlled trial of Bosentan in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Internet]. 2008 [cited 2017 May 21];177(1):75–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17901413.
56. King TE, Brown KK, Raghu G, et al. BUILD-3: a randomized, controlled trial of Bosentan in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Internet]. 2011 [cited 2017 May 21];184(1):92–9. https://doi.org/10.1164/rccm.201011-1874OC.
57. Raghu G, Million-Rousseau R, Morganti A, Perchenet L, Behr J, MUSIC Study Group. Macitentan for the treatment of idiopathic pulmonary fibrosis: the randomised controlled MUSIC trial. Eur Respir J [Internet]. 2013 [cited 2017 May 21];42(6):1622–32. https://doi.org/10.1183/09031936.00104612.
58. Raghu G, Behr J, Brown KK, et al. Treatment of idiopathic pulmonary fibrosis with Ambrisentan. Ann Intern Med [Internet]. 2013 [cited 2017 May 21];158(9):641. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23648946.
59. Daniels CE, Wilkes MC, Edens M, et al. Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis. J Clin Invest [Internet]. 2004 [cited 2017 May 22];114(9):1308–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15520863.
60. Daniels CE, Lasky JA, Limper AH, et al. Imatinib treatment for idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Internet]. 2010 [cited 2017 May 22];181(6):604–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20007927.
61. Hilberg O, Simonsen U, du Bois R, Bendstrup E. Pirfenidone: significant treatment effects in idiopathic pulmonary fibrosis. Clin Respir J [Internet]. 2012 [cited 2017 Jul 20];6(3):131–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22697264.
62. Conte E, Gili E, Fagone E, Fruciano M, Iemmolo M, Vancheri C. Effect of pirfenidone on proliferation, TGF-β-induced myofibroblast differentiation and fibrogenic activity of primary human lung fibroblasts. Eur J Pharm Sci [Internet]. 2014 [cited 2017 Jul 20];58:13–9. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0928098714000840.
63. Kakugawa T, Mukae H, Hayashi T, et al. Pirfenidone attenuates expression of HSP47 in murine bleomycin-induced pulmonary fibrosis. Eur Respir J [Internet]. 2004 [cited 2017 Jul 20];24(1):57–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15293605.
64. Misra HP, Rabideau C. Pirfenidone inhibits NADPH-dependent microsomal lipid peroxidation and scavenges hydroxyl radicals. Mol Cell Biochem [Internet]. 2000 [cited 2017 Jul 20];204(1–2):119–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10718632.
65. Raghu G, Johnson WC, Lockhart D, Mageto Y. Treatment of idiopathic pulmonary fibrosis with a new antifibrotic agent, pirfenidone: results of a prospective, open-label phase II study. Am J Respir Crit Care Med [Internet]. 1999 [cited 2017 Jul 20];159(4 Pt 1):1061–9. https://doi.org/10.1164/ajrccm.159.4.9805017.
66. Azuma A, Nukiwa T, Tsuboi E, et al. Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Internet]. 2005 [cited 2017 Feb 4];171(9):1040–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15665326.
67. Taniguchi H, Ebina M, Kondoh Y, et al. Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J [Internet]. 2010 [cited 2017 Feb 4];35(4):821–9. https://doi.org/10.1183/09031936.00005209.

68. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet [Internet]. 2011 [cited 2017 Jul 20];377(9779):1760–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21571362.
69. King TE, Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med [Internet]. 2014;370(22):2083–92. https://doi.org/10.1056/NEJMoa1402582.
70. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone for idiopathic pulmonary fibrosis: analysis of pooled data from three multinational phase 3 trials. Eur Respir J [Internet]. 2016 [cited 2017 Jul 20];47(1):243–53. https://doi.org/10.1183/13993003.00026-2015.
71. Nathan SD, Albera C, Bradford WZ, et al. Effect of pirfenidone on mortality: pooled analyses and meta-analyses of clinical trials in idiopathic pulmonary fibrosis. Lancet Respir Med [Internet]. 2017 [cited 2017 Jul 20];5(1):33–41. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2213260016303265.
72. Costabel U, Albera C, Bradford WZ, et al. Analysis of lung function and survival in RECAP: an open-label extension study of pirfenidone in patients with idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffus lung Dis Off J WASOG [Internet]. 2014 [cited 2017 Jul 20];31(3):198–205. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25363219.
73. Koschel D, Cottin V, Skold M, et al. Pirfenidone post-authorization safety registry (PASSPORT) – interim analysis of IPF treatment. Eur Respir J [Internet]. 2014 [cited 2017 Jul 20];44(Suppl 58):1904. Available from: http://erj.ersjournals.com/content/44/Suppl_58/1904.
74. Lancaster L, Albera C, Bradford WZ, et al. Safety of pirfenidone in patients with idiopathic pulmonary fibrosis: integrated analysis of cumulative data from 5 clinical trials. BMJ Open Respir Res [Internet]. 2016 [cited 2017 Jul 20];3(1):e000105. https://doi.org/10.1136/bmjresp-2015-000105.
75. Hilberg F, Roth GJ, Krssak M, et al. BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. Cancer Res [Internet]. 2008 [cited 2017 Jul 20];68(12):4774–82. https://doi.org/10.1158/0008-5472.CAN-07-6307.
76. Richeldi L, Costabel U, Selman M, et al. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med [Internet]. 2011 [cited 2017 Feb 4];365(12):1079–87. https://doi.org/10.1056/NEJMoa1103690.
77. Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med [Internet]. 2014 [cited 2017 Feb 4];370(22):2071–82. https://doi.org/10.1056/NEJMoa1402584.
78. Corte T, Bonella F, Crestani B, et al. Safety, tolerability and appropriate use of nintedanib in idiopathic pulmonary fibrosis. Respir Res [Internet]. 2015;16(1):116. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26400368.
79. Richeldi L, Cottin V, Flaherty KR, et al. Design of the INPULSIS™ trials: two phase 3 trials of nintedanib in patients with idiopathic pulmonary fibrosis. Respir Med [Internet]. 2014 [cited 2017 Jul 20];108(7):1023–30. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0954611114001565.
80. Richeldi L, Cottin V, du Bois RM, et al. Nintedanib in patients with idiopathic pulmonary fibrosis: combined evidence from the TOMORROW and INPULSIS?? Trials. Respir Med. 2015;113:74–9.
81. Kolb M, Richeldi L, Behr J, et al. Nintedanib in patients with idiopathic pulmonary fibrosis and preserved lung volume. Thorax [Internet]. 2017 [cited 2017 Jul 20];72(4):340–6. https://doi.org/10.1136/thoraxjnl-2016-208710.
82. Costabel U, Inoue Y, Richeldi L, et al. Efficacy of Nintedanib in idiopathic pulmonary fibrosis across Prespecified subgroups in INPULSIS. Am J Respir Crit Care Med [Internet]. 2016 [cited 2017 Jul 20];193(2):178–85. Available from: http://static.thoracic.org/podcast/AJRCCM-interviews/rccm-201503-0562OC.mp3.
83. Wuyts WA, Kolb M, Stowasser S, Stansen W, Huggins JT, Raghu G. First data on efficacy and safety of Nintedanib in patients with idiopathic pulmonary fibrosis and forced vital capacity of ≤50% of predicted value. Lung [Internet]. 2016 [cited 2017 Jul 20];194(5):739–43. https://doi.org/10.1007/s00408-016-9912-1.

84. Loveman E, Copley VR, Scott DA, Colquitt JL, Clegg AJ, O'Reilly KMA. Comparing new treatments for idiopathic pulmonary fibrosis – a network meta-analysis. BMC Pulm Med [Internet]. 2015 [cited 2017 Jul 20];15(1):37. https://doi.org/10.1186/s12890-015-0034-y.
85. Canestaro WJ, Forrester SH, Raghu G, Ho L, Devine BE. Drug treatment of idiopathic pulmonary fibrosis systematic review and network meta-analysis. Chest. 2016;149(3):756–66.
86. Linden PA, Gilbert RJ, Yeap BY, et al. Laparoscopic fundoplication in patients with end-stage lung disease awaiting transplantation. J Thorac Cardiovasc Surg [Internet]. 2006 [cited 2017 Jul 20];131(2):438–46. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0022522305017514.
87. Hoppo T, Jarido V, Pennathur A, et al. Antireflux surgery preserves lung function in patients with gastroesophageal reflux disease and end-stage lung disease before and after lung transplantation. Arch Surg [Internet]. 2011 [cited 2017 Jul 20];146(9):1041–7. https://doi.org/10.1001/archsurg.2011.216.
88. Raghu G, Morrow E, Collins BF, et al. Laparoscopic anti-reflux surgery for idiopathic pulmonary fibrosis at a single centre. Eur Respir J [Internet]. 2016 [cited 2017 Jul 20];48(3):826–32. https://doi.org/10.1183/13993003.00488-2016.
89. Lee JS, Ryu JH, Elicker BM, et al. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med [Internet]. 2011 [cited 2017 Jul 20];184(12):1390–4. https://doi.org/10.1164/rccm.201101-0138OC.
90. Lee JS, Collard HR, Anstrom KJ, et al. Anti-acid treatment and disease progression in idiopathic pulmonary fibrosis: an analysis of data from three randomised controlled trials. Lancet Respir Med. 2013;1(5):369–76.
91. Ghebremariam YT, Cooke JP, Gerhart W, et al. Pleiotropic effect of the proton pump inhibitor esomeprazole leading to suppression of lung inflammation and fibrosis. J Transl Med [Internet]. 2015 [cited 2017 Jul 20];13(1):249. Available from: http://www.translational-medicine.com/content/13/1/249.
92. Lee CM, Lee DH, Ahn BK, et al. Protective effect of proton pump inhibitor for survival in patients with gastroesophageal reflux disease and idiopathic pulmonary fibrosis. J Neurogastroenterol Motil [Internet]. 2016 [cited 2017 Jul 20];22(3):444–51. Available from: http://www.jnmjournal.org/journal/view.html?doi=10.5056/jnm15192
93. Liu B, Su F, Xu N, Qu T, Li M, Ju Y. Chronic use of anti-reflux therapy improves survival of patients with pulmonary fibrosis. Int J Clin Exp Med [Internet]. 2017 [cited 2017 Jul 20];10(3):5805–10. Available from: www.ijcem.com.
94. Kreuter M, Wuyts W, Renzoni E, Koschel D, Maher TM, Kolb M, Weycker D, Spagnolo P, Kirchgaessler KU, Herth FJ, Costabel U. Antacid therapy and disease outcomes in idiopathic pulmonary fibrosis: a pooled analysis. Lancet Respir Med. 2016;4(5):381–9. https://doi.org/10.1016/S2213-2600(16)00067-9.
95. Johannson KA, Strâmbu I, Ravaglia C, et al. Antacid therapy in idiopathic pulmonary fibrosis: more questions than answers? Lancet Respir Med [Internet]. 2017 [cited 2017 Jul 24];5(7):591–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2213260017302199.
96. Magnini D, Montemurro G, Iovene B, et al. Idiopathic pulmonary fibrosis: molecular endotypes of fibrosis stratifying existing and emerging therapies. Respiration [Internet]. 2017 [cited 2017 Aug 2];93(6):379–95. https://doi.org/10.1159/000475780.
97. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. Lancet [Internet]. 2017 [cited 2017 Aug 2];389(10082):1941–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28365056.
98. Lehtonen ST, Veijola A, Karvonen H, et al. Pirfenidone and nintedanib modulate properties of fibroblasts and myofibroblasts in idiopathic pulmonary fibrosis. Respir Res [Internet]. 2016;17(1):14. Available from: http://apps.webofknowledge.com.
99. Ogura T, Taniguchi H, Azuma A, et al. Safety and pharmacokinetics of nintedanib and pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J [Internet]. 2015;45(5):1382–92. Available from: http://erj.ersjournals.com/content/45/5/1382.

100. Jia G, Chandriani S, Abbas AR, et al. CXCL14 is a candidate biomarker for Hedgehog signalling in idiopathic pulmonary fibrosis. Thorax. [Internet] 2017 [cited 2017 Jul 20];thoraxjnl-2015-207682. Available from: http://thorax.bmj.com/lookup/doi/10.1136/thoraxjnl-2015-207682.
101. Leask A, Parapuram SK, Shi-Wen X, Abraham DJ. Connective tissue growth factor (CTGF, CCN2) gene regulation: a potent clinical bio-marker of fibroproliferative disease? J Cell Commun Signal [Internet]. 2009 [cited 2017 Jul 20];3(2):89–94. https://doi.org/10.1007/s12079-009-0037-7.
102. Murray LA, Argentieri RL, Farrell FX, et al. Hyper-responsiveness of IPF/UIP fibroblasts: interplay between TGF$β$1, IL-13 and CCL2. Int J Biochem Cell Biol [Internet]. 2008;40(10):2174–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18395486.
103. Bonniaud P, Martin G, Margetts PJ, et al. Connective tissue growth factor is crucial to inducing a profibrotic environment in "fibrosis-resistant" BALB/c mouse lungs. Am J Respir Cell Mol Biol [Internet]. 2004;31(5):510–6. Available from: http://www.atsjournals.org/doi/abs/10.1165/rcmb.2004-0158OC.
104. Wang X, Wu G, Gou L, et al. A novel single-chain-Fv antibody against connective tissue growth factor attenuates bleomycin-induced pulmonary fibrosis in mice. Respirology [Internet]. 2011 [cited 2017 Jul 20];16(3):500–7. https://doi.org/10.1111/j.1440-1843.2011.01938.x.
105. Bonniaud P, Kolb M, Galt T, et al. Smad3 null mice develop airspace enlargement and are resistant to TGF-beta-mediated pulmonary fibrosis. J Immunol [Internet]. 2004;173(3):2099–108. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15265946.
106. Allen JT, Knight RA, Bloor CA, Spiteri MA. Enhanced insulin-like growth factor binding protein–related protein 2 (connective tissue growth factor) expression in patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. Am J Respir Cell Mol Biol [Internet]. 1999;21(6):693–700. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10572066.
107. Leduc M, Tremblay M, Grouix B, et al. PBI-4050, a novel first-in-class anti-fibrotic compound, inhibits CTGF and collagen I production in human alveolar epithelial cells and fibroblasts, and reduces lung fibrosis in the bleomycin-induced lung fibrosis model. Am J Respir Crit Care Med. [Internet] 2014;189(A1998). https://doi.org/10.1164/ajrccm-conference.2014.189.1_MeetingAbstracts.A1998,
108. Goodwin A, Jenkins G. Role of integrin-mediated TGF$β$ activation in the pathogenesis of pulmonary fibrosis. Biochem Soc Trans [Internet]. 2009;37(4):849–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19614606.
109. Saini G, Porte J, Weinreb PH, et al. $α$v$β$6 integrin may be a potential prognostic biomarker in interstitial lung disease. Eur Respir J [Internet]. 2015;46(2):486–94. https://doi.org/10.1183/09031936.00210414.
110. Horan GS, Wood S, Ona V, et al. Partial inhibition of integrin alpha(v)beta6 prevents pulmonary fibrosis without exacerbating inflammation. Am J Respir Crit Care Med [Internet]. 2008 [cited 2017 Jul 24];177(1):56–65. https://doi.org/10.1164/rccm.200706-805OC.
111. Vancheri C. Common pathways in idiopathic pulmonary fibrosis and cancer. Eur Respir Rev [Internet]. 2013 [cited 2017 Jul 24];22(129):265–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23997054.
112. Conte E, Gili E, Fruciano M, et al. PI3K p110γ overexpression in idiopathic pulmonary fibrosis lung tissue and fibroblast cells: in vitro effects of its inhibition. Lab Investig [Internet]. 2013 [cited 2017 Jul 24];93(5):566–76. https://doi.org/10.1038/labinvest.2013.6.
113. Conte E, Fruciano M, Fagone E, et al. Inhibition of PI3K prevents the proliferation and differentiation of human lung fibroblasts into myofibroblasts: the role of class I P110 isoforms. PLoS One [Internet]. 2011 [cited 2017 Jul 24];6(10):e24663. https://doi.org/10.1371/journal.pone.0024663.
114. Mercer PF, Woodcock H V, Eley JD, et al. Exploration of a potent PI3 kinase/mTOR inhibitor as a novel anti-fibrotic agent in IPF. Thorax. [Internet] 2016;thoraxjnl--2015--207429--. Available from: http://thorax.bmj.com/content/early/2016/04/21/thoraxjnl-2015-207429?papetoc.

115. Jin X, Dai H, Ding K, Xu X, Pang B, Wang C. Rapamycin attenuates bleomycin-induced pulmonary fibrosis in rats and the expression of metalloproteinase-9 and tissue inhibitors of metalloproteinase-1 in lung tissue. Chin Med J [Internet]. 2014 [cited 2017 Jul 24];127(7):1304–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24709185.
116. Street CA, Bryan BA. Rho kinase proteins – pleiotropic modulators of cell survival and apoptosis. Anticancer Res [Internet]. 2011;31(11):3645–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22110183.
117. Le T-TT, Karmouty-Quintana H, Melicoff E, et al. Blockade of IL-6 trans signaling attenuates pulmonary fibrosis. J Immunol [Internet]. 2014 [cited 2017 Jul 24];193(7):3755–68. https://doi.org/10.4049/jimmunol.1302470.
118. Zanin-Zhorov A, Weiss JM, Nyuydzefe MS, et al. Selective oral ROCK2 inhibitor downregulates IL-21 and IL-17 secretion in human T cells via STAT3-dependent mechanism. Proc Natl Acad Sci U S A [Internet]. 2014;111(47):16814–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25385601.
119. Funke M, Zhao Z, Xu Y, Chun J, Tager AM. The lysophosphatidic acid receptor LPA1 promotes epithelial cell apoptosis after lung injury. Am J Respir Cell Mol Biol [Internet]. 2012 [cited 2017 Jul 24];46(3):355–64. https://doi.org/10.1165/rcmb.2010-0155OC.
120. Neidlinger NA, Larkin SK, Bhagat A, Victorino GP, Kuypers FA. Hydrolysis of phosphatidylserine-exposing red blood cells by secretory phospholipase A2 generates lysophosphatidic acid and results in vascular dysfunction. J Biol Chem [Internet]. 2006;281(2):775–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16278219.
121. Tager AM, LaCamera P, Shea BS, et al. The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. Nat Med [Internet]. 2008;14(1):45–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18066075.
122. Oikonomou N, Mouratis M-A, Tzouvelekis A, et al. Pulmonary Autotaxin expression contributes to the pathogenesis of pulmonary fibrosis. Am J Respir Cell Mol Biol [Internet]. 2012;47(5):566–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22744859.
123. Watterson KR, Lanning DA, Diegelmann RF, Spiegel S. Regulation of fibroblast functions by lysophospholipid mediators: potential roles in wound healing. Wound Repair Regen [Internet]. 2007;15(5):607–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17971005.
124. Swaney J, Chapman C, Correa L, et al. A novel, orally active LPA 1 receptor antagonist inhibits lung fibrosis in the mouse bleomycin modelb ph. Br J Pharmacol. 2010;160:1699–713.
125. Wygrecka M, Zakrzewicz D, Taborski B, et al. TGF-β1 induces tissue factor expression in human lung fibroblasts in a PI3K/JNK/Akt-dependent and AP-1-dependent manner. Am J Respir Cell Mol Biol [Internet]. 2012 [cited 2017 Jul 24];47(5):614–27. https://doi.org/10.1165/rcmb.2012-0097OC.
126. Cui Y, Osorio JC, Risquez C, et al. Transforming growth factor-β1 downregulates vascular endothelial growth factor-D expression in human lung fibroblasts via the Jun NH2-terminal kinase signaling pathway. Mol Med [Internet]. 2014 [cited 2017 Jul 24];20(1):120–34. Available from: http://www.molmed.org/content/pdfstore/13_123_Cui.pdf.
127. Königshoff M, Rojas M. Galectin-3: the bridge over troubled waters. Am J Respir Crit Care Med [Internet]. 2012;185(5):473–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22383566.
128. Mackinnon AC, Gibbons MA, Farnworth SL, et al. Regulation of transforming growth factor-β1-driven lung fibrosis by galectin-3. Am J Respir Crit Care Med [Internet]. 2012 [cited 2017 Jul 24];185(5):537–46. Available from: http://www.atsjournals.org/doi/abs/10.1164/rccm.201106-0965OC.
129. Hinz B. Mechanical aspects of lung fibrosis: a spotlight on the myofibroblast. Proc Am Thorac Soc [Internet]. 2012 [cited 2017 Jul 24];9(3):137–47. https://doi.org/10.1513/pats.201202-017AW.
130. Barry-Hamilton V, Spangler R, Marshall D, Scott McCauley HMR, Oyasu M, Mikels A, Vaysberg M, Ghermazien H, Carol Wai CAG, Velayo AC, Jorgensen B, Biermann D, Tsai D, Jennifer Green SZ-E, et al. Allosteric inhibition of lysyl oxidase–like-2 impedes the development of a pathologic microenvironment. Nat Med [Internet]. 2010;16(9):1009–17. Available from: http://www.nature.com/doifinder/10.1038/nm.2208.

131. Raghu G, Brown KK, Collard HR, et al. Efficacy of simtuzumab versus placebo in patients with idiopathic pulmonary fibrosis: a randomised, double-blind, controlled, phase 2 trial. Lancet Respir Med [Internet]. 2017 [cited 2017 Jul 24];5(1):22–32. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2213260016304210.
132. Peters-Golden M, Henderson WR. Leukotrienes. N Engl J Med [Internet]. 2007 [cited 2017 Jul 24];357(18):1841–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17978293.
133. Wilborn J, Bailie M, Coffey M, Burdick M, Strieter R, Peters-Golden M. Constitutive activation of 5-lipoxygenase in the lungs of patients with idiopathic pulmonary fibrosis. J Clin Invest [Internet]. 1996 [cited 2017 Jul 24];97(8):1827–36. Available from: http://www.jci.org/articles/view/118612.
134. Izumo T, Kondo M, Nagai A. Effects of a leukotriene B4 receptor antagonist on bleomycin-induced pulmonary fibrosis. Eur Respir J [Internet]. 2009 [cited 2017 Jul 24];34(6):1444–51. https://doi.org/10.1183/09031936.00143708.
135. May RD, Fung M. Strategies targeting the IL-4/IL-13 axes in disease. Cytokine [Internet]. 2015 [cited 2017 Jul 24];75(1):89–116. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1043466615002045.
136. Lee CG, Homer RJ, Zhu Z, et al. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor beta(1). J Exp Med [Internet]. 2001 [cited 2017 Jul 24];194(6):809–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11560996.
137. Park SW, Ahn MH, Jang HK, et al. Interleukin-13 and its receptors in idiopathic interstitial pneumonia: clinical implications for lung function. J Korean Med Sci [Internet]. 2009 [cited 2017 Jul 24];24(4):614–20. Available from: https://synapse.koreamed.org/DOIx.php?id=10.3346/jkms.2009.24.4.614.
138. Batra V, Musani AI, Hastie AT, et al. Bronchoalveolar lavage fluid concentrations of transforming growth factor (TGF)-beta1, TGF-beta2, interleukin (IL)-4 and IL-13 after segmental allergen challenge and their effects on alpha-smooth muscle actin and collagen III synthesis by primary human lu. Clin Exp Allergy [Internet]. 2004 [cited 2017 Jul 24];34(3):437–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15005738.
139. Darrell P, David R, Wang M, Ronkainen Sanna D, Crawford Jeff R, Travis Elizabeth L, Gomer RH. Reduction of bleomycin-induced pulmonary fibrosis by serum amyloid P. J Immunol. 2007;27(6):316–24.
140. Murray LA, Rosada R, Moreira AP, et al. Serum amyloid P therapeutically attenuates murine bleomycin-induced pulmonary fibrosis via its effects on macrophages. PLoS One. 2010;5(3).
141. Duffield JS, Lupher ML. PRM-151 (recombinant human serum amyloid P/pentraxin 2) for the treatment of fibrosis. Drug News Perspect [Internet]. 2010 [cited 2017 Jul 24];23(5):305–15. Available from: http://journals.prous.com/journals/servlet/xmlxsl/pk_journals.xml:summary_pr?p_JournalId=3&p_RefId=1444206&p_IsPs=N.
142. Dillingh MR, van den Blink B, Moerland M, et al. Recombinant human serum amyloid P in healthy volunteers and patients with pulmonary fibrosis. Pulm Pharmacol Ther [Internet]. 2013 [cited 2017 Jul 24];26(6):672–6. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1094553913000540.
143. Burlingham WJ, Love RB, Jankowska-Gan E, et al. IL-17-dependent cellular immunity to collagen type V predisposes to obliterative bronchiolitis in human lung transplants. J Clin Invest [Internet]. 2007 [cited 2017 Jul 24];117(11):3498–506. https://doi.org/10.1172/JCI28031.
144. Vittal R, Mickler EA, Fisher AJ, et al. Type V collagen induced tolerance suppresses collagen deposition, TGF-?? And associated transcripts in pulmonary fibrosis. PLoS One [Internet]. 2013 [cited 2017 Jul 24];8(10):e76451. https://doi.org/10.1371/journal.pone.0076451.
145. Wilkes DS, Chew T, Flaherty KR, et al. Oral immunotherapy with type v collagen in idiopathic pulmonary fibrosis. Eur Respir J [Internet]. 2015 [cited 2017 Jul 24];45(5):1393–402. https://doi.org/10.1183/09031936.00105314.
146. Feghali-Bostwick CA, Tsai CG, Valentine VG, et al. Cellular and humoral autoreactivity in idiopathic pulmonary fibrosis. J Immunol [Internet]. 2007 [cited 2017 Jul 24];179(4):2592–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17675522.
147. Yilmaz O, Oztay F, Kayalar O. Dasatinib attenuated bleomycin-induced pulmonary fibrosis in mice. Growth Factors [Internet]. 2015 [cited 2017 Jul 24];33(5–6):366–75. https://doi.org/10.3109/08977194.2015.1109511.

148. Cruz FF, Horta LFB, De Albuquerque Maia L, et al. Dasatinib reduces lung inflammation and fibrosis in acute experimental silicosis. PLoS One [Internet]. 2016 [cited 2017 Jul 24];11(1):e0147005. https://doi.org/10.1371/journal.pone.0147005.
149. Molyneaux PL, Maher TM. The role of infection in the pathogenesis of idiopathic pulmonary fibrosis. Eur Respir Rev [Internet]. 2013 [cited 2017 Feb 4];22(129):376–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23997064.
150. Yonemaru M, Kasuga I, Kusumoto H, et al. Elevation of antibodies to cytomegalovirus and other herpes viruses in pulmonary fibrosis. Eur Respir J [Internet]. 1997;10(9):2040–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9311499.
151. Manika K, Alexiou-Daniel S, Papakosta D, et al. Epstein-Barr virus DNA in bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis [Internet]. 2007 [cited 2017 Jul 24];24(2):134–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18496984.
152. Egan JJ, Adamali HI, Lok SS, Stewart JP, Woodcock AA. Ganciclovir antiviral therapy in advanced idiopathic pulmonary fibrosis: an open pilot study. Pulm Med [Internet]. 2011 [cited 2017 Jul 24];2011:240805. Available from: http://www.hindawi.com/journals/pm/2011/240805/.
153. Molyneaux PL, Cox MJ, Willis-Owen SAG, et al. The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2014;190(8):906–13.
154. Han MK, Zhou Y, Murray S, et al. Lung microbiome and disease progression in idiopathic pulmonary fibrosis: an analysis of the COMET study. Lancet Respir Med [Internet]. 2014 [cited 2017 Feb 4];2(7):548–56. Available from: http://www.thelancet.com/article/S2213260014700694/fulltext.
155. Shulgina L, Cahn AP, Chilvers ER, et al. Treating idiopathic pulmonary fibrosis with the addition of co-trimoxazole: a randomised controlled trial. Thorax [Internet]. 2013 [cited 2017 May 28];68(9):155–62. https://doi.org/10.1136/thoraxjnl-2012-202403.
156. Collard HR, Ryerson CJ, Corte TJ, et al. Acute exacerbation of idiopathic pulmonary fibrosis. An international working group report. Am J Respir Crit Care Med [Internet]. 2016 [cited 2017 Feb 4];194(3):265–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27299520.
157. Agarwal R, Jindal SK. Acute exacerbation of idiopathic pulmonary fibrosis: a systematic review. Eur J Intern Med [Internet]. 2008 [cited 2017 May 28];19(4):227–35. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0953620507003500.
158. Papiris SA, Manali ED, Kolilekas L, Triantafillidou C, Tsangaris I, Kagouridis K. Steroids in idiopathic pulmonary fibrosis acute exacerbation: defenders or killers? Am J Respir Crit Care Med [Internet]. 2012 [cited 2017 May 19];185(5):587–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22383571.
159. Kondoh Y, Taniguchi H, Kawabata Y, Yokoi T, Suzuki K, Takagi K. Acute exacerbation in idiopathic pulmonary fibrosis. Analysis of clinical and pathologic findings in three cases. Chest [Internet]. 1993 [cited 2017 May 28];103(6):1808–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8404104.
160. Song JW, Hong S-B, Lim C-M, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J [Internet]. 2011;37(2):356–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20595144.
161. Tajima S, Oshikawa K, Tominaga S, Sugiyama Y. The increase in serum soluble ST2 protein upon acute exacerbation of idiopathic pulmonary fibrosis. Chest [Internet]. 2003 [cited 2017 May 28];124(4):1206–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14555548.
162. Juarez MM, Chan AL, Norris AG, Morrissey BM, Albertson TE. Acute exacerbation of idiopathic pulmonary fibrosis-a review of current and novel pharmacotherapies. J Thorac Dis. 2015;7(3):499–519.
163. Ding J, Chen Z, Feng K. Procalcitonin-guided antibiotic use in acute exacerbations of idiopathic pulmonary fibrosis. Int J Med Sci. 2013;10(7):903–7.
164. Anderson R, Grabow G, Oosthuizen R, Theron A, Van Rensburg AJ. Effects of sulfamethoxazole and trimethoprim on human neutrophil and lymphocyte functions in vitro: in vivo

effects of co-trimoxazole. Antimicrob Agents Chemother [Internet]. 1980 [cited 2017 May 28];17(3):322–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7425598.
165. Kanoh S, Rubin BK. Mechanisms of action and clinical application of macrolides as immunomodulatory medications. Clin Microbiol Rev [Internet]. 2010 [cited 2017 May 28];23(3):590–615. https://doi.org/10.1128/CMR.00078-09.
166. Oda K, Yatera K, Fujino Y, et al. Efficacy of concurrent treatments in idiopathic pulmonary fibrosis patients with a rapid progression of respiratory failure: an analysis of a national administrative database in Japan. BMC Pulm Med [Internet]. 2016 [cited 2017 May 28];16(1):91. https://doi.org/10.1186/s12890-016-0253-x.
167. Eickelberg O, Pansky A, Koehler E, et al. Molecular mechanisms of TGF-(beta) antagonism by interferon (gamma) and cyclosporine A in lung fibroblasts. FASEB J [Internet]. 2001 [cited 2017 May 29];15(3):797–806. https://doi.org/10.1096/fj.00-0233com.
168. Homma S, Sakamoto S, Kawabata M, et al. Cyclosporin treatment in steroid-resistant and acutely exacerbated interstitial pneumonia. Intern Med [Internet]. 2005 [cited 2017 May 29];44(11):1144–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16357451.
169. Sakamoto S, Homma S, Miyamoto A, Kurosaki A, Fujii T, Yoshimura K. Cyclosporin A in the treatment of acute exacerbation of idiopathic pulmonary fibrosis. Intern Med [Internet]. 2010 [cited 2017 May 29];49(2):109–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20075573.
170. Inase N, Sawada M, Ohtani Y, et al. Cyclosporin A followed by the treatment of acute exacerbation of idiopathic pulmonary fibrosis with corticosteroid. Intern Med [Internet]. 2003 [cited 2017 May 29];42(7):565–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12879947.
171. Nagano J, Iyonaga K, Kawamura K, et al. Use of tacrolimus, a potent antifibrotic agent, in bleomycin-induced lung fibrosis. Eur Respir J [Internet]. 2006 [cited 2017 May 29];27(3):460–9. https://doi.org/10.1183/09031936.06.00070705.
172. Kino T, Hatanaka H, Miyata S, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. J Antibiot (Tokyo) [Internet]. 1987 [cited 2017 May 29];40(9):1256–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2445722.
173. Horita N, Akahane M, Okada Y, et al. Tacrolimus and steroid treatment for acute exacerbation of idiopathic pulmonary fibrosis. Intern Med [Internet]. 2011 [cited 2017 May 29];50(3):189–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21297319.
174. Nannini C, West CP, Erwin PJ, Matteson EL. Effects of cyclophosphamide on pulmonary function in patients with scleroderma and interstitial lung disease: a systematic review and meta-analysis of randomized controlled trials and observational prospective cohort studies. Arthritis Res Ther [Internet]. 2008 [cited 2017 May 29];10(5):R124. Available from: http://arthritis-research.biomedcentral.com/articles/10.1186/ar2534.
175. ovelli L, Ruggiero R, De Giacomi F, et al. Corticosteroid and cyclophosphamide in acute exacerbation of idiopathic pulmonary fibrosis: a single center experience and literature review. Sarcoidosis Vasc Diffus lung Dis Off J WASOG [Internet]. 2016 [cited 2017 May 29];33(4):385–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28079851.
176. Mendoza-Milla C, Valero Jiménez A, Rangel C, et al. Dehydroepiandrosterone has strong antifibrotic effects and is decreased in idiopathic pulmonary fibrosis. Eur Respir J [Internet]. 2013;42:1309–21. Available from: http://ow.ly/nD9sz.
177. Morawiec E, Tillie-Leblond I, Pansini V, Salleron J, Remy-Jardin M, Wallaert B. Exacerbations of idiopathic pulmonary fibrosis treated with corticosteroids and cyclophosphamide pulses. Eur Respir J [Internet]. 2011 [cited 2017 May 29];38(6):1487–9. Available from: http://erj.ersjournals.com/content/38/6/1487.short.
178. Donahoe M, Valentine VG, Chien N, Gibson KF, Raval JS, Saul M, et al. Autoantibody-targeted treatments for acute exacerbations of idiopathic pulmonary fibrosis. PLoS ONE. 2015;10(6):e0127771. https://doi.org/10.1371/journal.pone.0127771.
179. Tsushima K, Yamaguchi K, Kono Y, et al. Thrombomodulin for acute exacerbations of idiopathic pulmonary fibrosis: a proof of concept study. Pulm Pharmacol Ther [Internet]. 2014

[cited 2017 May 30];29(2):233–40. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1094553914000467.
180. Isshiki T, Sakamoto S, Kinoshita A, Sugino K, Kurosaki A, Homma S. Recombinant human soluble thrombomodulin treatment for acute exacerbation of idiopathic pulmonary fibrosis: a retrospective study. Respiration. 2015;89:201–7. https://doi.org/10.1159/000369828.
181. Kataoka K, Taniguchi H, Kondoh Y, et al. Recombinant human thrombomodulin in acute exacerbation of idiopathic pulmonary fibrosis. Chest [Internet]. 2015 [cited 2017 May 30];148(2):436–43. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0012369215503401.
182. Mitaka C, Tomita M. Polymyxin B-immobilized fiber column hemoperfusion therapy for septic shock. Shock [Internet]. 2011 [cited 2017 May 30];36(4):332–8. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00024382-201110000-00003.
183. Cruz DN, Perazella MA, Bellomo R, et al. Effectiveness of polymyxin B-immobilized fiber column in sepsis: a systematic review. Crit Care [Internet]. 2007 [cited 2017 May 30];11(2):R47. Available from: http://ccforum.biomedcentral.com/articles/10.1186/cc5780.
184. Enomoto N, Mikamo M, Oyama Y, et al. Treatment of acute exacerbation of idiopathic pulmonary fibrosis with direct hemoperfusion using a polymyxin B-immobilized fiber column improves survival. BMC Pulm Med [Internet]. 2015 [cited 2017 May 30];15(1):15. https://doi.org/10.1186/s12890-015-0004-4.
185. Abe S, Azuma A, Mukae H, et al. Polymyxin B-immobilized fiber column (PMX) treatment for idiopathic pulmonary fibrosis with acute exacerbation: a multicenter retrospective analysis. Intern Med [Internet]. 2012 [cited 2017 May 30];51(12):1487–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22728479.
186. Tachibana K, Inoue Y, Nishiyama A, et al. Polymyxin-B hemoperfusion for acute exacerbation of idiopathic pulmonary fibrosis: serum IL-7 as a prognostic marker. Sarcoidosis Vasc Diffus lung Dis Off J WASOG [Internet]. 2011 [cited 2017 May 30];28(2):113–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22117502.
187. Maher TM, Whyte MKB, Hoyles RK, et al. Development of a consensus statement for the definition, diagnosis, and treatment of acute exacerbations of idiopathic pulmonary fibrosis using the Delphi technique. Adv Ther [Internet]. 2015 [cited 2017 May 19];32(10):929–43. https://doi.org/10.1007/s12325-015-0249-6.

Chapter 14
Mimics of Idiopathic Pulmonary Fibrosis

Keith C. Meyer and Steven D. Nathan

Introduction

Many forms of interstitial lung disease (ILD), especially when accompanied by a significant degree of interstitial fibrosis, can have both a radiologic and/or histopathologic pattern that is consistent with or suggestive of the presence of usual interstitial pneumonia (UIP) [1, 2]. This may create considerable difficulty and confusion when clinicians attempt to differentiate such "mimics" from what we currently and commonly recognize as idiopathic pulmonary fibrosis (IPF) [3]. A comprehensive history obtained by the clinician is mandatory with close attention to symptoms, past medical history, family history, drug or environmental/occupational exposures, and physical abnormalities that a patient may have noticed such as rash or joint symptoms [4]. Next steps include performing a meticulous physical examination, ordering appropriate laboratory testing such as serologic markers that are associated with connective tissue disease (CTD), and obtaining thoracic imaging via high-resolution computed tomography (HRCT) (Fig. 14.1). If a careful evaluation of the patient combined with HRCT and appropriate laboratory testing do not result in a confident diagnosis, a biopsy, obtained either via bronchoscopy or surgical lung biopsy, may be needed to differentiate IPF from other disorders with a high degree of confidence. A key aspect of the diagnostic process may include multidisciplinary discussions (MDDs) among clinicians, radiologists, and pathologists to increase both the accuracy and confidence of a final diagnosis [5, 6]. Differentiation

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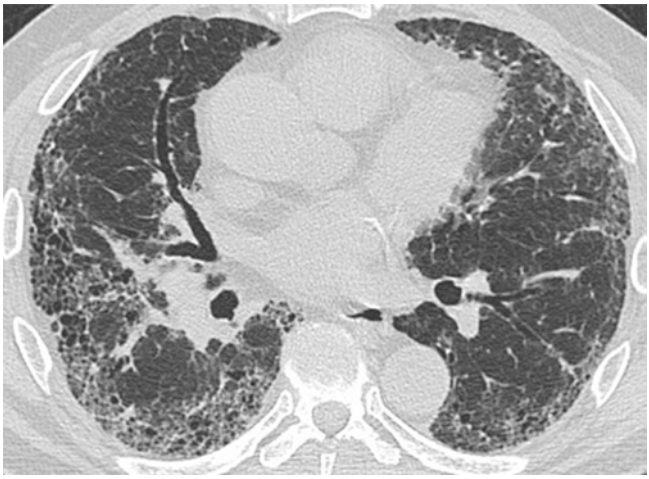

Fig. 14.1 HRCT cross-sectional view of a 71-year-old male patient with UIP/IPF. Note reticulation, peripheral pattern of subpleural honeycombing, and traction bronchiectasis

Table 14.1 Lung disorders that may present with a UIP or UIP-like pattern

Non-IPF forms of idiopathic interstitial pneumonia (IIP)
Connective tissue disease-associated ILD (CTD-ILD)
Systemic sclerosis
Rheumatoid arthritis
Antisynthetase syndrome
Sjögren's syndrome
Mixed connective tissue disease (MCTD)
Interstitial pneumonia with autoimmune features (IPAF)
Chronic hypersensitivity pneumonitis
Advanced pulmonary Langerhans cell histiocytosis (PLCH)
Hermansky-Pudlak syndrome
Drug-induced fibrosis
Asbestosis
Erdheim-Chester disease
Stage IV sarcoidosis
IgG4-related disease

among forms of idiopathic interstitial pneumonia (IIP) can be complicated by the existence of varying histopathologies in different regions of the same lung [7], and some cases must be categorized as undifferentiated IIP despite exhaustive attempts to make an ultimate diagnosis [1].

Many disorders can have features that mimic IPF (Table 14.1). Foremost among these are non-IPF forms of IIP such as NSIP, various forms of CTD with interstitial lung involvement, and chronic HP, but other entities such as asbestosis and adverse reactions to drugs that induce a fibrotic response can also be confused with IPF [2, 8]. Many of the disorders that can mimic IPF have findings on HRCT imaging or lung biopsy histopathology that suggest a non-IPF diagnosis (Table 14.2). Making an accurate diagnosis is key to providing personalized medicine that optimizes the

Table 14.2 Diagnostic clues that can be important in differentiating IPF MIMICS from IPF

Diagnostic entity	HRCT findings	Histopathologic findings
IPF	Definite or possible UIP pattern	Typical UIP pattern
CTD-ILD or IPAF	UIP or NSIP pattern most common	Prominent lymphoid hyperplasia
		Germinal center formation
		Pleuritis, pleural adhesions
Chronic HP	Mosaic perfusion	Airway-centered lesions (peribronchiolar interstitial pneumonia, peribronchiolar giant cells and poorly formed granulomas, and chronic bronchiolitis
	Air trapping	Centrilobular or airway-centered accentuation of fibrosis
	Relative sparing of the lung bases	Peribronchiolar metaplasia
Stage IV sarcoidosis	Upper lobe and peribronchovascular distribution	Centrilobular, lymphangitic, and/or mass-like areas of fibrosis
	Consolidative fibrotic masses	Residual granulomas or giant cells within areas of dense fibrosis
	Perilymphatic nodules	Honeycomb change and bronchiolectasis that is central (not subpleural as in UIP)
Advanced PLCH	Centrally located cysts	Nodular interstitial aggregations of Langerhans histiocytes
	Nodules with lucent centers	Dense fibrosis with stellate or starfish-like shapes
	Centrilobular fibrosis (inverted UIP appearance)	Relative sparing of subpleural parenchyma
Erdheim-Chester disease	Interlobular septal thickening that is smooth (in contrast to irregular reticulation seen in UIP/IPF)	Interstitial accumulations of non-Langerhans type histiocytes with lymphangitic distribution pattern
	Architectural distortion, honeycomb change, and traction bronchiectasis usually not present	Patchy fibrosis with lymphangitic distribution
		Absence of temporal heterogeneity
	Centrilobular ill-defined nodules	Lack of fibroblastic foci
Hermansky-Pudlak syndrome	Ground-glass opacities often present	Clear, vacuolated, ceroid-laden alveolar type 2 pneumocytes and alveolar macrophages present
	Peribronchovascular thickening	Fibrosis is patchy without a clear pattern of distribution
Asbestosis	Pleural plaque formation	Presence of asbestos bodies
		Pleural plaques with “basket-weave” pattern of hyalinized collagen

likelihood of attaining a therapeutic response for patients with IPF or non-IPF mimics. Equally important is avoiding potential adverse events if inappropriate therapies are initiated when a disorder has been misdiagnosed [9, 10]. Ensuring that an accurate diagnosis has been made is also key to enrolling patients in randomized clinical trials (RCTs) that seek to identify safe and efficacious novel therapies that target IPF or other forms of ILD [11].

Non-specific Interstitial Pneumonia

The term NSIP was coined and first described in 1994 to refer to a histopathologic pattern seen on surgical lung biopsy specimens that appeared distinct from previously described interstitial pneumonia patterns such as UIP and desquamative interstitial pneumonia (DIP) [12]. NSIP is characterized histopathologically by a diffuse, homogeneous infiltrative process that can have a predominance of cellular infiltrates, extensive fibrosis, or a mixture of both cellular infiltration and fibrosis [1]. In contrast to the older age and male predominance seen in IPF, NSIP tends to occur in younger patients and has a female predominance [13]. The clinical presentation is quite similar to that of patients with IPF with an insidious onset of dyspnea that may be associated with a chronic, nonproductive cough [13]. HRCT imaging patterns (Fig. 14.2) can be fairly suggestive of NSIP as a diagnosis [14], especially if a rim of subpleural interstitial sparing is seen [15]. However, the diagnosis of NSIP cannot be made with adequate confidence without sampling of lung tissue that definitively confirms the presence of a NSIP histopathologic pattern [1, 16]. Additionally, although a NSIP pattern of lung injury may be idiopathic and classified as a form of IIP, a NSIP pattern can be seen in other forms of ILD such as CTD-associated ILD [17, 18], idiopathic pneumonia with autoimmune features (IPAF) [19], or as a variant of chronic HP [20]. All forms of CTD with lung involvement can have a NSIP histopathologic pattern, and this is especially the case in patients with scleroderma, although it is uncommon in patients with rheumatoid arthritis where UIP is the predominant pattern [21]. Additionally, because other disorders such as drug toxicity, infection, or immunosuppression can be associated with a NSIP histologic pattern,

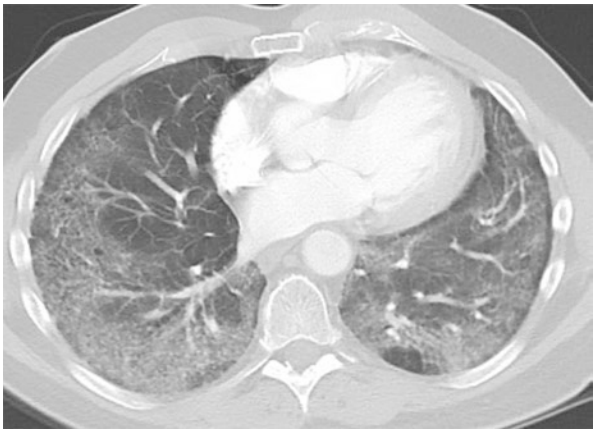

Fig. 14.2 HRCT cross-sectional view of a 74-year-old male with idiopathic NSIP. The patient noted gradual symptom onset and met requirements for supplemental oxygen at the time of diagnosis (just prior to lung biopsy). The patient slowly improved over a 6-month period on steroid-sparing therapy with mycophenolate, was able to cease using supplemental oxygen, and could resume climbing up one flight of stairs without oxyhemoglobin desaturation

it is incumbent upon the clinician to exclude such diagnoses as well as differentiate NSIP from IPF. More recent findings suggest that idiopathic NSIP is comprised of at least three differing phenotypes including NSIP associated with autoimmune features, significant emphysema, and familial interstitial pneumonia [FIP]. In addition, two major radiologic-pathologic profiles, an "inflammatory type" and a "highly fibrotic" type, have also been described [22]. The inflammatory type displays prominent lymphocytic inflammation in both tissue biopsy and bronchoalveolar lavage (BAL) and has a mixed NSIP/organizing pneumonia pattern on HRCT. On the other hand, the highly fibrotic type shows no BAL lymphocytosis and is characterized by prominent reticular changes and traction bronchiectasis on HRCT [22].

NSIP has a distinctly better prognosis and clinical course compared to IPF with a 5-year survival of approximately 80% [15], although patients who present with severe lung function impairment tend to have a worse prognosis that may be similar to patients with IPF [23]. Immunosuppressive therapy is widely regarded as standard of care for patients with NSIP [22]. This is in contrast to the treatment of IPF patients, for whom anti-fibrotic agents have emerged as the standard of care along with general recognition that immunosuppressive strategies may be deleterious [9]. NSIP patients with an inflammatory type of radiologic-pathologic profile tend to have a better response to corticosteroid or other immunosuppressive therapy, while the highly fibrotic cohort is less likely to respond to such therapy. However, while CTD-associated NSIP can respond to immunosuppressive therapy [24], adequately powered, placebo-controlled randomized clinical trials (RCTs) are lacking for treating idiopathic NSIP or other forms of NSIP with immunosuppressive agents. Studies are needed to define the role of anti-fibrotic therapy in patients with fibrotic NSIP, before such therapy can be endorsed, while lung transplantation should be considered for appropriate candidates with advanced disease [25].

Connective Tissue Disease-Associated Interstitial Lung Disease

Lung involvement is frequently observed in patients with CTD and can predate the onset of the underlying systemic disorder. ILD is the most common pulmonary manifestation [18], with NSIP or UIP patterns frequently identified on both HRCT imaging (Fig. 14.3) and histopathology. Prevalence estimates for CTD-associated ILD range from 40% to 100% for scleroderma, up to 60% for rheumatoid arthritis, up to 75% for antisynthetase syndromes (e.g., polymyositis/dermatomyositis), up to 25% for Sjögren's syndrome, and up to 8% for systemic lupus erythematosus [26–28]. ILD can also be observed in patients with autoimmune phenomena whose clinical presentation and CTD serologies do not meet criteria for a definitive CTD diagnosis; such patients can have NSIP or UIP lung lesions and meet criteria that categorize them as having IPAF [19]. Whether this newly defined entity has important prognostic and treatment distinctions from IPF or idiopathic NSIP remains to be determined. Patients who are found to have an isolated positive autoantibody

(e.g., rheumatoid factor or antinuclear antibody) but lack any other phenomena that meet criteria for a diagnosis of a CTD or IPAF can be regarded as having IPF if a comprehensive evaluation reveals findings consistent with UIP [29].

A comprehensive patient history and careful physical examination can provide important clues that suggest the possibility of an underlying CTD in patients lacking a preexistent CTD diagnosis. In some patients, the onset of respiratory symptoms associated with newly diagnosed ILD may indicate the onset of an underlying CTD. Another scenario can occur when patients are diagnosed with ILD but have no evidence of a CTD clinically or serologically at the time of their ILD diagnosis but develop manifestations of a CTD months to years later [30]. The radiographic patterns of CTD-ILD (Fig. 14.3) or IPAF are relatively non-specific and can show changes that are consistent with NSIP, UIP, or other interstitial pneumonia patterns such as organizing pneumonia or DIP. A thorough investigation is required to rule out the presence of a CTD when a radiographic and/or histopathologic UIP pattern is observed that may indicate a diagnosis of IPF or another mimic of IPF such as chronic HP [3].

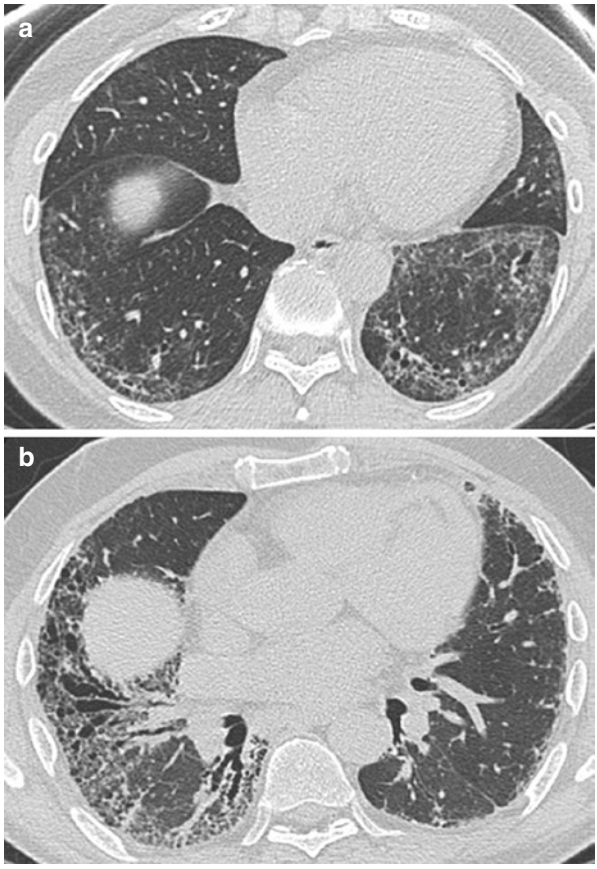

Fig. 14.3 HRCT cross-sectional views of two patients with scleroderma. (**a**) A 48-year-old female with scleroderma-associated NSIP. (**b**) A 56-year-old male with scleroderma-associated UIP

Chronic Hypersensitivity Pneumonitis

HP (also known as extrinsic allergic alveolitis) is an immune-mediated, complex pulmonary syndrome that occurs in response to inhalation of a variety of antigens to which an individual has been sensitized [31, 32]. HP has been described and classified as presenting in acute, subacute, or chronic forms, but widely accepted criteria to distinguish among these forms are lacking. Chronic HP with extensive fibrosis can be very difficult to differentiate from IPF. Many patients who present with chronic HP do not appear to have a history of prior acute or subacute forms of HP or documentation of exposure to an antigen known to be linked to induction of a HP response. Complicating the diagnosis further, precipitating antibodies to an offending antigen may not be detected despite extensive testing. Many potential exposures, almost exclusively to organic antigens (mammalian and avian proteins, fungi, thermophilic bacteria, mycobacteria) but occasionally to certain chemical compounds, can induce a HP response. The predominant causes of chronic HP include exposure to birds (bird fancier's lung), molds on decaying vegetation (farmer's lung caused by exposure to moldy hay, grains, or corn silage), or contaminated water reservoirs or forced air systems. However, many other potential exposures exist, and, therefore, a thorough occupational and social history is essential when evaluating any patient presenting with suspected ILD.

Patients with chronic HP can present in virtually identical fashion to those with IPF with the insidious, gradual onset of dyspnea. While worsening of dyspnea or cough in a specific environment in the workplace or home is an important clue for patients with acute/subacute HP, this is unlikely to be helpful in diagnosing chronic HP. HRCT imaging can show a variety of changes that include poorly formed bronchiolocentric nodules, patchy or diffuse ground-glass attenuation, peribronchiolar infiltrates, or areas of air trapping that may be optimally detected on expiratory views [33]. A UIP-like pattern on HRCT (Fig. 14.4) that appears to be consistent with a diagnosis of IPF can be seen in 37% of patients with fibrotic HP [34]. However, the presence of mosaic perfusion that corresponds to regions of air

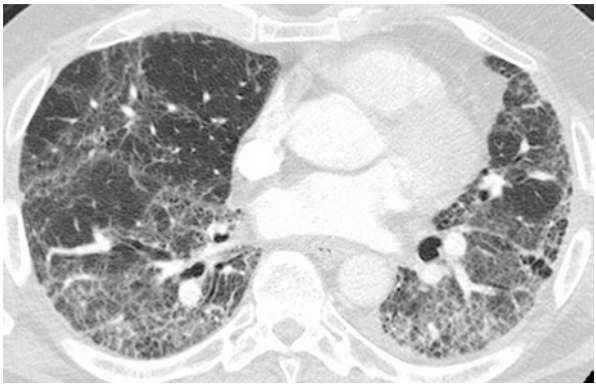

Fig. 14.4 HRCT cross-sectional view of a patient with chronic HP. A 62-year-old male diagnosed with IPF prior to referral. Review of surgical lung biopsy specimens showed a UIP pattern with focal superimposed organizing pneumonia, scattered poorly formed granulomas, and multinucleated giant cells

trapping on expiratory imaging, in association with fibrosis makes chronic HP a very likely diagnosis. Sparing of the lung bases might be another important clue to the presence of chronic HP. [33]. Poorly formed granulomas on lung histopathology, especially in a peribronchiolar distribution, are suggestive of HP, while peribronchiolar fibrosis might also suggest chronic HP. Other pathologies such as an NSIP-like or organizing pneumonia pattern may be present in patients with subacute/chronic forms of HP [34]. Evidence of extensive fibrosis on lung histopathology and/or HRCT is associated with worse survival, and late-stage fibrotic HP can appear histopathologically identical to UIP, fibrotic NSIP, or end-stage honeycomb lung [34–36]. Treatment should focus on the identification and avoidance of the implicated antigen, if one can be identified, and immunosuppressive therapy with corticosteroids with or without a steroid-sparing cytotoxic agent may also be clinically indicated [36, 37]. However, evidence guiding drug treatment for patients with chronic fibrotic HP is lacking, and whether anti-fibrotic agents have clinical efficacy for HP is unknown and remains to be investigated.

Other Fibrotic ILD

While cases of fibrotic NSIP, CTD-ILD, and chronic HP may be frequently encountered by clinicians and must be differentiated from IPF, UIP or UIP-like patterns on HRCT imaging as well as in surgical lung biopsy specimens can be seen in patients with other forms of ILD (Table 14.1). Although most cases of sarcoidosis will resolve spontaneously or respond to treatment, approximately 5% of patients with pulmonary sarcoidosis develop progressive fibrosis and declining lung function [38]. Approximately 50% of patients with advanced pulmonary sarcoidosis will have evidence of extrapulmonary involvement [39], which can aid in distinguishing advanced pulmonary sarcoidosis from IPF. Sarcoidosis is characterized histopathologically by the presence of well-formed granulomas with a lymphangitic distribution along bronchovascular bundles, interlobular septa, and pleura. However, these can become confluent and coalesce with disease progression. The invariable fibrosis, coupled with regression of the granulomatous infiltrates sets the stage for the emergence of a UIP pattern [2]. Findings of residual granulomas or giant cells and a central predilection for honeycomb change and bronchiectasis can help distinguish a UIP-like pattern of advanced sarcoidosis from IPF [2, 40].

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder associated with mutations of the *HPS* gene. Pulmonary fibrosis eventually develops in all individuals with HPS type 1, which is caused by a 16-base pair duplication within the *HPS1* gene, and can lead to respiratory failure and death within a few years of diagnosis [41]. Most cases occur in individuals of Puerto Rican descent and are characterized by a triad of lysosomal accumulation of ceroid lipofuscin, oculocutaneous albinism, and a platelet storage pool deficiency that causes a bleeding diathesis [41]. As is the case for UIP/IPF, reticulation and irregular septal thickening, subpleural cysts, and traction bronchiectasis are all described as HRCT findings in

HPS, although other features such as pleural thickening, a reticulonodular interstitial pattern, peribronchovascular thickening, and ground-glass opacities have also been described [42]. Pulmonary histopathologic manifestations of HPS include accumulations of ceroid lipofuscin within lung tissue with a background of prominent but patchy interstitial fibrosis with variable geographic distribution [2, 43]. Genetic testing of peripheral blood for molecular subtypes of HPS is now available and can be used to confirm a diagnosis.

Other forms of fibrotic ILD that may masquerade as UIP/IPF include Erdheim-Chester disease (ECD), advanced pulmonary Langerhans cell histiocytosis (PLCH), drug reactions, asbestosis, IgG4-related disease with lung involvement, and smoking-related interstitial fibrosis (SRIF) [2]. ECD, a rare, non-Langerhans histiocytosis that affects long bones with painful, osteosclerotic lesions in almost all cases, can involve the lungs and lead to advanced fibrosis that runs along the course of pulmonary lymphatics [44]. A common finding on HRCT is thickening of interlobular septa [45], while histopathologically there are interstitial accumulations of non-Langerhans histiocytes distributed along the lymphatics [46].

PLCH occurs primarily in smokers and is characterized by accumulations of Langerhans cells in airway-centered lesions that usually manifest as nodular and cystic lesions on HRCT imaging. These can rarely progress to extensive fibrosis that may simulate a UIP pattern [47]. The presence of nodules on HRCT and the more central location of cysts, which tend to be irregular in shape, can help distinguish PLCH from UIP/IPF. Langerhans histiocytes have a distinctive immunophenotype staining pattern and are found in nodular interstitial aggregates. These inflammatory histiocytic infiltrates can almost completely regress in older, persistent lesions as they are replaced by stellate configurations of dense fibrosis [2, 47].

Reactions to drugs that are potentially pneumotoxic can lead to a significant degree of pulmonary fibrosis [48]. Chief among these are bleomycin, methotrexate, amiodarone, and nitrofurantoin, but a large number of other drugs are also potential pneumotoxins (www.pneumotox.com). Various patterns on HRCT imaging or lung tissue histopathology can be seen that include UIP- or NSIP-like reactions, but a discussion of these patterns is beyond the scope of this chapter. Accurately diagnosing pulmonary fibrosis as a consequence of drug toxicity can be very challenging and requires taking a careful and comprehensive drug usage history to identify drug exposures that may correlate with fibrotic lung disease.

Individuals who have had significant occupational exposure to inhaled asbestos fibers are at risk to develop asbestosis, which can present clinically like other fibrotic ILDs including IPF. HRCT imaging can show a UIP pattern, although the presence of calcified pleural plaques greatly raises the suspicion for the presence of asbestosis. Histopathologic examination of lung tissue shows marked peribronchiolar fibrosis that can simulate a UIP pattern, but a careful examination of lung biopsy tissue should reveal the presence of asbestos bodies (asbestos fibers encrusted by iron) [49]. Additionally, asbestos-induced pleural plaques are not seen in UIP/IPF.

IgG4-related disease is a rare, multisystem disorder characterized by infiltration of involved organs with IgG4-positive plasma cells with associated lymphoplasmacytic inflammation and fibrosis that can involve either single or multiple anatomic

sites [50]. The lung can occasionally be involved with a NSIP or UIP pattern on thoracic HRCT [51]. The presence of elevated serum IgG4 levels and/or extrapulmonary manifestations can provide important clues to the diagnosis.

A histologically distinct pattern of interstitial fibrosis that occurs in smokers has been recently described and termed smoking-related interstitial fibrosis (SRIF) [52]. Fibrosis in SRIF is characterized by marked and relatively uniform alveolar septal thickening with dense hyalinization and hypocellularity that is most prominent in subpleural regions and associated with emphysema and respiratory bronchiolitis [52]. Chae et al. reported that HRCT imaging of biopsy-confirmed SRIF showed considerable similarity to that of patients with combined pulmonary fibrosis (UIP pattern) and emphysema (CPFE) as confirmed by the presence of UIP on surgical lung biopsy; however, 5-year survival for patients with SRIF was 85.7% versus 40.7% for patients with CPFE [53]. The difficulty in discerning SRIF from fibrotic NSIP or UIP with emphysema (CPFE) reinforces the need for an attentive clinical-radiologic-pathologic correlation to best enable an accurate diagnosis.

Unclassifiable Interstitial Lung Disease

A significant number of patients who present with ILD will elude a definitive diagnosis for a variety of reasons and be provided the rather unsatisfactory diagnosis of unclassifiable ILD. This classification likely represents a heterogeneous population of patients with fibrotic ILD that includes IPF, non-IPF IIP, chronic HP, and non-IIP disorders. Ryerson et al. found that ILD could not be classified in 10% ($N = 132$) of a total patient cohort of 1370 patients [54]. The major reason for patients to be given this classification was lack of a surgical lung biopsy due to high surgical risk (52%), while a definitive diagnosis could not be made despite performing HRCT and surgical lung biopsy in 18% of cases due to conflicting clinical, radiological, and histopathological data [54]. Other reasons included insufficient tissue obtained at surgical biopsy (8%), patient refusal to undergo surgical biopsy (8%), and the perception that the risk of surgical biopsy outweighed any potential benefit (9%). The risk of disease progression or death for those with unclassifiable disease correlates closely with baseline clinical and radiological features. Specifically, those patients with a radiologic diagnosis of UIP or possible UIP, a worse fibrosis score, or the presence of honeycomb change tend to have a worse prognosis [54].

Summary

Many non-IPF forms of fibrotic ILD can mimic IPF in their clinical presentation, radiologic imaging, and lung histopathology. Additionally, approximately 10% of ILD may be unclassifiable for a variety of reasons. If clinicians are to provide

precision medicine and personalized therapy approach to patients with fibrotic ILD, making an accurate and confident diagnosis of the specific entity at hand and differentiating IPF from non-IPF ILD are essential. Multidisciplinary discussions among clinicians, radiologists, and pathologists with expertise in ILD can maximize diagnostic confidence and should be undertaken whenever needed if feasible. The future work-up and management of ILDs will likely be facilitated by a greater understanding of the genetic underpinnings, genomic signatures, and disease-specific biomarkers of these disparate disorders. In addition to the current clinical-radiographic-histopathologic diagnostic paradigm, the future multidisciplinary approach will likely include biomarker profiling as a fourth dimension to further facilitate an accurate diagnosis. This will optimize management with more accurate prognostication and targeted therapeutic choices, including a pharmacogenomics approach with cell-based and other therapies.

Key Points

1. A NSIP pattern of ILD can be idiopathic but is more commonly seen in CTD-ILD and can occasionally be seen in HP.
2. Definitive diagnosis of NSIP requires a lung biopsy with adequate sampling of lung tissue.
3. ILD is common in patients with CTD and can be the initial manifestation of CTD. Patterns of NSIP, UIP, or organizing pneumonia may be present, and diagnosis of CTD-ILD requires correlation of clinical history, physical examination findings, serologic testing results, and thoracic imaging.
4. Chronic HP may be difficult to differentiate from fibrotic NSIP or UIP/IPF and can be seen when exposure to an identifiable antigen cannot be found. Although poorly formed granulomas are usually found in lung tissue biopsies, histopathologic patters of NSIP, organizing pneumonia, or UIP-like changes similar to that of patients with IPF without clear demonstration of granulomatous inflammation may be present.
5. When a diagnosis of UIP/IPF cannot be made via a combination of clinical presentation and HRCT imaging, adequate sampling of lung tissue via bronchoscopic cryobiopsy or surgical lung biopsy should be obtained unless such invasive procedures cannot be safely performed.
6. When clinicians encounter a patient with newly diagnosed fibrotic ILD, a number of clinical, HRCT imaging, and histopathologic clues can contribute to making an accurate and confident diagnosis.
7. Accurately discerning IPF from non-IPF fibrotic ILD is key to providing appropriate therapy for patients with IPF (e.g., anti-fibrotic agents, lung transplantation, avoidance of corticosteroids and cytotoxic agents). Anti-fibrotic agents have not as yet been adequately studied in patients with non-IPF ILD, but such patients may respond to immunosuppressive therapy.

References

1. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013;188(6):733–48.
2. Larsen BT, Smith ML, Elicker BM, Fernandez JM, de Morvil GAA, Pereira CAC, Leslie KO. Diagnostic approach to advanced fibrotic interstitial lung disease: bringing together clinical, radiologic, and histologic clues. Arch Pathol Lab Med. 2017;141(7):901–15.
3. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183:788–824.
4. Meyer KC. Diagnosis and management of interstitial lung disease. Transl Respir Med. 2014;2:4. https://doi.org/10.1186/2213-0802-2-4.
5. Flaherty KR, King TE Jr, Raghu G, Lynch JP 3rd, Colby TV, Travis WD, et al. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? Am J Respir Crit Care Med. 2004;170:904–10.
6. Walsh SL, Wells AU, Desai SR, Poletti V, Piciucchi S, Dubini A, et al. Multicentre evaluation of multidisciplinary team meeting agreement on diagnosis in diffuse parenchymal lung disease: a case-cohort study. Lancet Respir Med. 2016;4:557–65.
7. Flaherty KR, Travis WD, Colby TV, et al. Histopathologic variability in usual and nonspecific interstitial pneumonias. Am J Respir Crit Care Med. 2001;164(9):1722–7.
8. Meyer KC. Pulmonary fibrosis, part I: epidemiology, pathogenesis, and diagnosis. Expert Rev Respir Med. 2017;11(5):343–59.
9. Raghu G, Rochwerg B, Zhang Y, et al. An official ATS/ERS/JRS/ALAT clinical practice guideline: treatment of idiopathic pulmonary fibrosis. An update of the 2011 clinical practice guideline. Am J Respir Crit Care Med. 2015;192(2):e3–19.
10. Meyer KC. Pulmonary fibrosis, part II: state-of-the-art patient management. Expert Rev Respir Med. 2017;11(5):361–76.
11. Nathan SD, Meyer KC. IPF clinical trial design and endpoints. Curr Opin Pulm Med. 2014;20(5):463–71.
12. Katzenstein AL, Fiorelli RF. Nonspecific interstitial pneumonia/fibrosis. Histologic features and clinical significance. Am J Surg Pathol. 1994;18:136–47.
13. Glaspole I, Goh NS. Differentiating between IPF and NSIP. Chron Respir Dis. 2010;7(3):187–95.
14. MacDonald SL, Rubens MB, Hansell DM, et al. Nonspecific interstitial pneumonia and usual interstitial pneumonia: comparative appearances at and diagnostic accuracy of thin-section CT. Radiology. 2001;221(3):600–5.
15. Travis WD, Hunninghake G, King TE Jr, et al. Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. Am J Respir Crit Care Med. 2008;177:1338–47.
16. Flaherty KR, Martinez FJ. Nonspecific interstitial pneumonia. Semin Respir Crit Care Med. 2006;27(6):652–8.
17. Kim DS, Collard HR, King TE Jr. Classification and natural history of the idiopathic interstitial pneumonias. Proc Am Thorac Soc. 2006;3(4):285–92.
18. Gutsche M, Rosen GD, Swigris JJ. Connective tissue disease-associated interstitial lung disease: a review. Curr Respir Care Rep. 2012;1:224–32.
19. Fischer A, Antoniou KM, Brown KK, et al. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. Eur Respir J. 2015;46(4):976–87.
20. Vourlekis JS, Schwarz MI, Cool CD, Tuder RM, King TE, Brown KK. Nonspecific interstitial pneumonitis as the sole histologic expression of hypersensitivity pneumonitis. Am J Med. 2002;112(6):490–3.

21. Kim EJ, Collard HR, King TE Jr. Rheumatoid arthritis- associated interstitial lung disease: the relevance of histopathologic and radiographic pattern. Chest. 2009;136:1397–405.
22. Tomassetti S, Ryu JH, Piciucchi S, Chilosi M, Poletti V. Nonspecific interstitial pneumonia: what is the optimal approach to management? Semin Respir Crit Care Med. 2016;37(3):378–94.
23. Latsi PI, du Bois RM, Nicholson AG, et al. Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. Am J Respir Crit Care Med. 2003;168:531–7.
24. Fischer A, Brown KK, Du Bois RM, et al. Mycophenolate mofetil improves lung function in connective tissue disease-associated interstitial lung disease. J Rheumatol. 2013;40(5):640–6.
25. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014 – an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2015;34(1):1–15.
26. Castelino FV, Varga J. Interstitial lung disease in connective tissue diseases: evolving concepts of pathogenesis and management. Arthritis Res Ther. 2010;12:213.
27. Solomon J, Swigris JJ, Brown KK. Myositis-related interstitial lung disease and antisynthetase syndrome. J Bras Pneumol. 2011;37(1):100–9.
28. Suzuki A, Kondoh Y, Fischer A. Recent advances in connective tissue disease related interstitial lung disease. Expert Rev Respir Med. 2017;11(7):591–603.
29. Moua T, Maldonado F, Decker PA, Daniels CE, Ryu JH. Frequency and implication of autoimmune serologies in idiopathic pulmonary fibrosis. Mayo Clin Proc. 2014;89(3):319–26.
30. Vij R, Strek ME. Diagnosis and treatment of connective tissue disease-associated interstitial lung disease. Chest. 2013;143:814–24.
31. Selman M, Buendía-Roldán I. Immunopathology, diagnosis, and management of hypersensitivity pneumonitis. Semin Respir Crit Care Med. 2012;33:543–54.
32. Spagnolo P, Rossi G, Cavazza A, Bonifazi M, Paladini I, Bonella F, Sverzellati N, Costabel U. Hypersensitivity pneumonitis: a comprehensive review. J Investig Allergol Clin Immunol. 2015;25(4):237–50.
33. Silva CI, Müller NL, Lynch DA, et al. Chronic hypersensitivity pneumonitis: differentiation from idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia by using thin-section CT. Radiology. 2008;246:288–97.
34. Chung JH, Montner SM, Adegunsoye A, Oldham JM, Husain AN, Vij R, Noth I, Strek ME. CT findings associated with survival in chronic hypersensitivity pneumonitis. Eur Radiol. 2017;27(12):5127–35.
35. Selman M, Pardo A, King TE Jr. Hypersensitivity pneumonitis: insights in diagnosis and pathobiology. Am J Respir Crit Care Med. 2012;186(4):314–24.
36. Salisbury ML, Myers JL, Belloli EA, Kazerooni EA, Martinez FJ, Flaherty KR. Diagnosis and treatment of fibrotic hypersensitivity pneumonia. Where we stand and where we need to go. Am J Respir Crit Care Med. 2017;196(6):690–9.
37. Vasakova M, Morell F, Walsh S, Leslie K, Raghu G. Hypersensitivity pneumonitis: perspectives in diagnosis and management. Am J Respir Crit Care Med. 2017;196(6):680–9.
38. Shlobin OA, Nathan SD. Management of end-stage sarcoidosis: pulmonary hypertension and lung transplantation. Eur Respir J. 2012;39(6):1520–33.
39. Arcasoy SM, Christie JD, Pochettino A, Rosengard BR, Blumenthal NP, Bavaria JE, Kotloff RM. Characteristics and outcomes of patients with sarcoidosis listed for lung transplantation. Chest. 2001;120(3):873–80.
40. Xu L, Kligerman S, Burke A. End-stage sarcoid lung disease is distinct from usual interstitial pneumonia. Am J Surg Pathol. 2013;37(4):593–600.
41. El-Chemaly S, Young LR. Hermansky-Pudlak syndrome. Clin Chest Med. 2016 Sep;37(3):505–11.
42. Avila NA, Brantly M, Premkumar A, et al. Hermansky-Pudlak syndrome: radiography and CT of the chest compared with pulmonary function tests and genetic studies. AJR Am J Roentgenol. 2002 Oct;179(4):887–92.
43. Vicary GW, Vergne Y, Santiago-Cornier A, Young LR, Roman J. Pulmonary fibrosis in Hermansky-Pudlak syndrome. Ann Am Thorac Soc. 2016 Oct;13(10):1839–46.

44. Arnaud L, Pierre I, Beigelman-Aubry C, et al. Pulmonary involvement in Erdheim-Chester disease: a single-center study of thirty-four patients and a review of the literature. Arthritis Rheum. 2010;62(11):3504–12.
45. Brun AL, Touitou-Gottenberg D, Haroche J, et al. Erdheim-Chester disease: CT findings of thoracic involvement. Eur Radiol. 2010;20(11):2579–87.
46. Rush WL, Andriko JA, Galateau-Salle F, et al. Pulmonary pathology of Erdheim-Chester disease. Mod Pathol. 2000;13(7):747–54.
47. Sundar KM, Gosselin MV, Chung HL, et al. Pulmonary Langerhans cell histiocytosis: emerging concepts in pathobiology, radiology, and clinical evolution of disease. Chest. 2003;123(5):1673–83.
48. Camus P, Bonniaud P, Fanton A, et al. Drug-induced and iatrogenic infiltrative lung disease. Clin Chest Med. 2004;25(3):479–519.
49. Roggli VL, Gibbs AR, Attanoos R, et al. Pathology of asbestosis- an update of the diagnostic criteria: report of the asbestosis committee of the college of american pathologists and pulmonary pathology society. Arch Pathol Lab Med. 2010;134(3):462–80.
50. Yi ES, Sekiguchi H, Peikert T, et al. Pathologic manifestations of immunoglobulin(Ig) G4-related lung disease. Semin Diagn Pathol. 2012;29(4):219–25.
51. Schneider F, Veraldi KL, Levesque MC, Colby TV, S Yi E. IgG4-related lung disease associated with usual interstitial pneumonia. Open Rheumatol J. 2016;10:33–8.
52. Katzenstein AL. Smoking-related interstitial fibrosis (SRIF): pathologic findings and distinction from other chronic fibrosing lung diseases. J Clin Pathol. 2013;66(10):882–7.
53. Chae KJ, Jin GY, Jung HN, et al. Differentiating Smoking-Related Interstitial Fibrosis (SRIF) from Usual Interstitial Pneumonia (UIP) with emphysema using CT features based on pathologically proven cases. PLoS One. 2016;11(9):e0162231.
54. Ryerson CJ, Urbania TH, Richeldi L, et al. Prevalence and prognosis of unclassifiable interstitial lung disease. Eur Respir J. 2013;42:750–7.

Chapter 15
Gastroesophageal Reflux and IPF

Joyce S. Lee

Idiopathic pulmonary fibrosis (IPF) is a progressive form of lung fibrosis characterized by the usual interstitial pneumonia (UIP) pattern on high-resolution computed tomography scanning and/or surgical lung biopsy [1]. IPF is associated with a poor prognosis with a median survival of approximately 2–3 years [2]. In 2014, the Food and Drug Administration (FDA) approved two therapies, nintedanib and pirfenidone, for the treatment of IPF [3, 4]. While these drugs are not cures for the disease, their approval was an important first step for the treatment of IPF.

The etiology of IPF is unknown. There is an increasing recognition of the role of genetics [5], but several other risk factors have been described, including smoking, viral infection, and gastroesophageal reflux (GER) with microaspiration [1, 6]. Many patients with IPF have GER as a documented comorbidity. In a study using a large US claims database and ICD-9 codes, 9286 patients were identified to have IPF [7]. When compared to age- and gender-matched controls, patients with IPF had an increased risk for having GER (RR 2.42, 95% CI 2.10–2.79).

Although the relationship between GER, microaspiration, and IPF has been controversial, there are several appealing aspects to this relationship. First, nearly all patients with IPF have some degree of GER [8–10]. Second, GER and IPF share several risk factors including older age, smoking, and male gender [1, 11]. Last, and perhaps more significantly, there are proven medical and surgical treatments for GER [12]. The current state of IPF treatment focuses on slowing disease progression by limiting fibroproliferative processes. If there indeed is a relationship between GER, microaspiration, and IPF, the modification of GER and microaspiration may be potentially disease modifying by reducing the stimulus for further fibroproliferation.

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A Proposed Mechanism

The mechanism by which GER and microaspiration are thought to play a role in either the pathogenesis or progression of disease is likely complex. The original hypothesis was that GER is a sequela of IPF. Due to the decreased lung compliance in patients with IPF, the increased swings in pleural pressure caused mechanical traction on the lower esophageal sphincter leading to dysfunction and eventual GER.

Conversely, a competing hypothesis has also been suggested (Fig. 15.1) [6]. Stomach contents can enter the esophagus through a weakened lower esophageal sphincter. Reflux of these contents can occasionally reach the upper regions of the

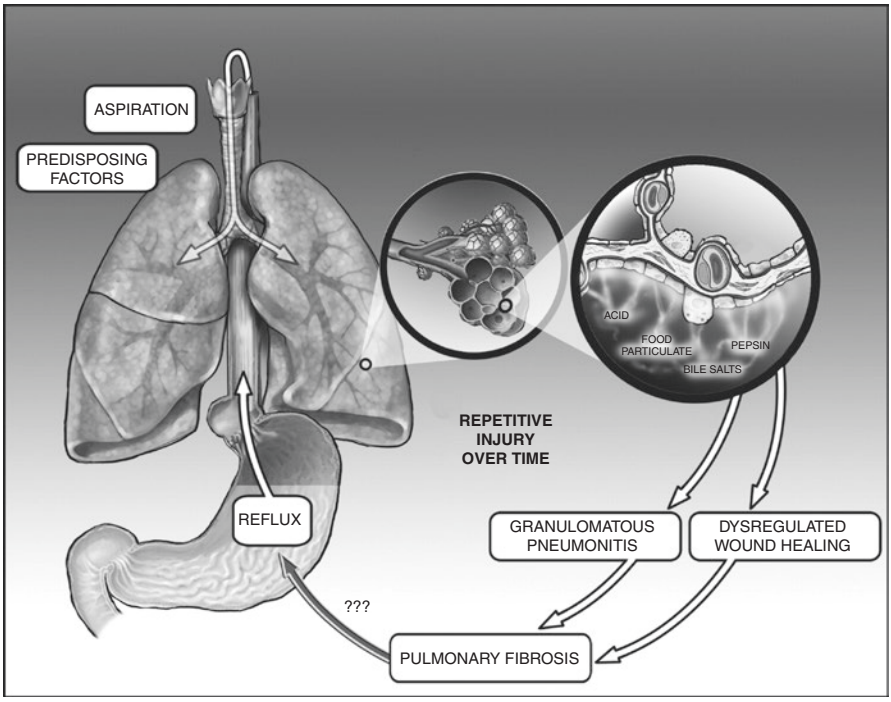

Fig. 15.1 Possible pathogenetic mechanism for chronic microaspiration in idiopathic pulmonary fibrosis. Gastric fluid can travel in a retrograde fashion through a weakened lower esophageal sphincter (eg, secondary to a hiatal hernia, traction from the diaphragm, or medications) up into the esophagus. The gastric refluxate can travel as high up as the cricopharyngeal region and enter the airway. Normal host defenses likely clear most gastric refluxate without clinical sequelae. However, in some cases, components of the gastric refluxate (eg, acid, bile, particulates) may directly injure the lung epithelium. In the genetically or otherwise predisposed patient, chronic microaspiration of gastric refluxate may cause repetitive injury over time, leading to granulomatous pneumonitis, dysregulated wound healing, and eventual lung fibrosis. Additionally, progressive pulmonary fibrosis may lead to distortion of the mediastinal structures and traction on the esophagus. This could cause additional weakening of the lower esophageal sphincter, which could in turn lead to microaspiration, lung injury, and the accelerated decline or acute respiratory decompensation seen in some patients with idiopathic pulmonary fibrosis

esophagus, allowing for penetration of these contents into the trachea. Most people can clear aspirated material without any clinical sequelae. However, in the genetically or otherwise predisposed host, this aspirated material can lead to inflammation and subsequent fibrosis. The development of lung fibrosis can further worsen this cycle by leading to retraction on the mediastinal structures, further weakening the lower esophageal sphincter. Thus, a feedback mechanism is created by which GER and secondary microaspiration can lead to repetitive lung injury, disease progression, and exacerbations. While this mechanistic link has not been proven, there are several basic and clinical studies that support a relationship between GER, microaspiration, and IPF.

Biologic Rationale

On a cellular level, stomach contents have been shown to influence pro-fibrotic pathways. For example, pepsin increased expression of alpha-smooth muscle actin (SMA), matrix glycoproteins, and matrix-degrading proteases when placed on lung epithelial cells in culture [13]. Additionally, bile acids have induced TGF-beta production in human airway epithelial cells and increased fibroblast proliferation [14].

In animals, aspirated gastric juice has been shown to distribute rapidly in the lungs, reaching the subpleural zones of dogs within 20 s [15]. Other rodent models of repetitive gastric fluid aspiration have shown the development of pneumonitis and parenchymal fibrosis [16, 17]. These models have also demonstrated increased expression of collagens III and IV and fibronectin in lung tissue along with increased TGF-beta production [17, 18].

Clinical Evidence

As stated previously, GER is common in patients with IPF. Over the years, several studies have been published describing the prevalence of GER disease (GERD) in IPF (Table 15.1). These studies differ in many ways, including study design, study population, and method of diagnosis of both GERD and IPF. However, what is similar across all the studies, despite these differences, is the generally high prevalence of GER. In addition, IPF patients with GER often do not have the classic symptoms of heartburn and reflux. The typical symptoms are present in only one third to half of IPF patients with documented GER by 24 h pH monitoring (sensitivity of 65% and specificity of 71%) [19]. Another important finding in one of these studies was that the severity of GER measured by 24 h pH monitoring did not correlate with the severity of IPF disease as measured by forced vital capacity [10].

There are some data to suggest that GER may impact disease progression in IPF. In a retrospective study of 32 asymmetric cases of IPF (asymmetry ratio determined by (most affected – least affected fibrosis score)/(most affected + least affected fibrosis score) >0.2), there was increased overt GERD in those with

Table 15.1 Studies describing the prevalence of gastroesophageal reflux disease in IPF

First author	Study location	Date of publication	Primary finding
Belcher [20]	London, UK	1949	Case reports of pulmonary fibrosis in patients with dysphagia
Pearson [21]	Bristol, UK	1971	4% of people with HH had diffuse pulmonary fibrosis
Mays [22]	San Francisco	1976	73% prevalence of HH and 44% prevalence of reflux in pulmonary fibrosis
Tobin [8]	Seattle	1998	94% prevalence of GERD in IPF
Patti [23]	San Francisco	2005	66% prevalence of GERD in IPF
Raghu [10]	Seattle	2006	87% prevalence of GERD in IPF
Salvioli [19]	Bologna, Italy	2006	67% prevalence of GERD in IPF
Sweet [9]	San Francisco	2007	67% prevalence of GERD in IPF
Noth [24]	Chicago	2012	39% prevalence of CT HH in IPF
Savarino [25]	Padua, Italy	2013	83% prevalence of GERD in IPF

CT computed tomography, *GERD* gastroesophageal reflux disease, *HH* hiatal hernia, *IPF* idiopathic pulmonary fibrosis

asymmetric disease vs. controls (63% vs. 31%, $p = 0.009$). Interestingly, those with asymmetric disease also reported sleeping on the more affected side [26]. These data are limited, however, by the retrospective nature of the study and patient recall bias.

GER and microaspiration have also been shown to be associated with acute exacerbation of IPF. Bronchoalveolar lavage (BAL) pepsin levels were measured in 24 cases of acute exacerbation of IPF and 30 stable IPF controls. BAL pepsin level was associated with acute exacerbation status ($p = 0.04$) but was not associated with survival status in those with an acute exacerbation [27].

Treatment of GER in IPF

There are now emerging data on the role of GER treatment in IPF. In addition, the current international treatment guidelines for IPF made a conditional recommendation for regular antiacid treatment in patients with IPF with low confidence in the estimates of effect given the data quality [28]. It is important to recognize that none of these are randomized controlled clinical trials specifically looking at the role of GER treatment in IPF (Table 15.2). Rather, they are either retrospective analyses or secondary data analyses of existing clinical trial data.

The first study to demonstrate a relationship between GER treatment and IPF was a case series of four patients with IPF. In this case series, patients had lung function stabilization when they were maintained on adequate treatment for their GER [29]. Another group published their experience with 14 IPF patients awaiting lung transplantation who had undergone laparoscopic Nissen fundoplication [30]. They

Table 15.2 Summary of published studies describing GER treatment in IPF

First author/date	Sample size	Type of GER treatment	Primary finding
Raghu 2006 [29]	4	Medical antiacid therapy and/or surgical fundoplication	Stabilization in lung function
Linden 2006 [30]	45 (31% had surgery)	Surgical fundoplication	Laparoscopic fundoplication was safe, and patients had stabilization of their oxygen requirements compared to those that did not have surgery
Lee 2011 [31]	204 (47% on antiacid therapy; 53% not on therapy)	Medical antiacid therapy	Antiacid therapy was associated with decreased radiologic fibrosis and was an independent predictor of longer survival time in patients with IPF ($p = 0.03$)
Lee 2013 [32]	242 (51% on antiacid therapy; 49% not on therapy)	Medical antiacid therapy	Antiacid therapy was associated with a smaller decline in FVC at 30 weeks ($p = 0.05$), estimated change at 52 weeks ($p = 0.04$), and acute exacerbation ($p = 0.017$). No difference in all-cause mortality ($p = 0.12$)
Ghebremariam 2015 [33]	215 (60% on antiacid therapy; 40% not on therapy)	Medical antiacid therapy	Patients on antiacid therapy had a longer survival time compared to those not on antiacid therapy ($p = 0.006$)
Kreuter 2016 [34]	624 (47% on antiacid therapy; 53% not on therapy)	Medical antiacid therapy	No difference between groups at 52 weeks for disease progression ($p = 0.48$), all-cause mortality ($p = 0.89$), or IPF-related mortality ($p = 0.43$)
Raghu 2016 [35]	27 (all had surgery)	Surgical fundoplication	The estimated benefit of laparoscopic anti-reflux surgery in this cohort was an FVC of 0.22 L (95% CI −0.06 to 0.49 L, $p = 0.12$)

found that those patients who had a Nissen fundoplication had more stable oxygen requirements compared to those who did not have the procedure.

This was followed by a retrospective cohort study looking specifically at the role of medical antiacid therapy with proton pump inhibitors and/or H2 blockers in IPF [31]. The authors assembled two large IPF cohorts ($n = 204$) and analyzed the relationship between antiacid therapy and survival. Patients who reported taking antiacid therapy at the baseline ILD clinic visit had a longer survival time compared to those not taking antiacid therapy at their baseline visit. This relationship was independent of disease severity, radiologic fibrosis, age, and sex. Those taking antiacid therapy were more likely to be women, report more frequent cough, and have less radiologic fibrosis at baseline.

Two subsequent studies looked at existing data within IPF clinical trials. The first was an analysis of the placebo arms of three IPFnet randomized controlled clinical

trials (STEP-IPF, ACE-IPF, and PANTHER-IPF) [36–38]. In these trials, there was prospective collection of antiacid therapy data (i.e., proton pump inhibitors, H2 blockers). Using these data, the relationship between antiacid therapy and change in FVC was analyzed in 242 patients with IPF. The authors found that antiacid therapy was associated with a slower decline in FVC (difference of 0.07 L, 95% CI 0–0.14, $p = 0.05$), and patients taking antiacid therapy had no acute exacerbations compared to nine events in the control group [32]. The subsequent study used the placebo arms of patients with IPF from three other clinical trials (CAPACITY 004, CAPACITY 006, and ASCEND) [4, 39]. In this analysis, the investigators found that of 624 patients, there was no difference in lung function decline between the patient cohort taking and the cohort not taking antiacid therapy at 52 weeks [34]. Thus, the differing findings in these two secondary data analyses make it unclear if antiacid treatment in IPF is associated with improved outcomes.

Additional Areas of Uncertainty

There remains equipoise in the field on the role of antiacid treatment in patients with IPF. There is an ongoing phase II NIH-sponsored study investigating the role of laparoscopic Nissen fundoplication in IPF. These results, while important, will not address the role of medical antiacid therapy in patients with IPF. In addition to the direct effects that antiacid therapy has on the acidity of aspirated gastric fluid, there are some recent data suggesting off-target effects of proton pump inhibitors. Specifically, proton pump inhibitors inhibit dimethylarginine dimethylaminohydrolase (DDAH), which regulates the metabolism of nitric oxide synthetase [40]. This inhibition blocks TGF-beta-induced collagen expression and may have additional antioxidant and anti-fibrotic effects [33].

The issues and confusion around treatment speak to our poor understanding of what it is about GER in IPF that is the problem. We don't know what is causing the injury (e.g., acid, bile, food particulates) and whether or not the injury has implications for disease pathogenesis and/or progression. A better understanding of what is causing the injury and why there is a discordance in the prevalence of GERD (20,000 per 100,000) [41] and the prevalence of IPF (14–43 per 100,000) [42] may help us better understand this relationship and test targeted therapy.

The final difficulty is how best to diagnose GER and microaspiration in IPF. GER is often diagnosed by 24 h pH monitoring and manometry. However, having GER does not equal microaspiration. Some radiologic studies have been used to assess for aspiration and risk for aspiration, including barium swallow, computed tomography scan, and scintigraphy. However, all of these studies have limitations in sensitivity, inter-observer variation, availability, and cost [6]. Biomarkers, such as bronchoalveolar lavage pepsin and bile salts, are being investigated as markers for aspiration given their specificity for the gastrointestinal tract. Further studies need to be done to validate these as clinically useful biomarkers.

Conclusion

In summary, the relationship between GER, microaspiration, and IPF is important. At this time, it remains unclear if there is a true pathogenetic link between these entities and what cofactors may be modifying this relationship. Further work needs to be done in order to understand this complex relationship. In addition, it will be important for us to determine if treatment (medical and/or surgical) of this common comorbidity will impact disease progression and symptoms in patients with IPF. Randomized controlled trials of medical and surgical therapy will need to be done in order for us to determine the role of GER treatment in IPF.

References

1. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. 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.
2. Bjoraker JA, Ryu JH, Edwin MK, Myers JL, Tazelaar HD, Schroeder DR, et al. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998;157(1):199–203.
3. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2071–82.
4. King TE Jr, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2083–92.
5. Spagnolo P, Cottin V. Genetics of idiopathic pulmonary fibrosis: from mechanistic pathways to personalised medicine. J Med Genet. 2017;54(2):93–9.
6. Lee JS, Collard HR, Raghu G, Sweet MP, Hays SR, Campos GM, et al. Does chronic microaspiration cause idiopathic pulmonary fibrosis? Am J Med. 2010;123(4):304–11.
7. Collard HR, Ward AJ, Lanes S, Cortney Hayflinger D, Rosenberg DM, Hunsche E. Burden of illness in idiopathic pulmonary fibrosis. J Med Econ. 2012;15(5):829–35.
8. Tobin RW, Pope CE 2nd, Pellegrini CA, Emond MJ, Sillery J, Raghu G. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998;158(6):1804–8.
9. Sweet MP, Patti MG, Leard LE, Golden JA, Hays SR, Hoopes C, et al. Gastroesophageal reflux in patients with idiopathic pulmonary fibrosis referred for lung transplantation. J Thorac Cardiovasc Surg. 2007;133(4):1078–84.
10. Raghu G, Freudenberger TD, Yang S, Curtis JR, Spada C, Hayes J, et al. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. Eur Respir J. 2006;27(1):136–42.
11. Sonnenberg A. Effects of environment and lifestyle on gastroesophageal reflux disease. Dig Dis. 2011;29(2):229–34.
12. Bredenoord AJ, Pandolfino JE, Smout AJ. Gastro-oesophageal reflux disease. Lancet. 2013;381(9881):1933–42.
13. Ahmad F, Ritzenthaler J, Roman J. Pepsin in gastric fluid promotes epithelial mesenchymal transformation: implications for understanding the role of reflux in pulmonary fibrosis. ATS: Am J Respir Crit Care Med. 2009;179:A5311.
14. Perng DW, Chang KT, Su KC, Wu YC, Wu MT, Hsu WH, et al. Exposure of airway epithelium to bile acids associated with gastroesophageal reflux symptoms: a relation to transforming growth factor-beta1 production and fibroblast proliferation. Chest. 2007;132(5):1548–56.

15. Hamelberg W, Bosomworth PP. Aspiration pneumonitis: experimental studies and clinical observations. Anesth Analg. 1964;43:669–77.
16. Downing TE, Sporn TA, Bollinger RR, Davis RD, Parker W, Lin SS. Pulmonary histopathology in an experimental model of chronic aspiration is independent of acidity. Exp Biol Med (Maywood). 2008;233:1202–12.
17. Appel JZ 3rd, Lee SM, Hartwig MG, Li B, Hsieh CC, Cantu E 3rd, et al. Characterization of the innate immune response to chronic aspiration in a novel rodent model. Respir Res. 2007;8:87.
18. Kwan M, Xu YD, Raghu G, Khalil N. Acid treatment of normal rat lungs releases transforming growth factor-beta1 (TGF-beta1) and increases connective tissue synthesis. Proceedings of the American Thoracic Society (PATS) AJRCCM. 2007. p. A967.
19. Salvioli B, Belmonte G, Stanghellini V, Baldi E, Fasano L, Pacilli AM, et al. Gastro-oesophageal reflux and interstitial lung disease. Dig Liver Dis. 2006;38(12):879–84.
20. Belcher JR. The pulmonary complications of dysphagia. Thorax. 1949;4(1):44–56.
21. Pearson JE, Wilson RS. Diffuse pulmonary fibrosis and hiatus hernia. Thorax. 1971;26(3):300–5.
22. Mays EE, Dubois JJ, Hamilton GB. Pulmonary fibrosis associated with tracheobronchial aspiration. A study of the frequency of hiatal hernia and gastroesophageal reflux in interstitial pulmonary fibrosis of obscure etiology. Chest. 1976;69(4):512–5.
23. Patti MG, Tedesco P, Golden J, Hays S, Hoopes C, Meneghetti A, et al. Idiopathic pulmonary fibrosis: how often is it really idiopathic? J Gastrointest Surg. 2005;9(8):1053–6. discussion 6–8
24. Noth I, Zangan SM, Soares RV, Forsythe A, Demchuk C, Takahashi SM, et al. Prevalence of hiatal hernia by blinded multidetector CT in patients with idiopathic pulmonary fibrosis. Eur Respir J. 2012;39(2):344–51.
25. Savarino E, Carbone R, Marabotto E, Furnari M, Sconfienza L, Ghio M, et al. Gastro-oesophageal reflux and gastric aspiration in idiopathic pulmonary fibrosis patients. Eur Respir J. 2013;42:1322–31.
26. Tcherakian C, Cottin V, Brillet PY, Freynet O, Naggara N, Carton Z, et al. Progression of idiopathic pulmonary fibrosis: lessons from asymmetrical disease. Thorax. 2011;66(3):226–31.
27. Lee JS, Song JW, Wolters PJ, Elicker BM, King TE Jr, Kim DS, et al. Bronchoalveolar lavage pepsin in acute exacerbation of idiopathic pulmonary fibrosis. Eur Respir J. 2011;39:352–8.
28. Raghu G, Rochwerg B, Zhang Y, Garcia CA, Azuma A, Behr J, et al. An Official ATS/ERS/JRS/ALAT clinical practice guideline: treatment of idiopathic pulmonary fibrosis. An update of the 2011 clinical practice guideline. Am J Respir Crit Care Med. 2015;192(2):e3–19.
29. Raghu G, Yang ST, Spada C, Hayes J, Pellegrini CA. Sole treatment of acid gastroesophageal reflux in idiopathic pulmonary fibrosis: a case series. Chest. 2006;129(3):794–800.
30. Linden PA, Gilbert RJ, Yeap BY, Boyle K, Deykin A, Jaklitsch MT, et al. Laparoscopic fundoplication in patients with end-stage lung disease awaiting transplantation. J Thorac Cardiovasc Surg. 2006;131(2):438–46.
31. Lee JS, Ryu JH, Elicker BM, Lydell CP, Jones KD, Wolters PJ, et al. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184(12):1390–4.
32. Lee JS, Collard HR, Anstrom KJ, Martinez F, Noth I, Roberts RS, et al. Anti-acid treatment and disease progression in idiopathic pulmonary fibrosis: an analysis of data from three randomized controlled trials. Lancet Respir Med. 2013;1(5):369–76.
33. Ghebremariam YT, Cooke JP, Gerhart W, Griego C, Brower JB, Doyle-Eisele M, et al. Pleiotropic effect of the proton pump inhibitor esomeprazole leading to suppression of lung inflammation and fibrosis. J Transl Med. 2015;13:249.
34. Kreuter M, Wuyts W, Renzoni E, Koschel D, Maher TM, Kolb M, et al. Anti-acid treatment in patients with IPF: interpret results from post-hoc, subgroup, and exploratory analyses with great caution – authors' reply. Lancet Respir Med. 2016;4(9):e48.
35. Raghu G, Morrow E, Collins BF, Ho LA, Hinojosa MW, Hayes JM, et al. Laparoscopic anti-reflux surgery for idiopathic pulmonary fibrosis at a single centre. Eur Respir J. 2016;48(3):826–32.

36. Zisman DA, Schwarz M, Anstrom KJ, Collard HR, Flaherty KR, Hunninghake GW. A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. N Engl J Med. 2010;363(7):620–8.
37. Noth I, Anstrom KJ, Calvert SB, de Andrade J, Flaherty KR, Glazer C, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186:88–95.
38. Idiopathic Pulmonary Fibrosis Clinical Research N, Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 2012;366(21):1968–77.
39. Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet. 2011;377(9779):1760–9.
40. Ghebremariam YT, LePendu P, Lee JC, Erlanson DA, Slaviero A, Shah NH, et al. Unexpected effect of proton pump inhibitors: elevation of the cardiovascular risk factor asymmetric dimethylarginine. Circulation. 2013;128(8):845–53.
41. Locke GR 3rd. Current medical management of gastroesophageal reflux disease. Thorac Surg Clin. 2005;15(3):369–75.
42. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006;174(7):810–6.

Chapter 16
The Role of Pulmonary Rehabilitation and Supplemental Oxygen Therapy in the Treatment of Patients with Idiopathic Pulmonary Fibrosis

Catherine Wittman and Jeffrey J. Swigris

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fibrosing lung disease with a median survival of 2–5 years. IPF is characterized by dyspnea, and many patients with IPF develop hypoxemia at rest, during sleep, or with exertion [1–3]. Fatigue, depression, and anxiety are underappreciated symptoms that, like dyspnea, may affect patients' quality of life (QOL).

Although, on average, nintedanib and pirfenidone slow the progression of IPF, neither have been shown to convincingly improve QOL or dyspnea [4, 5]. This is unfortunate, as dyspnea is the strongest determinant of reduced quality of life in patients with IPF [6]. As IPF progresses and dyspnea increases, physical functional capacity and strength decline [7]. This initiates a downward spiral of deconditioning, a physically inactive lifestyle, and increased dyspnea which puts patients at risk for social isolation and mood disturbance [8, 9]. In various studies, between 25% and 67% of IPF patients have reported depressive symptoms [10–12], and up to 50% have reported significant fatigue [13–15]. In patients with IPF, fatigue and exercise capacity are strong, independent predictors of physical activity (while adjusting for lung function) [16]. As with QOL and dyspnea, drugs have not been shown to improve fatigue or day-to-day physical functioning in patients with IPF. Given the limitations of current pharmacotherapy, complimentary approaches are needed to treat patients with IPF. In this chapter, we discuss the use of pulmonary rehabilitation and supplemental oxygen as adjunctive therapies for patients with IPF.

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Pulmonary Rehabilitation

Pulmonary rehabilitation is a program that combines exercise training, disease-specific education, and psychosocial support in an attempt to reduce symptoms, optimize functional status, increase participation in daily life activities, and improve emotional well-being [17]. Pulmonary rehabilitation programs were traditionally designed for people with chronic obstructive pulmonary disease (COPD) in whom completion of pulmonary rehabilitation has been observed to induce several beneficial effects: reduce respiratory rate (by prolonging expiration), increase tidal volume and oxygen saturation [18], improve cardiac conditioning, increase fat-free body mass [19], promote fatigue resistance in the quadriceps [20], and enhance the efficiency of skeletal muscle function at the cellular and molecular levels [17]. These effects translate to statistically significant and clinically meaningful improvements in exercise capacity, QOL, and dyspnea [17]. Despite the hope that anti-fibrotic therapies have fostered among IPF patients and the practitioners caring for them, the prognosis of IPF remains poor, and patients continue to suffer with intrusive symptoms and poor QOL. The wealth of data supporting the benefits of pulmonary rehabilitation in patients with COPD has prompted research into its effectiveness for IPF.

In fact, since 2008, there has been an explosion of interest in pulmonary rehabilitation for IPF, with the completion of five randomized, controlled trials and at least nine publications [16, 21–28]. In five studies of IPF outpatients, subjects were randomized to participate in 8–12 weeks of twice weekly, supervised exercise, consisting of strength and aerobic training. In some studies, the intervention also included an education component and flexibility or stretching exercises. In each, investigators assessed functional capacity with distance covered in a 6-min walk test (6MWD), dyspnea with the Borg Dyspnea Index (BDI) [16, 22, 24] or mMRC (modified Medical Research Council) [21, 23, 26, 28], and HRQOL (health-related quality of life) with the St. George's Respiratory Questionnaire (SGRQ) [16, 21, 22, 26, 28] or Chronic Respiratory Disease Questionnaire (CRQ) [23, 29]. In most studies, investigators followed subjects until the end of the intervention, but in a handful of studies, subjects were followed an additional 3 [7, 21, 22], 11 [25] or 30 months [27]. In two, results were presented for the subgroup of subjects with IPF [21, 23].

Pulmonary rehabilitation led to statically significant improvements in 6MWD [16, 21, 23, 28], HRQOL [16, 21–23, 28], and dyspnea [23, 28]. Interestingly, pulmonary rehabilitation improved dyspnea in two of the three studies in which the mMRC was used [21, 23, 28] but in neither study in which the Borg dyspnea scale was used [16, 22, 24]. The mMRC asks respondents for a global assessment of dyspnea on a day-to-day basis, and the Borg scale asks respondents to rate their shortness of breath at the very moment they are responding. Obviously, the Borg scale is dependent on how active the respondent has been in the seconds before responding as well as the energy demand of the activity. Thus, studies in which improvement was detected by the mMRC support beneficial effects of pulmonary rehabilitation on overall, day-to-day physical functional capacity rather than simply shortness of

breath after maximal or submaximal exertion. Disappointingly, none of the pulmonary rehabilitation-related gains in 6MWD, HRQOL, or dyspnea were durable in any study [21–23, 25], with the exception of HRQL at 11 months in the study by Vainshelboim and his colleagues [27]. Some investigators asked subjects to continue exercising at home after completion of the formal pulmonary rehabilitation program, but on average, as measured by International Physical Activity Questionnaire, subjects were no more active at home after the program than before [22, 25, 27].

The effects of pulmonary rehabilitation on emotional well-being, including anxiety and depression, are less clear. Each was assessed in two randomized, controlled trials of pulmonary rehabilitation in mixed cohorts of ILD patients [21, 23]. In a study by Dowman and co-investigators, there was no beneficial effect of pulmonary rehabilitation on anxiety or depression as measured by the HADS (Hospital Anxiety and Depression Scale); however, very few subjects had clinically significant depression or anxiety at baseline. In a study by Holland and her colleagues, pulmonary rehabilitation had no effect on IPF subjects' emotional well-being as measured by the CRQ [21, 23]. In other studies [30–37], results were mixed, ranging from no change to significant improvement in various emotional health domains [30–37]. Additional research is needed to confirm whether pulmonary rehabilitation has durable beneficial effects on emotional well-being in patients with IPF, particularly those with anxiety or mood disturbance at baseline.

Fatigue, an often overlooked but intrusive symptom of IPF, has been assessed in only a handful of studies: in most, pulmonary rehabilitation was associated with improvements [21, 23, 36, 37].

In summary, a growing body of literature suggests that, in patients with IPF, pulmonary rehabilitation improves exercise capacity, quality of life, dyspnea, and perhaps some aspects of emotional well-being. However, additional research is needed to better define the effects of pulmonary rehabilitation on other outcomes meaningful to patients with IPF. In IPF, a disease with very limited treatment options, we believe pulmonary rehabilitation can and should complement pharmacological and other therapies. When to start pulmonary rehabilitation, how long it should last, what components should be included, and whether standard pulmonary rehabilitation programs should be tweaked to better-suit patients with IPF are questions that currently do not have adequate answers and need further study.

When Should Pulmonary Rehabilitation Be Initiated in People with IPF and How Long Should It Last?

Given the variable disease course [38], short survival, and potentially disabling symptoms, it is unclear when pulmonary rehabilitation should be initiated to maximize its beneficial effects. Published data are limited and conflicting: Kozu and colleagues found that subjects with moderate or moderately severe dyspnea

(according to the original, 6-point MRC breathlessness scale) derived benefit from pulmonary rehabilitation, while subjects with severe or very severe dyspnea did not [32]. Holland and colleagues found that greater improvements in 6MWD at 6 months occurred in subjects with higher baseline FVC values and less profound exercise-induced oxygen desaturation. The same group also observed that subjects with worse baseline dyspnea had the greatest improvements in dyspnea at 6 months, which is in contrast to the findings of Kozu and colleagues [39]. Still other investigators have observed that subjects with lower functional capacity at baseline (as defined by 6MWD) experienced the greatest improvements in 6MWD after exercise training [21, 30, 31, 35]. Perhaps most importantly, in the overwhelming majority of studies, there was no 6MWD upper limit above which pulmonary rehabilitation was found to be ineffective [30, 35]; moreover, higher baseline walk distances did not preclude improvement in other end points [35].

In summary, the data in aggregate hint that patients with more severe dyspnea and worse exercise capacity will improve their 6MWD most from baseline, but all patients have the potential to benefit. Thus, we believe and strongly advocate that all patients with IPF should be given the opportunity to participate in pulmonary rehabilitation, regardless of disease severity.

There are few data on the ideal duration of pulmonary rehabilitation in IPF patients. In a longitudinal study in which outcomes were assessed at 12 and 24 weeks in 31 subjects with restrictive lung disease patients (11 with ILD, 6 with IPF), subjects with ILD improved their 6MWD from baseline to 12 weeks (mean 79 m) and had additional gains (mean 28 m) at 24 weeks [36]. In patients with COPD, a meta-analysis showed that longer-term pulmonary rehabilitation led to greater improvements in HRQOL than shorter programs [40]. Given this—and what is known about IPF—gains in HRQOL are not durable for long after completion of pulmonary rehabilitation [22, 23, 27] . In addition, the overwhelming majority of patients do not adhere to home exercise training after completing a pulmonary rehabilitation program [22, 25], and therefore it would seem that longer programs could increase the durability of beneficial effects. Given the lack of data, there is an urgent need to conduct studies to assess the optimal duration of pulmonary rehabilitation in patients with IPF.

Components of Pulmonary Rehabilitation for IPF

A comprehensive patient evaluation is a key element of pulmonary rehabilitation. At the commencement of the program, all participants should undergo a medical assessment, with particular attention paid to symptoms and signs of obstructive lung disease, gastroesophageal reflux, coronary artery disease (CAD), depression, anxiety, and pulmonary hypertension [41]. Patients with IPF are at risk for CAD [42–45] due to their age, relevant risk factors (e.g., a history of cigarette smoking, hypercholesterolemia), or perhaps circulating pro-fibrotic factors that promote CAD. Therefore consideration should be given to having patients undergo cardiac

stress testing prior to starting pulmonary rehabilitation. If impairments in diffusing capacity of the lung for carbon monoxide (DLCO), oxygen saturation during exertion, or exercise tolerance are out of proportion to the extent of fibrosis, an echocardiogram should be considered to screen for pulmonary hypertension. An objective measure of exercise tolerance such as a 6-min walk test or a formal cardiopulmonary exercise test is useful to determine a starting point for the aerobic component of the exercise regimen. Evaluation of exercise-induced oxyhemoglobin desaturation should be completed, and supplemental oxygen should be prescribed for use during training, if needed. Evaluation of HRQOL should be undertaken, using standardized measurement tools that are sensitive to change in IPF. There are several available. Other patient-reported outcome measures could be employed as dictated by intuition, interest, or research agendas. All participants should be re-evaluated after the pulmonary rehabilitation program to establish its effects on exercise capacity, symptoms, HRQOL, and mood and to plan for the ongoing care needs of the individual.

Special Considerations for Pulmonary Rehabilitation in IPF

Pulmonary rehabilitation was created for patients with COPD who have hyperinflation, musculoskeletal dysfunction, and impaired respiratory mechanics. While patients with IPF differ in the physiological basis for their exercise limitation, components of pulmonary rehabilitation have remained largely the same for all patients participating [36]. In the United States, pulmonary rehabilitation is conducted almost exclusively on an outpatient basis, while in Europe and Asia, some centers employ inpatient programs [31, 34]. In most outpatient programs, patients exercise two to three times a week for 6–12 weeks. Regimens typically encompass endurance and strength training [16, 21–28, 32, 33, 39]. Some programs also contain education components and flexibility or stretching exercises [16, 21, 22, 24–28, 30–37, 39].

Endurance training is accomplished in a variety of ways (walking, treadmill, stationary bike, step climbing) and often using combinations of modalities [21–28, 31, 36, 39]. It is unclear if patients with IPF might benefit more from interval training instead of endurance training [22, 25–28, 32, 36]. The intensity of the aerobic component is initially set at either 70–80% of average or peak walk speed on 6MWD [16, 21, 23, 25–28, 36, 39], 60–80% of the maximum predicted heart rate [22, 24, 37], or 60% of the cardiopulmonary test-derived maximum oxygen consumption. Resistance or strength training of the upper and lower limbs has been carried out using machines [37], elastic bands [16, 22, 24, 31, 34, 37], or weights [25–28, 31, 37]. We believe strengthening of the large muscle groups of the lower extremities should be a more prominent component of pulmonary rehabilitation in patients with IPF.

Like the rest of pulmonary rehabilitation, the educational component was originally developed for patients with COPD and may contain topics that are not relevant

to patients with IPF. Themes typically include medication and oxygen use, breathing techniques, nutrition, pacing, and energy conservation. In one study, 18 ILD patients (9 with IPF) were interviewed to help inform the development of ILD-specific educational content for pulmonary rehabilitation; they wanted information on the natural history of their disease, prognosis, and end-of-life planning [46]. In debriefing interviews, patients and caregivers mentioned feeling less isolated and having better perspective on their disease after completing an IPF educational program covering such topics as cause, pathophysiology, and treatment of IPF, cognitive behavior techniques, stress and depression, coping, end-of-life planning, symptoms management, energy conservation, oxygen therapy, and exercise [47].

Pulmonary hypertension is not rare in people with IPF [38, 48] and may worsen during exercise. Patients with pulmonary hypertension may require modifications to the standard exercise prescription, including a reduction in intensity of endurance and resistance exercises [49]. Clinicians should be trained to detect important signs and symptoms requiring cessation of exercise, including dizziness, hypotension, pre-syncope, excessive fatigue, palpitations, tachycardia, or chest pain. Some patients with advanced IPF will require very close supervision and support during all phases of exercise to achieve a sufficient training stimulus while maintaining safety, adequate oxygenation, and symptom control.

Supplemental Oxygen

Dyspnea is a major factor contributing to reduced HRQOL in patients with IPF. Scientific rationale and data from the COPD arena and other disease states suggest supplemental oxygen should play a role in treating dyspnea in patients with IPF. There are few studies of high methodological quality that inform the question of whether supplemental oxygen is beneficial to patients with IPF. In a randomized, placebo-controlled, crossover trial of Japanese IPF patients who desaturated (to less than 88%) during a 6MWD, investigators found no difference in dyspnea, walk distance, or leg fatigue in patients who used ambulatory air versus those who used 4 l of oxygen. Interestingly, 7 of the 20 patients in the study did have a one point or more reduction in their Borg dyspnea level with the use of oxygen. The study had limitations, including oxygen flow was not titrated, and the oxygen delivered did not correct hypoxemia (mean oxygen saturation immediately after the 6MWD with 4 l/min flow was 84%) [50]. In a landmark trial in patients with COPD and moderate exertional desaturation, supplemental oxygen did not improve outcomes, including mortality, time to first hospitalization, HRQOL, anxiety, depression, or 6MWD [51]. In two, small, single-center, retrospective studies that included subjects with various ILDs (34 of 52 had IPF or fibrotic nonspecific interstitial pneumonia (fNSIP)) [52, 53], optimization of ambulatory oxygen saturation (starting oxygen or increasing it to achieve saturations of >88 or 90%) was associated with increased 6MWD [52, 53]. With the use of oxygen, dyspnea improved significantly in the IPF subgroup [52]. Whether supplemental oxygen improves survival in IPF patients

with resting hypoxemia is unknown: no study has been conducted to answer this question. However, standard practice is to prescribe supplemental oxygen to such patients, as implemented for patients with COPD [54, 55]. Despite the absence of high-quality data, experts suggest IPF patients be assessed for supplemental oxygen requirements at each clinic visit [3].

We believe IPF patients with nocturnal or exertional hypoxemia should be prescribed supplemental oxygen after a careful discussion covering expected benefits and potential drawbacks (including the perceived stigma of wearing a cannula in public, feeling tethered to the delivery device, carrying added weight of the tanks, and constraints on leaving home or traveling). An understudied option for oxygen delivery available at some centers—and the one we endorse and discuss with our IPF patients—is a transtracheal catheter. Delivering oxygen directly into the trachea can relieve nasal drying and bleeding and allow flows to be lowered by an average of 50% at rest and 30% with exertion. Given the scant sputum they produce, many IPF patients make ideal candidates for transtracheal oxygen delivery.

Conclusion

IPF is a devastating disease with a median survival of 2–5 years after diagnosis. Two anti-fibrotic drugs may slow disease progression, but neither has been shown to improve HRQOL or dyspnea. Several studies have revealed that pulmonary rehabilitation does. Pulmonary rehabilitation should be offered to all patients with IPF (except for the rare patient with issues including active angina, musculoskeletal issues that make exercise unsafe), and patients should be encouraged to continue exercise training at home after completion of the program. Optimal duration of pulmonary rehabilitation has not been established, but available data hint that longer programs may lead to sustained benefits. Some of the educational components of traditional pulmonary rehabilitation may not be applicable to patients with IPF, and efforts are ongoing to develop disease-specific educational components.

Supplemental oxygen is routinely prescribed for resting hypoxia in IPF, though no study has assessed its benefits in this subgroup—and none is likely to do so. Some IPF patients with resting normoxia and exertional hypoxemia may derive benefit from using supplemental oxygen. However, realistic expectations must be established, and potential benefits must be weighed against monetary and potential hardships associated with ambulatory oxygen therapy. Research is ongoing to better understand how supplemental oxygen might help patients with IPF.

References

1. Meltzer EB, Noble PW. Idiopathic pulmonary fibrosis. Orphanet J Rare Dis. 2008;3:8. https://doi.org/10.1186/1750-1172-3-8.

2. Olson AL, Swigris JJ, Lezotte DC, Norris JM, Wilson CG, Brown KK. Mortality from pulmonary fibrosis increased in the United States from 1992 to 2003. Am J Respir Crit Care Med. 2007;176(3):277–84. https://doi.org/10.1164/rccm.200701-044OC.
3. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. 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. https://doi.org/10.1164/rccm.2009-040GL.
4. King TE Jr, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2083–92. https://doi.org/10.1056/NEJMoa1402582.
5. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2071–82. https://doi.org/10.1056/NEJMoa1402584.
6. Glaspole IN, Chapman SA, Cooper WA, Ellis SJ, Goh NS, Hopkins PM, et al. Health-related quality of life in idiopathic pulmonary fibrosis: data from the Australian IPF registry. Respirology. 2017;22(5):950–6. https://doi.org/10.1111/resp.12989.
7. Kozu R, Jenkins S, Senjyu H. Evaluation of activity limitation in patients with idiopathic pulmonary fibrosis grouped according to Medical Research Council dyspnea grade. Arch Phys Med Rehabil. 2014;95(5):950–5. https://doi.org/10.1016/j.apmr.2014.01.016.
8. Janssen DJ, Wouters EF, Spruit MA. Psychosocial consequences of living with breathlessness due to advanced disease. Curr Opin Support Palliat Care. 2015;9(3):232–7. https://doi.org/10.1097/SPC.0000000000000146.
9. Swigris JJ, Brown KK, Make BJ, Wamboldt FS. Pulmonary rehabilitation in idiopathic pulmonary fibrosis: a call for continued investigation. Respir Med. 2008;102(12):1675–80. https://doi.org/10.1016/j.rmed.2008.08.014.
10. Ryerson CJ, Arean PA, Berkeley J, Carrieri-Kohlman VL, Pantilat SZ, Landefeld CS, et al. Depression is a common and chronic comorbidity in patients with interstitial lung disease. Respirology. 2012;17(3):525–32. https://doi.org/10.1111/j.1440-1843.2011.02122.x.
11. Ryerson CJ, Berkeley J, Carrieri-Kohlman VL, Pantilat SZ, Landefeld CS, Collard HR. Depression and functional status are strongly associated with dyspnea in interstitial lung disease. Chest. 2011;139(3):609–16. https://doi.org/10.1378/chest.10-0608.
12. van Manen MJ, Kreuter M, van den Blink B, Oltmanns U, Palmowski K, Brunnemer E et al. What patients with pulmonary fibrosis and their partners think: a live, educative survey in the Netherlands and Germany. ERJ Open Res. 2017;3(1). https://doi.org/10.1183/23120541.00065-2016.
13. Milioli G, Bosi M, Poletti V, Tomassetti S, Grassi A, Riccardi S, et al. Sleep and respiratory sleep disorders in idiopathic pulmonary fibrosis. Sleep Med Rev. 2016;26:57–63. https://doi.org/10.1016/j.smrv.2015.03.005.
14. Swigris JJ, Stewart AL, Gould MK, Wilson SR. Patients' perspectives on how idiopathic pulmonary fibrosis affects the quality of their lives. Health Qual Life Outcomes. 2005;3:61. https://doi.org/10.1186/1477-7525-3-61.
15. Center for Drug Evaluation and Research (CDER), U.S. Food and Drug Administration, The voice of the patient: a series of reports from the U.S. Food and Drug Administration's (FDA's) patient-focused drug development initiative 2015. https://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM440829.pdf.
16. Nishiyama O, Kondoh Y, Kimura T, Kato K, Kataoka K, Ogawa T, et al. Effects of pulmonary rehabilitation in patients with idiopathic pulmonary fibrosis. Respirology. 2008;13(3):394–9. https://doi.org/10.1111/j.1440-1843.2007.01205.x.
17. Rochester CL, Vogiatzis I, Holland AE, Lareau SC, Marciniuk DD, Puhan MA, et al. An official American Thoracic Society/European Respiratory Society policy statement: enhancing implementation, use, and delivery of pulmonary rehabilitation. Am J Respir Crit Care Med. 2015;192(11):1373–86. https://doi.org/10.1164/rccm.201510-1966ST.

18. Bianchi R, Gigliotti F, Romagnoli I, Lanini B, Castellani C, Binazzi B, et al. Impact of a rehabilitation program on dyspnea intensity and quality in patients with chronic obstructive pulmonary disease. Respiration. 2011;81(3):186–95. https://doi.org/10.1159/000273675.
19. Bernard S, Whittom F, Leblanc P, Jobin J, Belleau R, Berube C, et al. Aerobic and strength training in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1999;159(3):896–901. https://doi.org/10.1164/ajrccm.159.3.9807034.
20. Mador MJ, Kufel TJ, Pineda LA, Steinwald A, Aggarwal A, Upadhyay AM, et al. Effect of pulmonary rehabilitation on quadriceps fatiguability during exercise. Am J Respir Crit Care Med. 2001;163(4):930–5. https://doi.org/10.1164/ajrccm.163.4.2006125.
21. Dowman LM, McDonald CF, Hill CJ, Lee AL, Barker K, Boote C, et al. The evidence of benefits of exercise training in interstitial lung disease: a randomised controlled trial. Thorax. 2017;72(7):610–9. https://doi.org/10.1136/thoraxjnl-2016-208638.
22. Gaunaurd IA, Gomez-Marin OW, Ramos CF, Sol CM, Cohen MI, Cahalin LP, et al. Physical activity and quality of life improvements of patients with idiopathic pulmonary fibrosis completing a pulmonary rehabilitation program. Respir Care. 2014;59(12):1872–9. https://doi.org/10.4187/respcare.03180.
23. Holland AE, Hill CJ, Conron M, Munro P, McDonald CF. Short term improvement in exercise capacity and symptoms following exercise training in interstitial lung disease. Thorax. 2008;63(6):549–54. https://doi.org/10.1136/thx.2007.088070.
24. Jackson RM, Gomez-Marin OW, Ramos CF, Sol CM, Cohen MI, Gaunaurd IA, et al. Exercise limitation in IPF patients: a randomized trial of pulmonary rehabilitation. Lung. 2014;192(3):367–76. https://doi.org/10.1007/s00408-014-9566-9.
25. Vainshelboim B, Fox BD, Kramer MR, Izhakian S, Gershman E, Oliveira J. Short-term improvement in physical activity and body composition after supervised exercise training program in idiopathic pulmonary fibrosis. Arch Phys Med Rehabil. 2016;97(5):788–97. https://doi.org/10.1016/j.apmr.2016.01.018.
26. Vainshelboim B, Kramer MR, Fox BD, Izhakian S, Sagie A, Oliveira J. Supervised exercise training improves exercise cardiovascular function in idiopathic pulmonary fibrosis. Eur J Phys Rehabil Med. 2017;53(2):209–18. https://doi.org/10.23736/S1973-9087.16.04319-7.
27. Vainshelboim B, Oliveira J, Fox BD, Soreck Y, Fruchter O, Kramer MR. Long-term effects of a 12-week exercise training program on clinical outcomes in idiopathic pulmonary fibrosis. Lung. 2015;193(3):345–54. https://doi.org/10.1007/s00408-015-9703-0.
28. Vainshelboim B, Oliveira J, Yehoshua L, Weiss I, Fox BD, Fruchter O, et al. Exercise training-based pulmonary rehabilitation program is clinically beneficial for idiopathic pulmonary fibrosis. Respiration. 2014;88(5):378–88. https://doi.org/10.1159/000367899.
29. Guyatt GH, Berman LB, Townsend M, Pugsley SO, Chambers LW. A measure of quality of life for clinical trials in chronic lung disease. Thorax. 1987;42(10):773–8.
30. Ferreira A, Garvey C, Connors GL, Hilling L, Rigler J, Farrell S, et al. Pulmonary rehabilitation in interstitial lung disease: benefits and predictors of response. Chest. 2009;135(2):442–7. https://doi.org/10.1378/chest.08-1458.
31. Huppmann P, Sczepanski B, Boensch M, Winterkamp S, Schonheit-Kenn U, Neurohr C, et al. Effects of inpatient pulmonary rehabilitation in patients with interstitial lung disease. Eur Respir J. 2013;42(2):444–53. https://doi.org/10.1183/09031936.00081512.
32. Kozu R, Jenkins S, Senjyu H. Effect of disability level on response to pulmonary rehabilitation in patients with idiopathic pulmonary fibrosis. Respirology. 2011;16(8):1196–202. https://doi.org/10.1111/j.1440-1843.2011.02029.x.
33. Naji NA, Connor MC, Donnelly SC, McDonnell TJ. Effectiveness of pulmonary rehabilitation in restrictive lung disease. J Cardpulm Rehabil. 2006;26(4):237–43.
34. Rammaert B, Leroy S, Cavestri B, Wallaert B, Grosbois JM. Home-based pulmonary rehabilitation in idiopathic pulmonary fibrosis. Rev Mal Respir. 2011;28(7):e52–7. https://doi.org/10.1016/j.rmr.2011.06.006.

35. Ryerson CJ, Cayou C, Topp F, Hilling L, Camp PG, Wilcox PG, et al. Pulmonary rehabilitation improves long-term outcomes in interstitial lung disease: a prospective cohort study. Respir Med. 2014;108(1):203–10. https://doi.org/10.1016/j.rmed.2013.11.016.
36. Salhi B, Troosters T, Behaegel M, Joos G, Derom E. Effects of pulmonary rehabilitation in patients with restrictive lung diseases. Chest. 2010;137(2):273–9. https://doi.org/10.1378/chest.09-0241.
37. Swigris JJ, Fairclough DL, Morrison M, Make B, Kozora E, Brown KK, et al. Benefits of pulmonary rehabilitation in idiopathic pulmonary fibrosis. Respir Care. 2011;56(6):783–9. https://doi.org/10.4187/respcare.00939.
38. Lettieri CJ, Nathan SD, Barnett SD, Ahmad S, Shorr AF. Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest. 2006;129(3):746–52. https://doi.org/10.1378/chest.129.3.746.
39. Holland AE, Hill CJ, Glaspole I, Goh N, McDonald CF. Predictors of benefit following pulmonary rehabilitation for interstitial lung disease. Respir Med. 2012;106(3):429–35. https://doi.org/10.1016/j.rmed.2011.11.014.
40. Beauchamp MK, Janaudis-Ferreira T, Goldstein RS, Brooks D. Optimal duration of pulmonary rehabilitation for individuals with chronic obstructive pulmonary disease – a systematic review. Chron Respir Dis. 2011;8(2):129–40. https://doi.org/10.1177/1479972311404256.
41. Garvey C. Interstitial lung disease and pulmonary rehabilitation. J Cardiopulm Rehabil Prev. 2010;30(3):141–6. https://doi.org/10.1097/HCR.0b013e3181c56b66.
42. Hubbard RB, Smith C, Le Jeune I, Gribbin J, Fogarty AW. The association between idiopathic pulmonary fibrosis and vascular disease: a population-based study. Am J Respir Crit Care Med. 2008;178(12):1257–61. https://doi.org/10.1164/rccm.200805-725OC.
43. Izbicki G, Ben-Dor I, Shitrit D, Bendayan D, Aldrich TK, Kornowski R, et al. The prevalence of coronary artery disease in end-stage pulmonary disease: is pulmonary fibrosis a risk factor? Respir Med. 2009;103(9):1346–9. https://doi.org/10.1016/j.rmed.2009.03.012.
44. Kizer JR, Zisman DA, Blumenthal NP, Kotloff RM, Kimmel SE, Strieter RM, et al. Association between pulmonary fibrosis and coronary artery disease. Arch Intern Med. 2004;164(5):551–6. https://doi.org/10.1001/archinte.164.5.551.
45. Nathan SD, Basavaraj A, Reichner C, Shlobin OA, Ahmad S, Kiernan J, et al. Prevalence and impact of coronary artery disease in idiopathic pulmonary fibrosis. Respir Med. 2010;104(7):1035–41. https://doi.org/10.1016/j.rmed.2010.02.008.
46. Holland AE, Fiore JF Jr, Goh N, Symons K, Dowman L, Westall G, et al. Be honest and help me prepare for the future: what people with interstitial lung disease want from education in pulmonary rehabilitation. Chron Respir Dis. 2015;12(2):93–101. https://doi.org/10.1177/1479972315571925.
47. Lindell KO, Olshansky E, Song MK, Zullo TG, Gibson KF, Kaminski N, et al. Impact of a disease-management program on symptom burden and health-related quality of life in patients with idiopathic pulmonary fibrosis and their care partners. Heart Lung. 2010;39(4):304–13. https://doi.org/10.1016/j.hrtlng.2009.08.005.
48. Glaser S, Noga O, Koch B, Opitz CF, Schmidt B, Temmesfeld B, et al. Impact of pulmonary hypertension on gas exchange and exercise capacity in patients with pulmonary fibrosis. Respir Med. 2009;103(2):317–24. https://doi.org/10.1016/j.rmed.2008.08.005.
49. Mereles D, Ehlken N, Kreuscher S, Ghofrani S, Hoeper MM, Halank M, et al. Exercise and respiratory training improve exercise capacity and quality of life in patients with severe chronic pulmonary hypertension. Circulation. 2006;114(14):1482–9. https://doi.org/10.1161/CIRCULATIONAHA.106.618397.
50. Nishiyama O, Miyajima H, Fukai Y, Yamazaki R, Satoh R, Yamagata T, et al. Effect of ambulatory oxygen on exertional dyspnea in IPF patients without resting hypoxemia. Respir Med. 2013;107(8):1241–6. https://doi.org/10.1016/j.rmed.2013.05.015.
51. Long-Term Oxygen Treatment Trial Research G, Albert RK, Au DH, Blackford AL, Casaburi R, Cooper JA Jr, et al. A randomized trial of long-term oxygen for COPD with moderate desaturation. N Engl J Med. 2016;375(17):1617–27. https://doi.org/10.1056/NEJMoa1604344.

52. Visca D, Montgomery A, de Lauretis A, Sestini P, Soteriou H, Maher TM, et al. Ambulatory oxygen in interstitial lung disease. Eur Respir J. 2011;38(4):987–90. https://doi.org/10.1183/09031936.00190710.
53. Frank RC, Hicks S, Duck AM, Spencer L, Leonard CT, Barnett E. Ambulatory oxygen in idiopathic pulmonary fibrosis: of what benefit? Eur Respir J. 2012;40(1):269–70. https://doi.org/10.1183/09031936.00007712.
54. Continuous or nocturnal oxygen therapy in hypoxemic chronic obstructive lung disease: a clinical trial. Nocturnal Oxygen Therapy Trial Group. Ann Intern Med. 1980;93(3):391–8.
55. Long term domiciliary oxygen therapy in chronic hypoxic cor pulmonale complicating chronic bronchitis and emphysema. Report of the Medical Research Council Working Party. Lancet. 1981;1(8222):681–6.

Chapter 17
Acute Exacerbation of Idiopathic Pulmonary Fibrosis

Joyce S. Lee and Harold R. Collard

A Case

A 78-year-old man was referred for surgical lung biopsy in the evaluation of his interstitial lung disease (ILD). At baseline, he reported mild dyspnea on exertion and a chronic, dry cough. His past medical history was significant for hypertension and gastroesophageal reflux (GER) disease. His medications included an antihypertensive medication and a proton-pump inhibitor. He was a lifelong non-smoker and worked as a dentist. He had no family history of ILD. His physical exam was significant for dry inspiratory crackles at both bases and normal resting oxygen saturation. His pulmonary function was abnormal with a forced vital capacity of 57% predicted and a diffusing capacity for carbon monoxide of 67% predicted. His high-resolution computed tomography (HRCT) scan demonstrated peripheral, subpleural predominant reticulation and traction bronchiectasis without honeycombing.

He was referred for surgical lung biopsy and had a video-assisted thoracic surgery procedure with biopsies obtained from the right lung. His perioperative course was uncomplicated. His pathology was reviewed and was consistent with a usual interstitial pneumonia (UIP) pattern, confirming the diagnosis of IPF. His initial postoperative course was uncomplicated, but approximately 5 days postoperatively, he developed increased dyspnea and cough with occasional production of clear sputum. He had new onset hypoxemia (88% on room air) with diffuse crackles to

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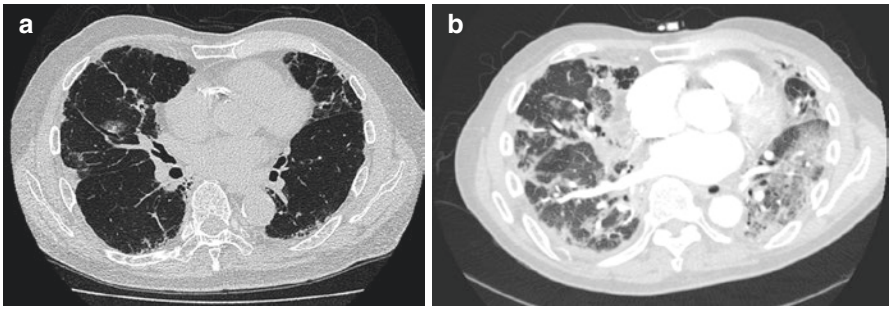

Fig. 17.1 (**a**) Pre-surgery high-resolution computed tomogram (HRCT) demonstrates peripheral reticulation and traction bronchiectasis without honeycombing (bottom left). (**b**) HRCT image obtained 5 days postoperatively demonstrates diffuse ground-glass opacities that are most prominent in the left lung

auscultation that were more prominent in the left chest. A repeat HRCT demonstrated new ground-glass opacities in the left lung (Fig. 17.1). All microbiologic data was negative, and there was no evidence of cardiac dysfunction or ischemia.

This case was thought to be due to an acute exacerbation (AEx) of IPF triggered by the surgical lung biopsy, possibly due to single-lung ventilation of the left lung. Unfortunately the patient progressively worsened despite supportive care and subsequently died from his AEx of IPF.

Epidemiology, Clinical Features, and Risk Factors

Our view of the natural history of IPF has changed over the last decade with the recognition that there are several distinct clinical courses that patients may follow [1]. Although most patients with IPF experience a steady decline in lung function over time, some will decline quickly, while others seem stable for many years. Increasingly, we recognize that some patients may also have a more unpredictable course [2]. These patients experience periods of relative stability followed by acute episodes of worsening in their respiratory status [3]. Episodes of acute respiratory decline in IPF can be secondary to complications such as infection, pulmonary embolism, pneumothorax, or heart failure [3, 4]. Such episodes of acute respiratory deterioration have been termed AEx of IPF when the cause for the acute worsening cannot be identified. Acute exacerbations likely comprise almost 50% of these acute respiratory events, and the clinical characteristics and prognosis are indistinguishable from acute exacerbations of known cause. This chapter will focus on AEx of IPF.

The phenomenon of AEx has been recognized since the late 1980s, when it was initially reported in the Japanese literature [5–8]. A survey of providers in the USA suggests that most clinicians believe AEx to be somewhat or very common [9]. The true incidence of AEx remains unknown, and the incidence may vary by country

due to different genetic and environmental factors. Largely due to differences in case definition, patient population, sample size, and duration of follow-up, the range of AEx incidence in clinical studies ranges anywhere from 1% to 43% [3, 4, 10–23]. In one study of 461 Korean patients with IPF in which patients were followed longitudinally over 3 years, a 1- and 3-year incidence of 14.2% and 20.7%, respectively, was found [4]. In a more ethnically diverse clinical trial population, the incidence of AEx among those in the placebo arm of the INPULSIS study (study drug: nintedanib) was 7.6% over a 52-week period [10].

The clinical presentation of AEx is generally quite dramatic and characterized by acute to subacute worsening of dyspnea over days to weeks [3]. Some patients experience symptoms of worsening cough, sputum production, and fever mimicking a respiratory tract infection [14, 24]. Most reported cases of AEx have required unscheduled medical attention (emergency room or hospital care), but there may well be less severe cases that do not get noted by patients and providers and, therefore, are not documented.

The occurrence of AEx is unpredictable and can sometimes be the presenting manifestation of IPF [14, 15, 25]. A few risk factors have been identified including those indicative of IPF disease severity. The most consistent risk factor for acute exacerbation is a low forced vital capacity (FVC) [4, 15, 18, 19, 21–23]. This is consistent with the increased incidence of AEx that was observed in the only study of advanced disease reported in the literature to date, namely, STEP-IPF [26]. Several other parameters reflecting disease severity have also been associated with an increased risk for AEx including low diffusing capacity for carbon monoxide (DLCO) [4, 18, 20, 21, 23], poor baseline oxygenation [23, 27], and recent decline in FVC [15, 27, 28]. Other risk factors associated with an increased risk for the development of AEx include higher body mass index [15] and younger age [18]. The data on the role of smoking and coexistent emphysema in AEx of IPF have been mixed [4, 17, 19, 20].

Acute exacerbations have also been described in non-IPF ILD, including nonspecific interstitial pneumonia (NSIP) [29], connective tissue disease-associated ILD [29–34], and hypersensitivity pneumonitis [35, 36]. Compared to IPF AEx, patients with an underlying NSIP pattern appeared to have a better prognosis following their AEx [29]. A UIP pattern may be a risk factor for AEx in the context of connective tissue disease-associated ILD and hypersensitivity pneumonitis, as the presence of a UIP pattern appeared to be a risk factor in some retrospective series [29, 33, 34, 36]. Whether AEx of non-IPF forms of ILD shares a similar pathobiology as AEx of IPF is unknown.

Etiology and Pathobiology

The etiology of AEx of IPF remains unknown. Several hypotheses have been proposed that include (1) AEx of IPF represents an abrupt acceleration of the patient's underlying disease; (2) AEx is a collection of occult, pathobiologically distinct

conditions (e.g., infection, heart failure); or (3) AEx is a combination of both processes that can serve as an occult trigger that leads to acceleration of the underlying fibroproliferative process.

Occult aspiration of gastric contents has been suggested as a possible trigger or cause of AEx of IPF. Gastroesophageal reflux is nearly universal in patients with IPF [37, 38] and is thought to be a risk factor for aspiration [39, 40]. Bronchoalveolar lavage pepsin levels, a biomarker for aspiration of gastric secretions, were shown to be elevated in a subset of patients with AEx of IPF [41]. In addition, patients with asymmetric IPF on HRCT scan had a higher rate of GER and AEx compared to patients with non-asymmetric disease, suggesting a role for GER and occult aspiration in a subset of patients with IPF [42].

Infection has also been suggested as a cause of AEx of IPF. Data in support of this hypothesis include animal studies [43] as well as some human studies [44, 45]. In one case series, 75.7% of 37 AEx cases occurred between December and May [24], lending further support to occult infection as a cause of AEx. However, in a prospective study of AEx of IPF (n = 47), acute viral infection, as determined by the most current genomics-based methodologies, was found in only 9% of this cohort [46]. While some cases may well have been missed (i.e., the virus had come and gone by the time testing was obtained), these data suggest that there are many cases of AEx that are not primarily due to occult viral infection. More recently, there are data describing a difference in the microbiome of IPF patients who are experiencing an AEx compared to stable patients. In a study of 20 AEx and 14 stable IPF patients matched for age, sex, smoking history, and baseline lung function, BAL bacterial burden was increased in AEx patients compared to stable patients [47].

Precipitating factors such as surgical lung biopsy and bronchoalveolar lavage (BAL) have also been reported [14, 48–58]. The occurrence of AEx after videoscopic-assisted surgical lung biopsy is particularly intriguing, as the exacerbation appears to be more pronounced in the lung that was ventilated (i.e., the nonsurgical side receiving single-lung ventilation) [52]. However, the precise relationship between these precipitating factors and AEx remains unclear.

An alternative explanation is that AEx of IPF is caused by an inherent acceleration of the pathobiology of IPF [3]. There is indirect evidence for this in several studies that evaluated serum biomarkers and gene expression in AEx. Serum biomarkers of alveolar epithelial cell injury/proliferation have been shown to be increased in AEx in a pattern that is qualitatively distinct from what is seen in acute lung injury (Table 17.1).

Gene expression studies performed in patients with AEx of IPF [60] have shown that patients have increased expression of genes encoding proteins involved in epithelial injury and proliferation including CCNA2 and alpha-defensins. Interestingly, there was no evidence from the same study for upregulation of genes commonly expressed in viral infection.

Table 17.1 This table summarizes serum biomarkers of alveolar epithelial cell injury/proliferation reported in acute exacerbation of idiopathic pulmonary fibrosis

Biomarker	Mechanism of action	Association with AEx of IPF	References
Alpha-defensin	Cationic proteins with antimicrobial activity found in neutrophils	Plasma levels higher in AEx compared to stable and seemed to correlate with disease course	[59, 60]
Annexin 1	Anti-inflammatory, antiproliferative, and pro-apoptotic calcium and phospholipid-binding protein that regulates differentiation; found in alveolar type II cells and alveolar macrophages	Associated with antibody production and CD4+ T-cell response in AEx	[61]
Circulating fibrocytes	Circulating mesenchymal cell progenitors involved in tissue repair and fibrosis	Increased levels of circulating fibrocytes in AEx compared to stable IPF	[62]
Heat shock protein 47 (HSP47)	Collagen-specific molecular chaperone essential in the biosynthesis and secretion of collagen molecules	Serum levels of HSP47 were higher in AEx compared to stable IPF	[63]
High-mobility group protein B1 (HMGB1)	Nuclear nonhistone protein involved in endogenous danger signaling and a mediator of systemic inflammation; can bind to RAGE to promote chemotaxis and production of cytokines via NF-kB activation	Serum HMGB1 levels are higher in AEx requiring mechanical ventilation compared to stable IPF; BAL HMGB1 gradually increases during AEx, which correlated with monocyte chemotactic protein-1 (MCP-1)	[64, 65]
IL-6	Cytokine involved in a broad range of cellular responses including inflammation	Higher levels in AEx vs. stable	[66]
KL-6	Marker of alveolar type II cell injury and/or proliferation	Plasma levels higher in AEx of IPF compared to stable; serial serum KL-6 levels increased in patients who died of their AEx; baseline serum KL-6 levels predicted future development of AEx in IPF	[19, 66, 67]
Leptin	Regulation of energy balance and other physiological processes, including the immune response, the inflammatory reactions, and the development of carcinomas	Plasma leptin levels were higher in AEx vs. stable and in decedents vs. survivors of IPF	[68]
PAI-1	Principal inhibitor of tissue plasminogen activator and urokinase	Higher plasma levels in AEx compared to stable	[66]

(continued)

Table 17.1 (continued)

Biomarker	Mechanism of action	Association with AEx of IPF	References
Protein C	The activated form regulates blood clotting, inflammation, and cell death	Higher plasma % in AEx compared to stable	[66]
RAGE	Marker of alveolar type I cell injury and/or proliferation	No difference in plasma levels between stable and AEx of IPF	[66]
ST2	Predominantly expressed in Th2 cells and induced by proinflammatory cytokines	Higher serum levels in AEx compared to stable with a sensitivity of 71% and specificity of 92%	[69]
SP-D	Marker of alveolar type II cell injury and/or proliferation	Plasma levels higher in AEx compared to stable	[66]
Thrombomodulin	Membrane protein expressed on the surface of endothelial cells which serves as a receptor for thrombin	Plasma levels higher in AEx compared to stable, and log change in thrombomodulin was predictive of survival	[66]
Von Willebrand factor	Marker of endothelial cell injury and is involved in hemostasis	Higher plasma % in AEx compared to stable	[66]

Abbreviations:AEx acute exacerbation, *IPF* idiopathic pulmonary fibrosis, *KL-6* Krebs von den Lungen-6, *PAI-1* plasminogen activator inhibitor-1, *RAGE* receptor for advanced glycation end products, *NF-kB* nuclear factor-kB, *ST-2*, *SP-D* surfactant protein D

Work-Up and Diagnostic Criteria

Laboratory Evaluation

There are no specific laboratory tests that aid in the evaluation and diagnosis of AEx of IPF. Often, patients are found to have impaired gas exchange with a decrease in their arterial oxygen tension [24]. In patients that can tolerate bronchoscopy with lavage, an increase in BAL neutrophils has been reported [14, 70]. Non-specific elevations in serum lactate dehydrogenase (LDH) and C-reactive protein (CRP) have also been observed [24]. Serial levels of serum KL-6 and baseline thrombomodulin may help identify patients at increased risk for death from AEx [66, 67]. Although many experimental biomarkers have been investigated, as shown in Table 17.1, none are routinely used in clinical practice.

Radiologic Evaluation

High-resolution CT scans are often obtained during AEx of IPF. The findings include new, generally bilateral, ground-glass opacities and/or consolidation superimposed on the underlying UIP pattern [71]. The pattern of ground-glass changes during an AEx may have prognostic significance, with more diffuse abnormality correlating with worse outcomes [71].

Histopathologic Evaluation

Surgical lung biopsy is not frequently obtained during AEx of IPF. A small case series of seven patients who had a surgical lung biopsy during their AEx demonstrated primarily diffuse alveolar damage (DAD) associated with underlying changes typical for UIP (Fig. 17.2) [72]. One case had organizing pneumonia and UIP, and another case had DAD without underlying UIP. Autopsy series and other case series have demonstrated similar findings [6, 14, 70, 73–75].

Diagnostic Criteria

Several definitions have been used over the last decade to define AEx of IPF [3, 6, 75]. In order to standardize these criteria, a consensus definition was proposed by the National Institutes of Health-funded US IPF Network (IPFNet) in 2007 (Table 17.2) [3]. Other definitions that have been described are generally similar; however, they often include a reduction in PaO_2 as one of their criteria as well as bilateral chest x-ray abnormalities (instead of a HRCT scan) [6, 75].

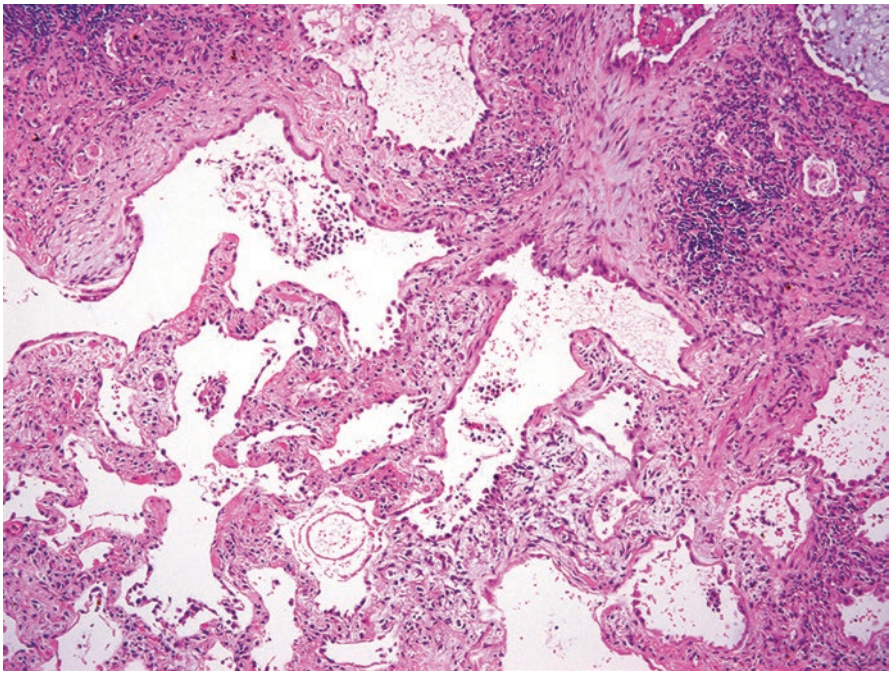

Fig. 17.2 Histopathologic section from the lung explanted at the time of lung transplant shows subpleural fibrosis with honeycombing that is typical of usual interstitial pneumonia. The central lung tissue shows diffuse alveolar septal thickening by edema and type II pneumocyte hyperplasia and airspace consolidation due to edema and fibrin deposition (H&E, 100×). (Figure courtesy of Kirk Jones, MD)

Table 17.2 This table details the original IPFNet consensus criteria for acute exacerbation of idiopathic pulmonary fibrosis

IPFNet consensus criteria for acute exacerbation of idiopathic pulmonary fibrosis [3]
Previous or concurrent diagnosis of idiopathic pulmonary fibrosis
Unexplained development or worsening of dyspnea within 30 days
High-resolution computed tomography with new bilateral ground-glass abnormality and/or consolidation superimposed on a background reticular or honeycomb pattern consistent with usual interstitial pneumonia
No evidence of pulmonary infection by endotracheal aspirate or bronchoalveolar lavage
Exclusion of alternative causes, including left heart failure, pulmonary embolism, and other identifiable causes of acute lung injury

*Patients who do not meet all five criteria should be termed "suspected acute exacerbation"

Table 17.3 This table details the revised definition and diagnostic criteria for acute exacerbation of idiopathic pulmonary fibrosis

Proposed revised definition and diagnostic criteria for acute exacerbation of idiopathic pulmonary fibrosis [76]
Definition: an acute, clinically significant respiratory deterioration characterized by evidence of new widespread alveolar abnormality
Criteria:
Previous or concurrent diagnosis of idiopathic pulmonary fibrosis
Acute worsening or development of dyspnea typically <1 month duration
Computed tomography with new bilateral ground-glass opacity and/or consolidation superimposed on a background pattern consistent with usual interstitial pneumonia pattern
Deterioration not fully explained by cardiac failure or volume overload

*Patients who do not meet all four diagnostic criteria due to missing computed tomography data should be termed "suspected acute exacerbation"

While the IPFNet criteria have helped to standardize the definition of AEx of IPF, satisfaction of all criteria was quite difficult to achieve in many clinical settings. In addition, there was increasing evidence to suggest that the constraints of an "idiopathic" label and a set time interval of 30 days were unnecessarily restrictive and arbitrary. As a result, a new, international working group came together to propose a new conceptual framework for acute respiratory deterioration in IPF [76]. Their revised definition and diagnostic criteria are outlined in Table 17.3.

The revised definition and criteria were developed to better reflect the current state of knowledge, as well as improve the feasibility of studying the epidemiology of acute exacerbation in future research. As with any set of criteria, fundamental assumptions made in the development of criteria, whether it is for clinical or research purposes, should be reassessed periodically in order to incorporate the emerging data and knowledge in the field. This reassessment of the diagnostic criteria for AEx of IPF simplifies the requirement to exclude certain triggers of respiratory deterioration, such as aspiration and infection. Instead, it recognizes that distinguishing between triggered and so-called idiopathic acute exacerbations of IPF has little clinical or biological support. The hope is that these criteria will provide an improved framework for studying the etiology, pathobiology, and clinical management of AEx of IPF.

Management and Prognosis

There is no known effective treatment for preventing or improving outcomes in AEx of IPF.

Prevention

While thereare no data to support efficacy, vaccination and treatment of comorbidities like heart disease and GER seem prudent as measures that could prevent episodes of acute decline in respiratory function due to known causes such as infection, heart failure, and aspiration. In a retrospective analysis of the placebo arms of the three IPFNet studies, patients who were on antiacid therapy had a lower incidence (0%) of AEx of IPF compared to those who were not on antiacid therapy (8%) during the trial period [77].

Some novel therapies have suggested a reduction in AEx in clinical trials; these include warfarin [78], pirfenidone [79], and nintedanib [11]. Unfortunately, both warfarin and pirfenidone have subsequently been shown to have no impact on the rate of AEx, suggesting that the initial observations may be inaccurate [80, 81]. The two follow-up and parallel phase-3 clinical trials using nintedanib had mixed results in regard to prevention of AEx [10]. Interestingly, a secondary data analysis from three IPF clinical trials suggested that pirfenidone was associated with a lower risk of respiratory-related hospitalization compared to placebo, but not all-cause or non-respiratory-related hospitalization [82]. In addition, those hospitalized for any reason had lower risk of death if they were on pirfenidone. While these events were not specific for AEx of IPF, these data suggest that pirfenidone may have an impact on the risk and severity of respiratory deterioration, including AEx, in IPF.

Medical Therapy During AEx

Although commonly prescribed for the treatment of AEx of IPF, there have been no controlled trials assessing the efficacy of high-dose corticosteroids. Recent international guidelines on IPF management suggested that the majority of IPF patients with AEx could be treated with corticosteroids [83]; however, approaches to dosing, route, and duration of therapy were not provided.

Although most clinicians would treat patients who develop an AEx of IPF with high-dose corticosteroids, the efficacy of this treatment is unclear. Perhaps we should be more critical of the use of corticosteroids to treat AEx of IPF. There are two distinct viewpoints regarding the role of corticosteroids in AEx of IPF. The first viewpoint is that AEx of IPF is histopathologically similar to acute respiratory distress syndrome (ARDS) characterized by DAD and acute lung injury [84] and should, therefore, be treated similarly to ARDS. In the ARDS literature, the

mortality benefit of corticosteroids is unclear [85–90]. In one study, increased mortality was observed in ARDS patients treated with delayed corticosteroids (after 14 days) [90]. If we were to follow the ARDS paradigm, most clinicians would not use corticosteroids in the treatment of AEx of IPF. A second viewpoint for the role of corticosteroids in IPF is that some patients with AEx of IPF have organizing pneumonia on biopsy [74]. Organizing pneumonia is generally thought to be steroid responsive, and it may be that the pathobiology is different enough between ARDS and AEx of IPF to warrant continued use of corticosteroids. There remains equipoise on the efficacy of corticosteroids in AEx of IPF, and this treatment intervention should be studied more carefully [66].

The use of another immunosuppressant, cyclosporine A, to treat AEx of IPF has been reported. These studies suggest some benefit to the use of cyclosporine A plus corticosteroids [91–93]. However, conclusions that can be made from these data are limited by problems with study design and small sample size, and benefit has not yet been validated in a randomized controlled trial. Other experimental therapies that have reported possible efficacy to treat AEx of IPF include cyclophosphamide [30, 71, 94, 95], tacrolimus [96], hemoperfusion with polymyxin B-immobilized fiber column [65, 97–102], sivelestat [103], rituximab and plasma exchange [104], and thrombomodulin [105–107]. These investigations were all limited by small numbers and suboptimal study design.

Supportive Therapy During AEx

Supportive therapy is the standard of care in AEx of IPF. Supportive care for respiratory failure almost always requires higher oxygen supplementation and consideration of additional means of ventilatory support including mechanical ventilation (see discussion below) and noninvasive positive-pressure ventilation (NIPPV). Yokoyama et al. described the outcomes of patients with AEx of IPF treated with NIPPV to avoid intubation in acute respiratory failure [94]. In this retrospective case series of 11 patients, 6 patients failed a NIPPV trial and subsequently succumbed to respiratory failure. The other five patients survived more than 3 months after the onset of their AEx. However, the use of ventilatory support in AEx (both mechanical ventilation and NIPPV) has never been studied in a randomized controlled trial.

Lung Transplantation

A few select centers have experience with emergent transplantation for AEx of IPF [108–111]. These critically ill IPF patients have generally been bridged to lung transplant with extracorporeal membrane oxygenation (ECMO) and/or mechanical ventilation [109]. Outcomes of patients who have undergone emergent transplantation have been mixed [110, 111]. Emergent lung transplantation requires careful patient selection and is not done at all transplant centers.

Prognosis

The prognosis of AEx of IPF is poor, with most case series reporting very high short-term mortality rates [14, 112–116]. This is particularly true for those patients requiring mechanical ventilation. A systematic review of mechanical ventilation in IPF and respiratory failure (n = 135), including AEx, reported a hospital mortality of 87% [114]. Short-term mortality (within 3 months of hospital discharge) was 94%. Risk factors associated with mortality in AEx of IPF include lower baseline FVC and DLCO [4, 15, 24], more extensive CT scan abnormalities at the time of the AEx [14, 21, 71, 95], worse oxygenation [4, 102], and bronchoalveolar lavage neutrophilia [4].

The routine use of mechanical ventilation in patients with AEx of IPF is not recommended in the international consensus guidelines because of its low likelihood of benefit and high risk of complications and further suffering [83]. Careful consideration regarding intubation and goals of care must be made, given the poor prognosis associated with this condition. Ideally, a discussion concerning end-of-life issues should be held between the patient and their provider in the outpatient setting with the inclusion of the patient's family, if applicable.

Summary

Acute exacerbation of IPF is responsible for substantial morbidity and mortality in patients with IPF. We suggest that AEx of IPF represents an acute acceleration of the fibroproliferative process (i.e., the underlying pathobiology of IPF) that is triggered by some generally occult stress or insult to the lung (e.g., infection, aspiration, mechanical stretch from ventilation or lavage, high-inspired oxygen concentration during surgery). We propose that the prevention and treatment of AEx of IPF must focus on both disease-specific (e.g., anti-fibrotic therapies) and non-disease-specific (e.g., vaccination, prevention of stress) areas. The next decade will hopefully answer many of the unresolved questions concerning AEx of IPF.

References

1. Kim DS, Collard HR, King TE Jr. Classification and natural history of the idiopathic interstitial pneumonias. Proc Am Thorac Soc. 2006;3(4):285–92.
2. 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.
3. Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, King TE Jr, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2007;176(7):636–43.
4. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J. 2011;37(2):356–63.
5. Suga T, Sugiyama Y, Ohno S, Kitamura S. Two cases of IIP which developed acute exacerbation after bronchoalveolar lavage. Nihon Kyobu Shikkan Gakkai Zasshi. 1994;32(2):174–8.

6. Kondoh Y, Taniguchi H, Kawabata Y, Yokoi T, Suzuki K, Takagi K. Acute exacerbation in idiopathic pulmonary fibrosis. Analysis of clinical and pathologic findings in three cases. Chest. 1993;103(6):1808–12.
7. Kondo A, Saiki S. In: Harasawa M, Fukuchi Y, Morinari H, editors. Acute exacerbation in idiopathic interstitial pneumonia (IIP). Tokyo: University of Tokyo Press; 1989.
8. Horio H, Nomori H, Morinaga S, Fuyuno G, Kobayashi R, Iga R. Exacerbation of idiopathic interstitial pneumonia after lobectomy for lung cancer. Nihon Kyobu Shikkan Gakkai Zasshi. 1996;34(4):439–43.
9. Collard HR, Loyd JE, King TE Jr, Lancaster LH. Current diagnosis and management of idiopathic pulmonary fibrosis: a survey of academic physicians. Respir Med. 2007;101(9):2011–6.
10. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2071–82.
11. Richeldi L, Costabel U, Selman M, Kim DS, Hansell DM, Nicholson AG, et al. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med. 2011;365(12):1079–87.
12. Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, Suga M, et al. Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J. 2010;35(4):821–9.
13. Fernandez Perez ER, Daniels CE, Schroeder DR, St Sauver J, Hartman TE, Bartholmai BJ, et al. Incidence, prevalence, and clinical course of idiopathic pulmonary fibrosis: a population-based study. Chest. 2010;137(1):129–37.
14. Kim DS, Park JH, Park BK, Lee JS, Nicholson AG, Colby T. Acute exacerbation of idiopathic pulmonary fibrosis: frequency and clinical features. Eur Respir J. 2006;27(1):143–50.
15. Kondoh Y, Taniguchi H, Katsuta T, Kataoka K, Kimura T, Nishiyama O, et al. Risk factors of acute exacerbation of idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis. 2010;27(2):103–10.
16. Tomassetti S, Ruy JH, Gurioli C, Ravaglia C, Buccioli M, Tantalocco P, et al. The effect of anticoagulant therapy for idiopathic pulmonary fibrosis in real life practice. Sarcoidosis Vasc Diffuse Lung Dis. 2013;30(2):121–7.
17. Sugino K, Gocho K, Kikuchi N, Shibuya K, Uekusa T, Homma S. Acute exacerbation of combined pulmonary fibrosis and emphysema associated with Hermansky-Pudlak syndrome. Respirol Case Rep. 2016;4(1):13–5.
18. Schupp JC, Binder H, Jager B, Cillis G, Zissel G, Muller-Quernheim J, et al. Macrophage activation in acute exacerbation of idiopathic pulmonary fibrosis. PLoS One. 2015;10(1):e0116775.
19. Ohshimo S, Ishikawa N, Horimasu Y, Hattori N, Hirohashi N, Tanigawa K, et al. Baseline KL-6 predicts increased risk for acute exacerbation of idiopathic pulmonary fibrosis. Respir Med. 2014;108(7):1031–9.
20. Mura M, Porretta MA, Bargagli E, Sergiacomi G, Zompatori M, Sverzellati N, et al. Predicting survival in newly diagnosed idiopathic pulmonary fibrosis: a 3-year prospective study. Eur Respir J. 2012;40(1):101–9.
21. Kishaba T, Tamaki H, Shimaoka Y, Fukuyama H, Yamashiro S. Staging of acute exacerbation in patients with idiopathic pulmonary fibrosis. Lung. 2014;192(1):141–9.
22. Johannson KA, Vittinghoff E, Lee K, Balmes JR, Ji W, Kaplan GG, et al. Acute exacerbation of idiopathic pulmonary fibrosis associated with air pollution exposure. Eur Respir J. 2014;43(4):1124–31.
23. Collard HR, Yow E, Richeldi L, Anstrom KJ, Glazer C, Investigators IP. Suspected acute exacerbation of idiopathic pulmonary fibrosis as an outcome measure in clinical trials. Respir Res. 2013;14:73.
24. Simon-Blancal V, Freynet O, Nunes H, Bouvry D, Naggara N, Brillet PY, et al. Acute exacerbation of idiopathic pulmonary fibrosis: outcome and prognostic factors. Respiration. 2012;83(1):28–35.
25. Sakamoto K, Taniguchi H, Kondoh Y, Ono K, Hasegawa Y, Kitaichi M. Acute exacerbation of idiopathic pulmonary fibrosis as the initial presentation of the disease. Eur Respir Rev. 2009;18(112):129–32.

26. Zisman DA, Schwarz M, Anstrom KJ, Collard HR, Flaherty KR, Hunninghake GW. A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. N Engl J Med. 2010;363(7):620–8.
27. Kondoh Y, Taniguchi H, Ebina M, Azuma A, Ogura T, Taguchi Y, et al. Risk factors for acute exacerbation of idiopathic pulmonary fibrosis--extended analysis of pirfenidone trial in Japan. Respir Investig. 2015;53(6):271–8.
28. Reichmann WM, Yu YF, Macaulay D, Wu EQ, Nathan SD. Change in forced vital capacity and associated subsequent outcomes in patients with newly diagnosed idiopathic pulmonary fibrosis. BMC Pulm Med. 2015;15:167.
29. Park IN, Kim DS, Shim TS, Lim CM, Lee SD, Koh Y, et al. Acute exacerbation of interstitial pneumonia other than idiopathic pulmonary fibrosis. Chest. 2007;132(1):214–20.
30. Tachikawa R, Tomii K, Ueda H, Nagata K, Nanjo S, Sakurai A, et al. Clinical features and outcome of acute exacerbation of interstitial pneumonia: collagen vascular diseases-related versus idiopathic. Respiration. 2012;83(1):20–7.
31. Suda T, Kaida Y, Nakamura Y, Enomoto N, Fujisawa T, Imokawa S, et al. Acute exacerbation of interstitial pneumonia associated with collagen vascular diseases. Respir Med. 2009;103(6):846–53.
32. Toyoda Y, Hanibuchi M, Kishi J, Kawano H, Morizumi S, Sato S, et al. Clinical features and outcome of acute exacerbation of interstitial pneumonia associated with connective tissue disease. J Med Investig. 2016;63(3–4):294–9.
33. Okamoto M, Fujimoto K, Sadohara J, Furuya K, Kaieda S, Miyamura T, et al. A retrospective cohort study of outcome in systemic sclerosis-associated interstitial lung disease. Respir Investig. 2016;54(6):445–53.
34. Hozumi H, Nakamura Y, Johkoh T, Sumikawa H, Colby TV, Kono M, et al. Acute exacerbation in rheumatoid arthritis-associated interstitial lung disease: a retrospective case control study. BMJ Open. 2013;3(9):e003132.
35. Olson AL, Huie TJ, Groshong SD, Cosgrove GP, Janssen WJ, Schwarz MI, et al. Acute exacerbations of fibrotic hypersensitivity pneumonitis: a case series. Chest. 2008;134(4):844–50.
36. Miyazaki Y, Tateishi T, Akashi T, Ohtani Y, Inase N, Yoshizawa Y. Clinical predictors and histologic appearance of acute exacerbations in chronic hypersensitivity pneumonitis. Chest. 2008;134(6):1265–70.
37. Tobin RW, Pope CE 2nd, Pellegrini CA, Emond MJ, Sillery J, Raghu G. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998;158(6):1804–8.
38. Raghu G, Freudenberger TD, Yang S, Curtis JR, Spada C, Hayes J, et al. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. Eur Respir J. 2006;27(1):136–42.
39. Marik PE. Aspiration pneumonitis and aspiration pneumonia. N Engl J Med. 2001;344(9):665–71.
40. Lee JS, Collard HR, Raghu G, Sweet MP, Hays SR, Campos GM, et al. Does chronic microaspiration cause idiopathic pulmonary fibrosis? Am J Med. 2010;123(4):304–11.
41. Lee JS, Song JW, Wolters PJ, Elicker BM, King TE Jr, Kim DS, et al. Bronchoalveolar lavage pepsin in acute exacerbation of idiopathic pulmonary fibrosis. Eur Respir J. 2012;39(2):352–8.
42. Tcherakian C, Cottin V, Brillet PY, Freynet O, Naggara N, Carton Z, et al. Progression of idiopathic pulmonary fibrosis: lessons from asymmetrical disease. Thorax. 2011;66(3):226–31.
43. McMillan TR, Moore BB, Weinberg JB, Vannella KM, Fields WB, Christensen PJ, et al. Exacerbation of established pulmonary fibrosis in a murine model by gammaherpesvirus. Am J Respir Crit Care Med. 2008;177(7):771–80.
44. Tomioka H, Sakurai T, Hashimoto K, Iwasaki H. Acute exacerbation of idiopathic pulmonary fibrosis: role of Chlamydophila pneumoniae infection. Respirology. 2007;12(5):700–6.
45. Huie TJ, Olson AL, Cosgrove GP, Janssen WJ, Lara AR, Lynch DA, et al. A detailed evaluation of acute respiratory decline in patients with fibrotic lung disease: aetiology and outcomes. Respirology. 2010;15(6):909–17.

46. Wootton SC, Kim DS, Kondoh Y, Chen E, Lee JS, Song JW, et al. Viral infection in acute exacerbation of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;183(12):1698–702.
47. Molyneaux PL, Cox MJ, Wells AU, Kim HC, Ji W, Cookson WO, et al. Changes in the respiratory microbiome during acute exacerbations of idiopathic pulmonary fibrosis. Respir Res. 2017;18(1):29.
48. Zegdi R, Azorin J, Tremblay B, Destable MD, Lajos PS, Valeyre D. Videothoracoscopic lung biopsy in diffuse infiltrative lung diseases: a 5-year surgical experience. Ann Thorac Surg. 1998;66(4):1170–3.
49. Yuksel M, Ozyurtkan MO, Bostanci K, Ahiskali R, Kodalli N. Acute exacerbation of interstitial fibrosis after pulmonary resection. Ann Thorac Surg. 2006;82(1):336–8.
50. Utz JP, Ryu JH, Douglas WW, Hartman TE, Tazelaar HD, Myers JL, et al. High short-term mortality following lung biopsy for usual interstitial pneumonia. Eur Respir J. 2001;17(2):175–9.
51. Kumar P, Goldstraw P, Yamada K, Nicholson AG, Wells AU, Hansell DM, et al. Pulmonary fibrosis and lung cancer: risk and benefit analysis of pulmonary resection. J Thorac Cardiovasc Surg. 2003;125(6):1321–7.
52. Kondoh Y, Taniguchi H, Kitaichi M, Yokoi T, Johkoh T, Oishi T, et al. Acute exacerbation of interstitial pneumonia following surgical lung biopsy. Respir Med. 2006;100(10):1753–9.
53. Hiwatari N, Shimura S, Takishima T, Shirato K. Bronchoalveolar lavage as a possible cause of acute exacerbation in idiopathic pulmonary fibrosis patients. Tohoku J Exp Med. 1994;174(4):379–86.
54. Samejima J, Tajiri M, Ogura T, Baba T, Omori T, Tsuboi M, et al. Thoracoscopic lung biopsy in 285 patients with diffuse pulmonary disease. Asian Cardiovasc Thorac Ann. 2015;23(2):191–7.
55. Sakamoto K, Taniguchi H, Kondoh Y, Wakai K, Kimura T, Kataoka K, et al. Acute exacerbation of IPF following diagnostic bronchoalveolar lavage procedures. Respir Med. 2012;106(3):436–42.
56. Ghatol A, Ruhl AP, Danoff SK. Exacerbations in idiopathic pulmonary fibrosis triggered by pulmonary and nonpulmonary surgery: a case series and comprehensive review of the literature. Lung. 2012;190(4):373–80.
57. Bando M, Ohno S, Hosono T, Yanase K, Sato Y, Sohara Y, et al. Risk of acute exacerbation after video-assisted thoracoscopic lung biopsy for interstitial lung disease. J Bronchology Interv Pulmonol. 2009;16(4):229–35.
58. Sakamoto S, Homma S, Mun M, Fujii T, Kurosaki A, Yoshimura K. Acute exacerbation of idiopathic interstitial pneumonia following lung surgery in 3 of 68 consecutive patients: a retrospective study. Intern Med. 2011;50(2):77–85.
59. 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.
60. 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.
61. Kurosu K, Takiguchi Y, Okada O, Yumoto N, Sakao S, Tada Y, et al. Identification of annexin 1 as a novel autoantigen in acute exacerbation of idiopathic pulmonary fibrosis. J Immunol. 2008;181(1):756–67.
62. 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.
63. 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.
64. Ebina M, Taniguchi H, Miyasho T, Yamada S, Shibata N, Ohta H, et al. Gradual increase of high mobility group protein b1 in the lungs after the onset of acute exacerbation of idiopathic pulmonary fibrosis. Pulm Med. 2011;2011:916486.

65. Abe S, Hayashi H, Seo Y, Matsuda K, Kamio K, Saito Y, et al. Reduction in serum high mobility group box-1 level by polymyxin B-immobilized fiber column in patients with idiopathic pulmonary fibrosis with acute exacerbation. Blood Purif. 2011;32(4):310–6.
66. 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 Phys Lung Cell Mol Phys. 2010;299(1):L3–7.
67. Yokoyama A, Kohno N, Hamada H, Sakatani M, Ueda E, Kondo K, et al. Circulating KL-6 predicts the outcome of rapidly progressive idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998;158(5 Pt 1):1680–4.
68. Cao M, Swigris JJ, Wang X, Cao M, Qiu Y, Huang M, et al. Plasma leptin is elevated in acute exacerbation of idiopathic pulmonary fibrosis. Mediat Inflamm. 2016;2016:6940480.
69. Tajima S, Oshikawa K, Tominaga S, Sugiyama Y. The increase in serum soluble ST2 protein upon acute exacerbation of idiopathic pulmonary fibrosis. Chest. 2003;124(4):1206–14.
70. Ambrosini V, Cancellieri A, Chilosi M, Zompatori M, Trisolini R, Saragoni L, et al. Acute exacerbation of idiopathic pulmonary fibrosis: report of a series. Eur Respir J. 2003;22(5):821–6.
71. Akira M, Kozuka T, Yamamoto S, Sakatani M. Computed tomography findings in acute exacerbation of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2008;178(4):372–8.
72. Parambil JG, Myers JL, Ryu JH. Histopathologic features and outcome of patients with acute exacerbation of idiopathic pulmonary fibrosis undergoing surgical lung biopsy. Chest. 2005;128(5):3310–5.
73. Rice AJ, Wells AU, Bouros D, du Bois RM, Hansell DM, Polychronopoulos V, et al. Terminal diffuse alveolar damage in relation to interstitial pneumonias. An autopsy study. Am J Clin Pathol. 2003;119(5):709–14.
74. Churg A, Muller NL, Silva CI, Wright JL. Acute exacerbation (acute lung injury of unknown cause) in UIP and other forms of fibrotic interstitial pneumonias. Am J Surg Pathol. 2007;31(2):277–84.
75. Akira M, Hamada H, Sakatani M, Kobayashi C, Nishioka M, Yamamoto S. CT findings during phase of accelerated deterioration in patients with idiopathic pulmonary fibrosis. AJR Am J Roentgenol. 1997;168(1):79–83.
76. Collard HR, Ryerson CJ, Corte TJ, Jenkins G, Kondoh Y, Lederer DJ, et al. Acute exacerbation of idiopathic pulmonary fibrosis. An International Working Group Report. Am J Respir Crit Care Med. 2016;194(3):265–75.
77. Lee JS, Collard HR, Anstrom KJ, Martinez F, Noth I, Roberts RS, et al. Anti-acid treatment and disease progression in idiopathic pulmonary fibrosis: an analysis of data from three randomized controlled trials. Lancet Respir Med. 2013;1(5):369–76.
78. Kubo H, Nakayama K, Yanai M, Suzuki T, Yamaya M, Watanabe M, et al. Anticoagulant therapy for idiopathic pulmonary fibrosis. Chest. 2005;128(3):1475–82.
79. Azuma A, Nukiwa T, Tsuboi E, Suga M, Abe S, Nakata K, et al. Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2005;171(9):1040–7.
80. Noth I, Anstrom KJ, Calvert SB, de Andrade J, Flaherty KR, Glazer C, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186(1):88–95.
81. Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet. 2011;377(9779):1760–9.
82. Ley B, Swigris J, Day BM, Stauffer JL, Raimundo K, Chou W, et al. Pirfenidone reduces respiratory-related hospitalizations in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2017;196(6):756–61.
83. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. 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.
84. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med. 2000;342(18):1334–49.

85. Weigelt JA, Norcross JF, Borman KR, Snyder WH 3rd. Early steroid therapy for respiratory failure. Arch Surg. 1985;120(5):536–40.
86. Peter JV, John P, Graham PL, Moran JL, George IA, Bersten A. Corticosteroids in the prevention and treatment of acute respiratory distress syndrome (ARDS) in adults: meta-analysis. BMJ. 2008;336(7651):1006–9.
87. Meduri GU, Golden E, Freire AX, Taylor E, Zaman M, Carson SJ, et al. Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest. 2007;131(4):954–63.
88. Luce JM, Montgomery AB, Marks JD, Turner J, Metz CA, Murray JF. Ineffectiveness of high-dose methylprednisolone in preventing parenchymal lung injury and improving mortality in patients with septic shock. Am Rev Respir Dis. 1988;138(1):62–8.
89. Bernard GR, Luce JM, Sprung CL, Rinaldo JE, Tate RM, Sibbald WJ, et al. High-dose corticosteroids in patients with the adult respiratory distress syndrome. N Engl J Med. 1987;317(25):1565–70.
90. Steinberg KP, Hudson LD, Goodman RB, Hough CL, Lanken PN, Hyzy R, et al. Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. N Engl J Med. 2006;354(16):1671–84.
91. Sakamoto S, Homma S, Miyamoto A, Kurosaki A, Fujii T, Yoshimura K. Cyclosporin A in the treatment of acute exacerbation of idiopathic pulmonary fibrosis. Intern Med. 2010;49(2):109–15.
92. Inase N, Sawada M, Ohtani Y, Miyake S, Isogai S, Sakashita H, et al. Cyclosporin A followed by the treatment of acute exacerbation of idiopathic pulmonary fibrosis with corticosteroid. Intern Med. 2003;42(7):565–70.
93. Homma S, Sakamoto S, Kawabata M, Kishi K, Tsuboi E, Motoi N, et al. Cyclosporin treatment in steroid-resistant and acutely exacerbated interstitial pneumonia. Intern Med. 2005;44(11):1144–50.
94. Yokoyama T, Kondoh Y, Taniguchi H, Kataoka K, Kato K, Nishiyama O, et al. Noninvasive ventilation in acute exacerbation of idiopathic pulmonary fibrosis. Intern Med. 2010;49(15):1509–14.
95. Fujimoto K, Taniguchi H, Johkoh T, Kondoh Y, Ichikado K, Sumikawa H, et al. Acute exacerbation of idiopathic pulmonary fibrosis: high-resolution CT scores predict mortality. Eur Radiol. 2012;22(1):83–92.
96. Horita N, Akahane M, Okada Y, Kobayashi Y, Arai T, Amano I, et al. Tacrolimus and steroid treatment for acute exacerbation of idiopathic pulmonary fibrosis. Intern Med. 2011;50(3):189–95.
97. Seo Y, Abe S, Kurahara M, Okada D, Saito Y, Usuki J, et al. Beneficial effect of polymyxin B-immobilized fiber column (PMX) hemoperfusion treatment on acute exacerbation of idiopathic pulmonary fibrosis. Intern Med. 2006;45(18):1033–8.
98. Miyamoto K, Tasaka S, Hasegawa N, Kamata H, Shinoda H, Kimizuka Y, et al. Effect of direct hemoperfusion with a polymyxin B immobilized fiber column in acute exacerbation of interstitial pneumonia and serum indicators. Nihon Kokyuki Gakkai Zasshi. 2009;47(11):978–84.
99. Enomoto N, Suda T, Uto T, Kato M, Kaida Y, Ozawa Y, et al. Possible therapeutic effect of direct haemoperfusion with a polymyxin B immobilized fibre column (PMX-DHP) on pulmonary oxygenation in acute exacerbations of interstitial pneumonia. Respirology. 2008;13(3):452–60.
100. Tachibana K, Inoue Y, Nishiyama A, Sugimoto C, Matsumuro A, Hirose M, et al. Polymyxin-B hemoperfusion for acute exacerbation of idiopathic pulmonary fibrosis: serum IL-7 as a prognostic marker. Sarcoidosis Vasc Diffuse Lung Dis. 2011;28(2):113–22.
101. Oishi K, Mimura-Kimura Y, Miyasho T, Aoe K, Ogata Y, Katayama H, et al. Association between cytokine removal by polymyxin B hemoperfusion and improved pulmonary oxygenation in patients with acute exacerbation of idiopathic pulmonary fibrosis. Cytokine. 2013;61(1):84–9.

102. Abe S, Azuma A, Mukae H, Ogura T, Taniguchi H, Bando M, et al. Polymyxin B-immobilized fiber column (PMX) treatment for idiopathic pulmonary fibrosis with acute exacerbation: a multicenter retrospective analysis. Intern Med. 2012;51(12):1487–91.
103. Nakamura M, Ogura T, Miyazawa N, Tagawa A, Kozawa S, Watanuki Y, et al. Outcome of patients with acute exacerbation of idiopathic interstitial fibrosis (IPF) treated with sivelestat and the prognostic value of serum KL-6 and surfactant protein D. Nihon Kokyuki Gakkai Zasshi. 2007;45(6):455–9.
104. Donahoe M, Valentine VG, Chien N, Gibson KF, Raval JS, Saul M, et al. Autoantibody-targeted treatments for acute exacerbations of idiopathic pulmonary fibrosis. PLoS One. 2015;10(6):e0127771.
105. Tsushima K, Yamaguchi K, Kono Y, Yokoyama T, Kubo K, Matsumura T, et al. Thrombomodulin for acute exacerbations of idiopathic pulmonary fibrosis: a proof of concept study. Pulm Pharmacol Ther. 2014;29(2):233–40.
106. Kataoka K, Taniguchi H, Kondoh Y, Nishiyama O, Kimura T, Matsuda T, et al. Recombinant human Thrombomodulin in acute exacerbation of idiopathic pulmonary fibrosis. Chest. 2015;148(2):436–43.
107. Isshiki T, Sakamoto S, Kinoshita A, Sugino K, Kurosaki A, Homma S. Recombinant human soluble thrombomodulin treatment for acute exacerbation of idiopathic pulmonary fibrosis: a retrospective study. Respiration. 2015;89(3):201–7.
108. Gottlieb J, Warnecke G, Hadem J, Dierich M, Wiesner O, Fuhner T, et al. Outcome of critically ill lung transplant candidates on invasive respiratory support. Intensive Care Med. 2012;38(6):968–75.
109. Fuehner T, Kuehn C, Hadem J, Wiesner O, Gottlieb J, Tudorache I, et al. Extracorporeal membrane oxygenation in awake patients as bridge to lung transplantation. Am J Respir Crit Care Med. 2012;185(7):763–8.
110. Boussaud V, Mal H, Trinquart L, Thabut G, Danner-Boucher I, Dromer C, et al. One-year experience with high-emergency lung transplantation in France. Transplantation. 2012;93(10):1058–63.
111. Bermudez CA, Rocha RV, Zaldonis D, Bhama JK, Crespo MM, Shigemura N, et al. Extracorporeal membrane oxygenation as a bridge to lung transplant: midterm outcomes. Ann Thorac Surg. 2011;92(4):1226–31; discussion 31–2.
112. Saydain G, Islam A, Afessa B, Ryu JH, Scott JP, Peters SG. Outcome of patients with idiopathic pulmonary fibrosis admitted to the intensive care unit. Am J Respir Crit Care Med. 2002;166(6):839–42.
113. Mollica C, Paone G, Conti V, Ceccarelli D, Schmid G, Mattia P, et al. Mechanical ventilation in patients with end-stage idiopathic pulmonary fibrosis. Respiration. 2010;79(3):209–15.
114. Mallick S. Outcome of patients with idiopathic pulmonary fibrosis (IPF) ventilated in intensive care unit. Respir Med. 2008;102(10):1355–9.
115. Blivet S, Philit F, Sab JM, Langevin B, Paret M, Guerin C, et al. Outcome of patients with idiopathic pulmonary fibrosis admitted to the ICU for respiratory failure. Chest. 2001;120(1):209–12.
116. Stern JB, Mal H, Groussard O, Brugiere O, Marceau A, Jebrak G, et al. Prognosis of patients with advanced idiopathic pulmonary fibrosis requiring mechanical ventilation for acute respiratory failure. Chest. 2001;120(1):213–9.

Chapter 18
Lung Transplantation for Idiopathic Pulmonary Fibrosis

Daniela J. Lamas and David J. Lederer

Background

Lung transplantation is a surgical procedure during which one or both diseased lungs are replaced by organs from a deceased organ donor (or, less commonly, by lobes from living donors) (Table 18.1). Although survival time after lung transplantation is typically limited, transplantation can confer substantial benefits, including prolongation of life, to selected candidates with advanced lung diseases such as idiopathic pulmonary fibrosis (IPF) [1]. Between 1995 and June 2016, there were 60,107 lung transplant procedures performed worldwide, of which 16,442 (27%)

Publications based on data in Ref. [3] (Valapour M, Lehr CJ, Skeans MA, Smith JM, Carrico R, Uccellini K, et al. OPTN/SRTR 2016 Annual Data Report. *Lung. Am J Transplant*. 2018;18 Suppl 1:363–433) must include the following statement: The data and analyses reported in the 2016 Annual Data Report of the US Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients have been supplied by the United Network for Organ Sharing and the Minneapolis Medical Research Foundation under contract with HHS/HRSA. The authors alone are responsible for reporting and interpreting these data; the views expressed herein are those of the authors and not necessarily those of the US Government.

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Table 18.1 Types of lung transplant procedures

Procedure	Description	Number performed in the United States in 2016
Single-lung transplantation	Replacement of a single lung with a deceased donor lung	588
Bilateral sequential lung transplantation	Replacement of both lungs with deceased donor lungs with two main stem bronchial anastomoses	1741
Heart-lung transplantation	Replacement of both lungs and the heart with deceased donor lungs and heart	18
Living-donor lung transplantation	Replacement of both lungs with lobes from two living donors	0

From Valapour et al. [3]

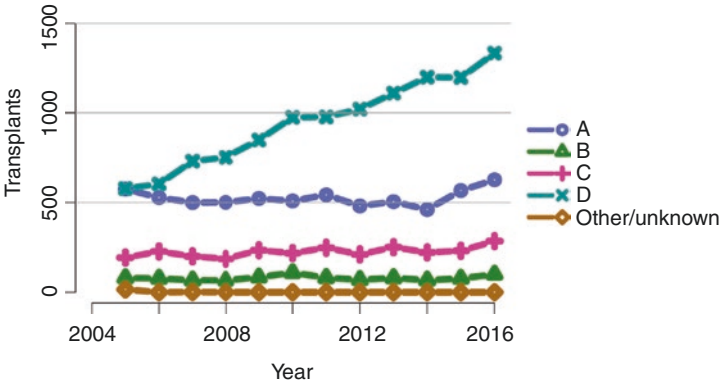

Fig. 18.1 Total lung transplants in the United States stratified by LAS diagnostic group, 2004–2016. Group A, obstructive lung disease; Group B, pulmonary vascular disease; Group C, cystic fibrosis; Group D, restrictive lung disease including IPF. (From: Valapour et al. [3])

were performed for IPF[1] [2]. Over the past decade, the proportion of lung transplant procedures performed for IPF in the United States has increased, and in 2006, IPF surpassed chronic obstructive pulmonary disease as the leading indication for lung transplantation in the United States (Fig. 18.1) [3]. In 2016, 57% of US lung transplant procedures were performed for IPF [3].

In this chapter, we will review the role of lung transplantation for patients with IPF, including candidate selection criteria, the evaluation process, organ allocation in the United States, and outcomes and complications of transplantation.

[1] The use of the term idiopathic pulmonary fibrosis (IPF) in transplant registries has historically referred to a variety of form of interstitial lung disease rather than IPF alone. In this chapter, usage of the term IPF includes interstitial lung diseases other than IPF.

Timing of Referral of IPF Patients for Lung Transplant Evaluation

IPF has been estimated to affect as many as 100,000 Americans [4]. Yet, in 2016, only 1331 adults underwent lung transplantation for IPF in the United States [3]. While some patients with IPF do not meet criteria for lung transplantation or may be too well for the procedure, the surprisingly small number of patients with IPF undergoing transplantation annually largely reflects the scarcity of suitable lungs from deceased organ donors. While there were in excess of 14,000 deceased donor kidney transplants performed in the United States in 2017, only 2449 lungs from deceased organ donors were used for transplantation. This discrepancy is largely due to unsuitable pulmonary conditions at the time of death in the majority of donors, such as pneumonia, ARDS, and pulmonary contusion [5].

In the face of this organ shortage, lung transplant providers must not only balance the risks and benefits of lung transplantation for individual patients, but they must also attempt to allocate deceased donor organs in a fashion that maximizes the overall public good achieved through transplantation (a utilitarian approach to the principle of distributive justice) [6]. Therefore, patients who stand to benefit from transplantation but who are also at exceedingly high risk of early death after transplantation should not undergo lung transplantation in geographic regions where a donor shortage exists. Stated simply, a patient must be "sick enough" to warrant transplantation but also "well enough" to tolerate the procedure and potentially enjoy many years of additional life after transplantation.

For these reasons, the selection of appropriate candidates for lung transplantation is challenging. In 2014, the International Society for Heart and Lung Transplantation (ISHLT) published guidelines (Tables 18.2 and 18.3) to aid in the selection of candidates for lung transplantation [7]. In general, these guidelines recommend that patients be considered for transplant evaluation when it is estimated that a patient has only a 50% chance of surviving the next 2 years, has a high (>80%) likelihood of surviving at least 90 days after transplant, and has a high likelihood of 5-year posttransplant survival from a general medical perspective [7]. Given the poor prognosis of patients with IPF, the guidelines specifically recommend that patients with IPF be referred for lung transplantation upon identification of "histolopathologic or radiographic evidence of UIP or fibrosing NSIP regardless of lung function" [7]. In a joint statement, the American Thoracic Society, the European Respiratory Society,

Table 18.2 ISHLT recommendations for the timing of listing for lung transplantation in IPF

Decline in FVC >10% during 6 months of follow-up
Decline in diffusing capacity >15% during 6 months of follow-up
Desaturation to <88% or distance <250 m on a 6 min walk test or >50 m decline in a 6 min walk distance over a 6-month period
Pulmonary hypertension on right heart catheterization or echocardiography
Hospitalization because of respiratory decline, pneumothorax, or acute exacerbation

From Weill et al. [37]

Table 18.3 Contraindications to lung transplantation

Absolute contraindications
Malignancy in the last 2 years, with the exception of non-melanoma skin cancer that has been treated appropriately. In general, a 5-year disease-free interval is prudent
Untreatable advanced dysfunction of another major organ system (e.g., heart, liver, or kidney) unless combined transplantation can be performed
Uncorrected atherosclerotic disease with suspected or confirmed end-organ ischemia or dysfunction and/or coronary artery disease not amenable to revascularization
Acute medial instability
Uncorrectable bleeding diathesis
Chronic infection with highly virulent and/or resistant microbes that are poorly controlled
Significant chest wall or spinal deformity
Body mass index greater than 35 kg/m^2
Current nonadherence to medical therapy or a history of repeated or prolonged episodes of nonadherence
Psychiatric or psychological condition associated with the inability to cooperate or comply with medical therapy
Absence of an adequate or reliable social support system
Severely limited functional status
Substance abuse or dependence (e.g., alcohol, tobacco, or narcotics)
Relative contraindications
Age older than 65 years in association with low physiologic reserve and/or other relative contraindications
Critical or unstable clinical condition (e.g., shock, mechanical ventilation, or extracorporeal membrane oxygenation)
Body mass index of 30–35 kg/m^2
Progressive or severe malnutrition
Extensive prior chest surgery with lung resection
Mechanical ventilation and/or extracorporeal life support
Colonization with highly resistant or highly virulent bacteria, fungi, or mycobacteria
Atherosclerotic disease burden sufficient to put the candidate at risk for end-organ disease after lung transplantation
Severe or symptomatic osteoporosis
Oher medical conditions that have not resulted in end-stage organ damage, such as diabetes mellitus, systemic hypertension, epilepsy, central venous obstruction, peptic ulcer disease, or gastroesophageal reflux, should be optimally treated before transplantation

From Weil et al. [37]

the Japanese Respiratory Society, and the Latin American Thoracic Association have recommended that IPF patients undergo transplant evaluation "at the first sign of objective deterioration" but do not provide details or specific criteria for "deterioration" [8].

While these recommendations have strong face validity, current evidence suggests that many patients are not referred for subspecialty or transplant care early in the course of their disease. Two prior studies have shown that the median delay between symptom onset and accessing subspecialty pulmonary care (by an ILD or

transplant pulmonologist) is 2 years [9, 10] and that longer delays are associated with a higher risk of death independent of lung function and age [10].

While some clinicians have used a failure of a trial of corticosteroids as an indication for transplant referral (as prior guidelines have suggested [11]), the "triple therapy" arm, consisting of prednisone, azathioprine, and N-acetylcysteine, of the PANTHER-IPF trial was halted early when an interim efficacy analysis indicated that increased mortality, hospitalizations, and adverse events were observed among study participants allocated to a combination of prednisone, azathioprine, and N-acetylcysteine [12]. Neither should a trial of pirfenidone nor nintedanib, one of two anti-fibrotic agents available in many parts of the world, delay referral for lung transplantation, since neither of these drugs improve lung function.

Early referral for lung transplant evaluation allows sufficient time for a thorough evaluation of the medical, surgical, and psychosocial candidacy of the patient, permits longitudinal evaluation of progression by the transplant team, ensures adequate transplant-specific education, and avoids high-risk emergent transplantation of patients with severe hypoxemic respiratory failure. It is our recommendation that patients with IPF be referred for lung transplantation as soon as the diagnosis is made. In cases where delayed referral is favored by providers, it is our opinion that referral should occur no later than upon determination that supplemental oxygen is required during ambulation and/or exercise.

Contraindications to Lung Transplantation

ISHLT-recommended contraindications to lung transplantation are listed in Table 18.3 [7]. There is general agreement that malignancy, severe chronic comorbid illness, psychosocial barriers, and the other absolute contraindications in Table 18.3 should prohibit lung transplantation for most candidates. On the other hand, the barrier that each of the relative contraindications listed in Table 18.3 poses to transplantation will vary according to candidate- and center-specific characteristics. These relative contraindications are largely factors reflecting body composition and surgical suitability that increase the risk of complications after lung transplantation.

Older age is associated with shorter survival time after lung transplantation [13]. The 5-year survival for adults over age 65 is less than 50%, comparing unfavorably to the nearly 70% 5-year survival for 35–49-year-olds (Fig. 18.2) [3]. Despite this increased risk, the proportion of lung transplants performed for older individuals has increased over time: in 2016, 30% of all lung transplant procedures in the United States were performed for adults 65 years of age and older [3]. The ISHLT guidelines state that age alone should not be used as the sole criterion to deny lung transplantation, but age instead should be considered as one of many factors when determining suitability for transplantation.

Obesity has been implicated as a marker for poor outcomes after lung transplantation. In early studies, a body mass index (BMI) greater than 30 kg/m^2 was identified as a risk factor for early mortality after lung transplantation. More recent work has

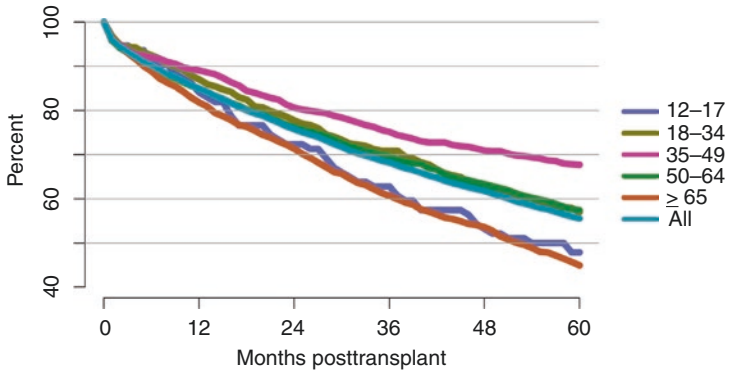

Fig. 18.2 Patient survival among lung transplant recipients aged 12 years or older, 2009–2011, stratified by age. (From: Valapour et al. [3])

found that those with a BMI between 30 and 35 kg/m^2 are not at increased risk of early mortality, perhaps due to the finding that BMI is a poor measure of total body fat. Obesity has also been shown to be an independent risk factor for primary graft dysfunction after lung transplantation [14, 15]. The mechanisms underlying these findings are not yet clear, but these may involve secretion of pro-inflammatory mediators from macrophages in adipose tissue [16]. Based on these risks, mild elevations in BMI should not prohibit lung transplantation in all candidates, but instead the risks associated with obesity should be balanced with other risk factors and the potential benefit of transplantation for each individual candidate. In some cases, it may be reasonable to withhold lung transplantation from severely obese candidates until weight loss has been achieved. Health-care providers should provide counseling and, when indicated, interventions in order to achieve a healthy weight for all patients with IPF, regardless of disease severity.

Candidate Evaluation and Timing of Listing for Lung Transplantation

Once referred for lung transplant evaluation, patients with IPF should undergo a thorough evaluation to determine if they are suitable candidates for lung transplantation based on the selection criteria described above and in Table 18.3. There are few published descriptions of the required elements of the evaluation of a lung transplant candidate, making the evaluation largely center-specific. Candidate evaluation typically begins with a review of medical records to determine if any absolute contraindications exist. If none are identified, the candidate meets with a transplant pulmonologist, thoracic surgeon, and/or transplant coordinator during which an extensive history and physical examination is performed, and the patient and his or her family are educated about the evaluation process, transplant procedure, postoperative expectations, complications, posttransplant lifestyle changes,

and survival statistics. In addition, this opportunity is taken to individualize the discussion of risks and benefits of transplantation and to discuss the patient's specific barriers to transplantation (such as obesity, underweight, poor functional status, and comorbidities), and recommendations to improve candidacy are made.

Following the initial consultation, patients typically perform an extensive evaluation to determine their suitability for lung transplantation (Table 18.4). Once the evaluation has been completed, the patient's case is discussed at a multidisciplinary team selection meeting. If deemed a suitable candidate for transplantation, the patient is placed on the active waiting list for transplantation. Commonly, patients will not be deemed candidates until they complete missing components of the evaluation and achieve strict health-related goals (such as weight loss and participation in pulmonary rehabilitation) or until additional follow-up shows signs of disease progression. The timing of listing for lung transplantation is based largely on the estimated risk of respiratory failure and death for patients with IPF. Table 18.2 shows known predictors of an increased risk of death in IPF that are recommended by the ISHLT for listing for lung transplantation [7]. In addition to these criteria,

Table 18.4 Suggested evaluation of lung transplant candidates

Radiologic and functional studies
Chest radiograph and high-resolution chest computed tomography scan
Quantitative ventilation/perfusion lung scan
Complete pulmonary function tests with arterial blood gas
Cardiopulmonary exercise testing (if deemed necessary)
6-minute walk test
Echocardiogram and electrocardiogram
Right heart catheterization
Left heart catheterization with coronary angiography in patients above age 45 or with risk factors for CAD
Bone densitometry
Barium esophagram
Laboratory evaluation
Complete blood count, electrolytes, BUN/creatinine, liver function studies, fasting lipid profile, quantitative immunoglobulin levels, viral serologies (HIV, HBsAg, HBsAb, HBcAb, HCV, HSV, CMV, EBV, VZV), toxoplasma antibody, aspergillus antibodies, blood type and screen, urinalysis, MDRD calculation of creatinine clearance, prostate-specific antigen (males over the age of 40), panel reactive antibody testing, and identification of specific anti-HLA antibodies
PPD testing or ELISPOT-based testing
Consultations
Psychosocial evaluation is completed by a transplant social worker and, if deemed necessary, supplemented by psychiatric evaluation
Rehabilitation medicine
Nutritionist, if deemed necessary on the initial nutritional screening
Dental evaluation
Ophthalmologic evaluation
Age- and gender-appropriate cancer screening

patients with IPF who have an interval increase in oxygen requirements or develop pulmonary hypertension should also be considered for active listing for lung transplantation. Additional factors that might favor earlier listing for lung transplantation (depending on local donor availability) include pre-sensitization to human leukocyte antigens, need for bilateral transplantation, and short stature [17].

Deceased Donor Lung Allocation in the United States

Prior to 2005, allocation of deceased donor lungs in the United States was based on waiting time, with the highest priority given to those with the longest waiting time. Aside from a 90-day credit for patients with IPF, disease severity was not a factor in determining waiting list priority. In 1999, the US Department of Health and Human Services issued the "Final Rule," which requires that deceased organ allocation systems de-emphasize waiting time and instead allocate organs based on "objective and measureable medical criteria… ordered from most to least medically urgent…." [18]. In response, the United Network for Organ Sharing/Organ Procurement and Transplantation Network (UNOS/OPTN) and the Scientific Registry of Transplant Recipients (SRTR) developed the Lung Allocation Score (LAS) system, which was put into place on May 4, 2005 [19]. The LAS system prioritizes waiting list candidates based on two criteria: medical urgency (the predicted risk of dying within 1 year) and estimated transplant benefit (the number of additional days of life expected from lung transplantation during the next year). Transplant benefit is calculated as the difference between expected survival time after lung transplantation and expected waiting list survival time (medical urgency). Medical urgency and expected survival after lung transplantation are estimated from multivariable regression models that contain the predictors in Table 18.5. The LAS, which varies from

Table 18.5 Variables included in the LAS calculation

Category	Variable
Disease severity	Forced vital capacity
	Mechanical ventilation
	Diagnosis
	Oxygen requirement
	Pulmonary artery pressure
	Cardiac index
	Central venous pressure
	Serum creatinine
	Serum bilirubin
	Partial pressure of carbon dioxide in arterial blood
Physiologic reserve	Age
	Functional status
	Diabetes mellitus
	Body mass index
	6-minute walk distance

0 to 100, is then derived from output of these models. Those with the greater medical urgency and expected transplant benefit receive higher LAS. After accounting for other criteria (geographic proximity to the donor, pediatric age, and blood type), deceased donor lungs are offered first to those with higher LAS. The LAS has been updated since its inception to include the addition of the partial pressure of carbon dioxide in arterial blood and the addition of serum bilirubin is planned (to aid the estimation of medical urgency for those with right heart failure due to pulmonary arterial hypertension).

The LAS system has had a number of notable consequences overall and for patients with IPF in particular. First, the transplantation rate for actively listed patients has increased dramatically with the greatest increase observed among those with IPF (Fig. 18.3), leading to IPF becoming the leading indication for lung transplantation in the United States (Fig. 18.1) [3, 20]. Second, waiting list mortality rates, which were decreasing prior to institution of the LAS system, increased over the past decade, particularly for patients with IPF (Fig. 18.4) [3]. Whether this increase in waiting list mortality is due to removal of healthier patients from the waiting list, due to listing of more severely affected patients, or due to an inadequate number of donors remains to be determined. Third, as discussed above, older patients are now being considered more commonly for transplantation, opening up this treatment modality to a wider pool of patients with IPF.

While the LAS appears to have increased the availability of transplantation for patients with IPF, concern remains that the scoring system – by preferentially emphasizing pretransplant urgency – may be prioritizing those at highest risk for poor posttransplant outcomes. Indeed, one study suggested there might be higher rates of primary graft dysfunction and longer intensive care unit stays under the LAS

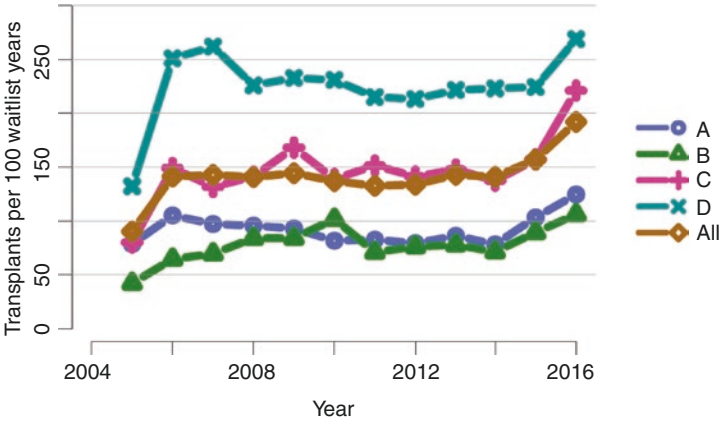

Fig. 18.3 Rate of lung transplantation for waiting list candidates in the United States stratified by LAS diagnostic group, 2004–2016. Group A, obstructive lung disease; Group B, pulmonary vascular disease; Group C, cystic fibrosis; Group D, restrictive lung disease including IPF. (From: Valapour et al. [3])

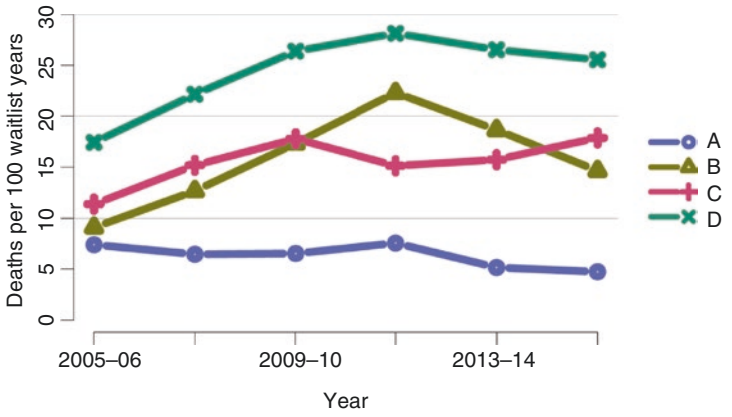

Fig. 18.4 Mortality rate of waiting list candidates aged 12 years and older on the lung transplant waiting list, by LAS diagnosis group, 2005–2015. Group A, obstructive lung disease; Group B, pulmonary vascular disease; Group C, cystic fibrosis; Group D, restrictive lung disease including IPF. (From: Valapour et al. [3])

system [21]. Two studies have also suggested that higher LAS are associated with higher mortality rates after lung transplantation [22, 23]. These studies raise questions about the utility of a system that grants organs to the sickest patients, increasing the likelihood of performing "futile" transplantation (i.e., transplantation of a donor organ without a consequent prolongation of life). However, one recent study demonstrates that a majority of adults undergoing transplantation experience a survival benefit and that the greatest potential benefit in fact comes to those with higher LAS or restrictive lung disease or cystic fibrosis – those patients who are the sickest entering the transplant [24]. Development of innovative methods to predict perioperative and posttransplant risk is underway and may ultimately lead to improved allocation methods and aid in optimizing the timing of lung transplantation.

Types of Transplant Procedures

While five different lung transplant procedureshave been developed (Table 18.1), the vast majority of lung transplant procedures performed in the modern era are either bilateral sequential lung transplantation or single-lung transplantation. In general, bilateral lung transplantation is indicated for patients with septic lung disease (such as bronchiectasis) and is preferred in patients with moderate-to-severe pulmonary hypertension. In IPF, many patients are candidates for either a bilateral or single-lung transplant procedure, and there are advantages to each procedure: bilateral transplantation confers greater improvement in lung mechanics and avoids native lung complications (such as malignancy), while single-lung transplantation is a simpler, shorter operation with a shorter waiting time that leaves the recipient with native lung function that may aid gas exchange during allograft complications, such as primary graft dysfunction [25].

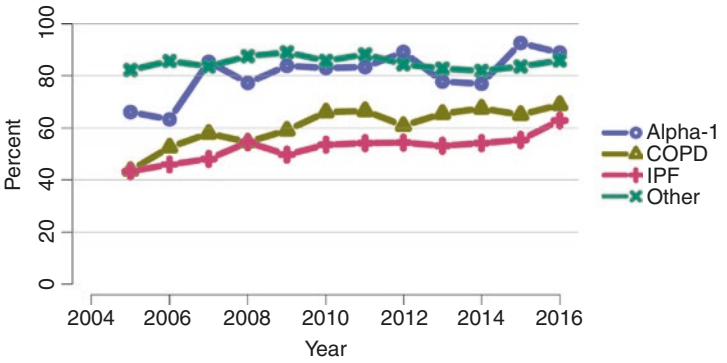

Fig. 18.5 Distribution of single- and bilateral lung transplantation in the United States, aged 12 and older, 2004–2016. (From: Valapour et al. [3])

The first isolated lung transplant procedures were single-lung transplant procedures for IPF and other interstitial lung diseases [26, 27]. Over time, bilateral lung transplantation has become the preferred procedure for IPF in the United States (Fig. 18.5) [28], yet controversy remains regarding whether one procedure confers a survival benefit over the other. Observational studies of treatments are typically confounded by the indication for the treatment itself [29], limiting the ability to make confident inferences from studies comparing single to bilateral lung transplantation. An older well-performed study using US nationwide registry found similar overall mortality rates among bilateral and single-lung transplant recipients with IPF [28], yet a more recent study using data following institution of the LAS system found that double-lung transplantation was associated with better graft survival than single-lung transplantation in patients with IPF [30].

In clinical practice, the decision to offer single- or bilateral lung transplantation to patients with IPF is often informed by the presence of pulmonary hypertension and the candidate's perceived surgical suitability for one procedure or the other. For candidates thought to be eligible for either procedure, single-lung transplantation should be preferred since the other lung could be used to transplant a second candidate, and available data suggest overall outcomes are similar between procedures. Indeed, patients with IPF listed for single-lung transplantation have higher transplantation rates and lower waiting list mortality rates than those listed for bilateral lung transplantation [31].

Outcomes and Complications of Lung Transplantation

Overall survival after lung transplantation has improved over time, with the median survival time improving from 4.2 years in the 1990 to 1998 ISHLT cohort to 6.1 years in the 1999–2008 ISHLT cohort [2]. For patients with IPF, the historical median survival time is 4.9 years [2], and patients with IPF unfortunately have the lowest 5-year survival rates compared to patients with other diagnoses [3]. Risk

factors for 1-year mortality after lung transplantation for patients with IPF include older age, donor age, total bilirubin, donor height, height difference, and transplant center volume [2]. Despite these risks, observational studies suggest that, on average, lung transplantation prolongs life for patients with IPF [1, 32].

Most lung transplant recipients have improved functional status with over 80% of surviving lung transplant recipients having no or only mild activity limitation at 1, 3, or 5 years after transplantation [2], suggesting a significant personal benefit of lung transplantation to many recipients.

Despite these benefits, lung transplantation carries significant risk. During the first posttransplant year, 28% of lung transplant recipients experience an episode of acute rejection, and over 50% are re-hospitalized, most commonly for infection or rejection [2]. Metabolic and cardiovascular complications are also common, with 23% developing diabetes, 26% developing chronic kidney disease, approximately one-third developing hyperlipidemia, and approximately 20% developing diabetes within 1 year of lung transplantation [2, 33]. The leading causes of death in the 1st year after transplantation are graft failure and infection [2].

The most feared complication of lung transplantation is chronic lung allograft dysfunction (CLAD), which includes both bronchiolitis obliterans syndrome (BOS) and restrictive allograft disorder (RAD). BOS manifests as airflow obstruction and occurs in 57% of lung transplant recipients by 5 years [3]. CLAD also frequently manifests as a fibrotic restrictive disease [34, 35]. CLAD is likely a final common pathway of multiple causes of airway injury, including alloimmune-mediated inflammation, infection, and gastroesophageal reflux [36], suggesting a variety of methods to potentially prevent CLAD. Nevertheless, once CLAD is present, there are (by definition) no known methods to improve lung function. CLAD is often progressive and is the leading cause of death after the 1st year of transplantation [2].

Summary

Lung transplantation is an effective therapy for highly selected patients with advanced IPF. Early referral to a lung transplant program should be considered for all patients with IPF. Since selection criteria continue to evolve, referring clinicians should consider referral of patients who may not have been candidates in past years, such as adults over the age of 70 and those with acute illness.

References

1. Hosenpud JD, Bennett LE, Keck BM, Edwards EB, Novick RJ. Effect of diagnosis on survival benefit of lung transplantation for end-stage lung disease. Lancet. 1998;351(9095):24–7.
2. Chambers DC, Yusen RD, Cherikh WS, Goldfarb SB, Kucheryavaya AY, Khusch K, et al. The registry of the international society for heart and lung transplantation: thirty-fourth adult lung and heart-lung transplantation report-2017; focus theme: allograft ischemic time. J Heart Lung Transplant. 2017;36(10):1047–59.

3. Valapour M, Lehr CJ, Skeans MA, Smith JM, Carrico R, Uccellini K, Lehman R, Robinson A, Israni AK, Snyder JJ, Kasiske BL. OPTN/SRTR 2016 annual data report: lung. Am J Transplant. 2018;18(Suppl 1):363–433.
4. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006;174(7):810–6.
5. Organ Procurement and Transplantation Network data as of March 12, 2018. Available from: http://optn.transplant.hrsa.gov/data/.
6. Persad G, Wertheimer A, Emanuel EJ. Principles for allocation of scarce medical interventions. Lancet. 2009;373(9661):423–31.
7. Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, et al. A consensus document for the selection of lung transplant candidates: 2014 – an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2015;34(1):1–15.
8. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. 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.
9. King TE Jr, Tooze JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med. 2001;164(7):1171–81.
10. Lamas DJ, Kawut SM, Bagiella E, Philip N, Arcasoy SM, Lederer DJ. Delayed access and survival in idiopathic pulmonary fibrosis: a cohort study. Am J Respir Crit Care Med. 2011;184(7):842–7.
11. Maurer JR, Frost AE, Estenne M, Higenbottam T, Glanville AR. International guidelines for the selection of lung transplant candidates. The International Society for Heart and Lung Transplantation, the American Thoracic Society, the American Society of Transplant Physicians, the European Respiratory Society. J Heart Lung Transplant. 1998;17(7):703–9.
12. Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 2012;366(21):1968–77.
13. Gutierrez C, Al-Faifi S, Chaparro C, Waddell T, Hadjiliadis D, Singer L, et al. The effect of recipient's age on lung transplant outcome. Am J Transplant. 2007;7(5):1271–7.
14. Lederer DJ, Wilt JS, D'Ovidio F, Bacchetta MD, Shah L, Ravichandran S, et al. Obesity and underweight are associated with an increased risk of death after lung transplantation. Am J Respir Crit Care Med. 2009;180(9):887–95.
15. Lederer DJ, Kawut SM, Wickersham N, Winterbottom C, Bhorade S, Palmer SM, et al. Obesity and primary graft dysfunction after lung transplantation: the Lung Transplant Outcomes Group Obesity Study. Am J Respir Crit Care Med. 2011;184(9):1055–61.
16. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003;112(12):1796–808.
17. Sell JL, Bacchetta M, Goldfarb SB, Park H, Heffernan PV, Robbins HA, et al. Short stature and access to lung transplantation in the United States. A Cohort Study. Am J Respir Crit Care Med. 2016;193(6):681–8.
18. "Allocation of Organs" Code of Federal Regulations Title 42, Pt. 121.8, 1999 ed.
19. Egan TM, Murray S, Bustami RT, Shearon TH, McCullough KP, Edwards LB, et al. Development of the new lung allocation system in the United States. Am J Transplant. 2006;6(5 Pt 2):1212–27.
20. Cantu E, Diamond JM, Suzuki Y, Lasky J, Schaufler C, Lim B, et al. Quantitative evidence for revising the definition of primary graft dysfunction after lung transplant. Am J Respir Crit Care Med. 2018;197(2):235–43.
21. Kozower BD, Meyers BF, Smith MA, De Oliveira NC, Cassivi SD, Guthrie TJ, et al. The impact of the lung allocation score on short-term transplantation outcomes: a multicenter study. J Thorac Cardiovasc Surg. 2008;135(1):166–71.
22. Russo MJ, Iribarne A, Hong KN, Davies RR, Xydas S, Takayama H, et al. High lung allocation score is associated with increased morbidity and mortality following transplantation. Chest. 2010;137(3):651–7.

23. Liu V, Zamora MR, Dhillon GS, Weill D. Increasing lung allocation scores predict worsened survival among lung transplant recipients. Am J Transplant. 2010;10(4):915–20.
24. Vock DM, Durheim MT, Tsuang WM, Finlen Copeland CA, Tsiatis AA, Davidian M, et al. Survival benefit of lung transplantation in the modern era of lung allocation. Ann Am Thorac Soc. 2017;14(2):172–81.
25. Hadjiliadis D, Angel LF. Controversies in lung transplantation: are two lungs better than one? Semin Respir Crit Care Med. 2006;27(5):561–6.
26. Toronto Lung Transplant Group. Unilateral lung transplantation for pulmonary fibrosis. N Engl J Med. 1986;314(18):1140–5.
27. Grossman RF, Frost A, Zamel N, Patterson GA, Cooper JD, Myron PR, et al. Results of single-lung transplantation for bilateral pulmonary fibrosis. The Toronto Lung Transplant Group. N Engl J Med. 1990;322(11):727–33.
28. Thabut G, Christie JD, Ravaud P, Castier Y, Dauriat G, Jebrak G, et al. Survival after bilateral versus single-lung transplantation for idiopathic pulmonary fibrosis. Ann Intern Med. 2009;151(11):767–74.
29. Mauri L. Why we still need randomized trials to compare effectiveness. N Engl J Med. 2012;366(16):1538–40.
30. Schaffer JM, Singh SK, Reitz BA, Zamanian RT, Mallidi HR. Single- vs double-lung transplantation in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis since the implementation of lung allocation based on medical need. JAMA. 2015;313(9):936–48.
31. Nathan SD, Shlobin OA, Ahmad S, Burton NA, Barnett SD, Edwards E. Comparison of wait times and mortality for idiopathic pulmonary fibrosis patients listed for single or bilateral lung transplantation. J Heart Lung Transplant. 2010;29(10):1165–71.
32. Titman A, Rogers CA, Bonser RS, Banner NR, Sharples LD. Disease-specific survival benefit of lung transplantation in adults: a national cohort study. Am J Transplant. 2009;9(7):1640–9.
33. Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dobbels F, Kirk R, et al. The registry of the international society for heart and lung transplantation: twenty-eighth adult lung and heart-lung transplant report – 2011. J Heart Lung Transplant. 2011;30(10):1104–22.
34. Martinu T, Howell DN, Davis RD, Steele MP, Palmer SM. Pathologic correlates of bronchiolitis obliterans syndrome in pulmonary retransplant recipients. Chest. 2006;129(4):1016–23.
35. Sato M, Waddell TK, Wagnetz U, Roberts HC, Hwang DM, Haroon A, et al. Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction. J Heart Lung Transplant. 2011;30(7):735–42.
36. Bowdish ME, Arcasoy SM, Wilt JS, Conte JV, Davis RD, Garrity ER, et al. Surrogate markers and risk factors for chronic lung allograft dysfunction. Am J Transplant. 2004;4(7):1171–8.
37. Weill D, Benden C, Corris P, Dark J, David RD, Keshavjee S, Lederer DJ, et al. A consensus document for the selection of lung transplant candidates: 2014 – an update from the pulmonary transplantation council of the international society for heart and lung transplantation. J Heart Lung Transplant. 2015;34(1):1–12.

Chapter 19
Clinical Trials in IPF: What Are the Best Endpoints?

Paolo Spagnolo, Elisabetta Cocconcelli, and Vincent Cottin

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease of unknown etiology characterized anatomically by scarring of the lung parenchyma, physiologically by progressive lung function deterioration, and clinically by troublesome cough and progressive shortness of breath, resulting in early death [1]. Within this framework, however, various clinical phenotypes exist with respect to disease extent, functional decline, and survival [2]. Despite the recent approval worldwide of two drugs that are able to slow down the pace of functional decline and disease progression (i.e., pirfenidone and nintedanib), IPF lacks a curative treatment. At present, the only cure for patients with IPF is lung transplantation, which unfortunately is a viable therapeutic option for only a small minority of highly selected patients. Lung transplantation is also associated with its own inherent complications, constraints, and limitations [3]. Accordingly, there is an urgent need for medical treatments that can truly modify the natural history of the disease and prolong survival with an acceptable safety and tolerability profile. This can only be achieved through well-designed and adequately conducted clinical trials of pharmacological interventions.

A number of promising candidate drugs are currently being tested; yet, while all of the efficacy and safety endpoints in a clinical trial provide important information,

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whether a trial is positive or negative is determined by the effect of the intervention on the predefined *primary* endpoint (Table 19.1). Therefore, a key determinant of the success of a trial is the choice of the optimal endpoints to match the design of the study. Both pirfenidone and nintedanib have been approved by the US Food and Drug Administration (FDA) based on slowed decline in forced vital capacity (FVC). The FDA noted that "the relationship between FVC and mortality trends in both sets of clinical trials strengthened our ability to rely on FVC as a clinically relevant efficacy measure in IPF" [29], thus implicitly acknowledging FVC as the preferred endpoint in IPF clinical trials. However, "preferred" does not mean "ideal," and what constitutes the most clinically meaningful efficacy variable for IPF clinical development programs remains uncertain and highly debated. Incomplete knowledge of disease pathogenesis along with the highly variable and unpredictable disease course on an individual basis contributes substantially to such uncertainty [30]. There is a general agreement that the optimal outcome measure should be reliable, reproducible, responsive to changes in disease status, clinically meaningful, predictive of clinical outcome, responsive to treatment effect, equally applicable to all IPF phenotypes, and easy to measure. However, none of the outcomes utilized over the last decade of clinical trials of IPF meets all these criteria. In an inexorably progressive and ultimately fatal disease such as IPF, some have advocated mortality as the unequivocal and most clinically important measure of efficacy [31], but such a study is likely to be impractical due to the large number of patients and long duration that would be required [32].

In this chapter, we summarize the various endpoints that have been used thus far in clinical trials of IPF and discuss the drawbacks that trialists should consider when designing such studies.

Forced Vital Capacity

Change in FVC has been the most widely used primary endpoint in clinical trials of IPF, the rationale being that, due to the archetypal pathophysiology of IPF (i.e., a fibrotic process that reduces the size of the lung), decline in FVC over time is likely to represent disease progression in most cases. FVC is reliable (values are stable when repeated at different time points) and responsive (as measured by the correlations between change in FVC and changes in other clinically relevant parameters). Importantly, categorical decrements of FVC are powerful predictors of mortality. Specifically, an absolute decline in % predicted FVC $\geq$10% (i.e., from 60% predicted to 50% predicted) at 24 weeks is associated with a nearly fivefold increase in the risk of mortality over the subsequent year [33, 34]. Moreover, it has been shown that using the relative 10% change in FVC (i.e., from 60% predicted to 54% predicted) maximizes the chance of identifying a meaningful decline in FVC without sacrificing prognostic accuracy [35].

Notably, the 10% threshold for an FVC change to be "significant" is not arbitrary, but it was originally designated to deal with the confounding effect of measurement

Table 19.1 Overview of the largest clinical trials in IPF and relative primary endpoints

Study agent/study acronym	Primary endpoint/number of patients	Outcome/comment	References
Pirfenidone/SP2	Difference in the change in the lowest oxygen saturation during a 6-min exercise test from baseline to 6 months/*n* = 102	The primary outcome was not met. Pirfenidone improved VC and prevented acute exacerbation of IPF during the 9 months of follow-up	[4]
Pirfenidone/SP3	Change in VC at week 52/*n* = 275	The study met the primary outcome. The primary endpoint was changed before unblinding	[5]
Pirfenidone/ CAPACITY 1, CAPACITY 2	Change in percentage predicted FVC at week 72/*n* = 344 and *n* = 435	The primary outcome was met in CAPACITY 2 but not in CAPACITY 1	[6]
Pirfenidone/ ASCEND	Change from baseline to week 52 in the percentage of the predicted FVC/*n* = 555	The primary outcome was achieved	[7]
Nintedanib/ TOMORROW	Annual rate of decline in FVC/*n* = 432	Nintedanib was associated with a trend toward a reduction in the decline in lung function	[8]
Nintedanib/ INPULSIS-1, INPULSIS-2	Annual rate of decline in FVC/*n* = 513 and *n* = 548	Both studies met the primary outcome	[9]
Ambrisentan/ ARTEMIS	Time to disease progression (death, decline in FVC ≥10%, decline in DL_{CO} ≥15%, or acute exacerbation)/*n* = 492	The study was terminated early due to increased risk for disease progression and hospitalization	[10]
Azathioprine + prednisone + NAC/IFIGENIA	Changes between baseline and month 12 in vital capacity and in DLco	No true placebo arm. The study met the primary outcome	[11]
	n = 236		
Azathioprine + prednisone + NAC/PANTHER	Change in FVC over 60 weeks	The combination therapy arm was terminated early due to increased rate of death and hospitalization	[12]
	n = 236		
Bosentan/ BUILD-1	Change from baseline up to month 12 in 6MWT/*n* = 158	The primary endpoint was not achieved	[13]
Bosentan/ BUILD-3	Time to IPF worsening (decline in FVC ≥10% and decline in DL_{CO} ≥15% or acute exacerbation) or death/*n* = 616	No difference between treatment groups in the primary endpoint analysis	[14]
Carlumab	Rate of percentage change in FVC/*n* = 126	Terminated prematurely due to lack of efficacy	[15]
Co-trimoxazole	Change in FVC over 1 year/*n* = 181	Co-trimoxazole was added to *standard treatment*	[16]
		No effect on lung function	

(continued)

Table 19.1 (continued)

Study agent/study acronym	Primary endpoint/number of patients	Outcome/comment	References
Etanercept	Changes from baseline in % predicted FVC, DL_{CO} % predicted, and P(A-a)O_2 at rest over 48 weeks (n = 88)	No differences in the predefined endpoints	[17]
Everolimus	Time to disease progression (time to the second of any two of 10% decline in FVC or TLC, 15% decline in DL_{CO}, 4% decline in room air oxygen saturation)/n = 89	Everolimus treatment was associated with increased disease progression and higher frequency of adverse events	[18]
Imatinib	Time to disease progression (10% decline in FVC from baseline) or death/n = 119	No effect of imatinib on survival or lung function	[19]
Interferon γ-1b	Progression-free survival (time to disease progression or death)/n = 330	No effect on progression-free survival	[20]
Interferon γ-1b/ INSPIRE	Overall survival n = 826	The study was terminated at the second interim analysis due to lack of benefit compared with placebo	[21]
Macitentan/ MUSIC	Change in FVC from baseline up to month 12/n = 178	The primary outcome was not met	[22]
NAC/PANTHER	Change in FVC over 60 weeks n = 264	No effect of NAC in reducing FVC decline	[23]
Pamrevlumab	Lung function, HRCT, and measures of health-related quality of life/n = 89	Open-label. No placebo arm. A subset of patients showed an increase of FVC and/or reduced reticular fibrosis on HRCT	[24]
Sildenafil/STEP	Proportion of patients with an increase in the 6-min walk distance of ≥20%/n = 180	The study enrolled patients with advanced IPF (DL_{CO} <35% of the predicted value) No benefit for sildenafil for the primary outcome	[25]
Simtuzumab/ RAINIER	Progression-free survival, defined as time to all-cause death or a categorical decrease from baseline in % predicted FVC/n = 544	Patients were stratified by baseline serum LOXL2 concentrations. The study did not achieve the primary outcome	[26]
Tralokinumab	Change from baseline to week 52 in % predicted FVC/n = 176	Subgroups defined by periostin baseline concentration. The primary outcome was not achieved	[27]
Warfarin/ACE	Composite endpoint (time to death, hospitalization, or ≥10% decline in FVC)/n = 145	The study was terminated early due to increased mortality in the warfarin arm	[28]

variation. Nevertheless, Zappala and colleagues have shown that a change as small as 5% might also have significant prognostic implications, suggesting that changes in % predicted FVC that were previously regarded as evidence of functionally stable disease are actually clinically relevant and worthy of further evaluation [36]. However, a 5% (marginal) decline in FVC was not significantly associated with increased risk of death in a large cohort of placebo-treated patients pooled from six pirfenidone and nintedanib FDA registration trials (n = 1132), although this was probably due to the shorter duration of observation [37]. In the same study, Paterniti and colleagues evaluated the association between FVC decline and mortality and explored the risk of death caused by acute exacerbations [37]. They showed that subjects experiencing one or more acute exacerbations had an increased risk of death (hazard ratio 10.3). In addition, consistent with previous studies, an absolute decline in FVC of >10% (at any time point during follow-up) significantly increased the risk of death.

A large body of evidence demonstrates that declines in FVC predict subsequent mortality in untreated cohorts and in patients randomized to placebo in clinical trials of IPF [33, 34, 36, 38–44]. On the other hand, the parallel benefits of antifibrotic therapy on FVC decline and mortality suggest that slowed functional deterioration may favorably impact survival. While these observations make FVC change a valid surrogate endpoint for death in IPF trials, its surrogacy may be dependent on the mechanism of action of the drug being tested [45]. Ideally, a therapeutic effect on FVC should parallel an effect on other clinically meaningful endpoints, such as mortality, acute exacerbations, or hospitalization. Therefore, as with all surrogate endpoints, FVC should be subjected to ongoing validation and scrutiny. Moreover, it has been argued that FVC is not a "patient-centered" outcome, as a pharmacologically induced reduction in the rate of functional decline may not be perceived as a tangible benefit from the patient's perspective [46].

6-Minute Walk Test

The 6-min walk test (6MWT) is a practical measure of exercise tolerance in patients with a variety of cardiac and pulmonary diseases [47]. Owing to its role in staging disease severity and predicting prognosis, it has also been used as a primary or secondary endpoint in several clinical trials of IPF. Until recently, however, studies evaluating the performance characteristics of the 6MWT in IPF were limited by small sample size or enrollment of narrowly defined patient subgroups and, because of these limitations, have generally yielded conflicting results [48–51]. From a clinical standpoint, the 6MWT has the advantages of being practical and safe; indeed, no special equipment or advanced training are required, and unlike maximal cardiopulmonary exercise testing, it can be performed by all but the most severely impaired patients. In addition, it provides clinically meaningful information. Data analysis from a large population of patients (n = 822) completing a 6MWT in the INSPIRE trial of interferon γ-1 showed that a 24-week reduction of >50 m in the walked

distance was associated with a fourfold increase in the risk of death over the following year [52]. Moreover, among patients listed for lung transplant, 6MWT distance may be a better predictor of 6-month mortality than the FVC [51]. Changes in 6MWT distance were weakly correlated with other measures of physiologic function (i.e., FVC, diffusing capacity of the lung for carbon monoxide [DL_{CO}], resting alveolar-arterial gradient of partial pressure of oxygen), dyspnea, and health-related quality of life [HRQL]), while values were consistently and significantly lower for patients with the poorest status [52]. A similar analysis that assessed 338 patients from the placebo groups of the CAPACITY trials confirmed these findings [53]. The minimal clinically important difference (MCID) in 6MWT distance has been estimated at 24–45 m [52].

The 6MWT was a secondary endpoint in the phase 3 CAPACITY and ASCEND trials with controversial findings. As compared to placebo, pirfenidone significantly reduced the decline in 6MWT distance in CAPACITY 2 [6] and ASCEND [7], but these results were not replicated in CAPACITY 1. In clinical trials of IPF, the mean change in the distance walked between the treatment and placebo arms has been commonly employed as the outcome measure, although other readily measurable 6MWT parameters such as oxygen desaturation and heart rate recovery (HRR) may predict outcomes better than the distance itself [54, 55]. Categorical change may represent a more informative analysis, especially if used in the context of a composite endpoint. Whether this should be a specific distance (e.g., 50 m) or a percentage change on the individual patient level is unclear [56]. In ASCEND, a confirmed decrease of 50 m or more was used as a secondary endpoint. This categorical change in the 6MWT distance was also one of three components of the composite "progression-free survival" outcome, the other two being a 10% decrease in percent predicted FVC and death [7]. The implementation of a composite endpoint that includes 6MWT results is an attractive alternative. For event-driven clinical trials, a low distance threshold would capture a higher number of events, which however may not reflect meaningful change. Conversely, a high distance threshold would result in fewer events detected. An alternative approach could be to employ other 6MWT-derived variables as an internal validation measure [56]. For instance, any change in the 6MWT distance would be more likely to be clinically meaningful if accompanied by increased desaturation, worsening dyspnea, or reduced HRR.

The 6MWT is a reliable, valid, and responsive measure of disease status in patients with IPF. However, the test does not provide insight into the mechanisms of exercise limitation. In addition, its results can be affected by factors unrelated to the underlying disease, including age, weight, peripheral arterial disease, musculoskeletal problems, and cognitive function [57].

Hospitalization

Respiratory, all-cause, and IPF-related hospitalizations are clinically significant events associated with high in-hospital mortality and limited survival beyond discharge [33]. In a recent post hoc analysis, Ley and colleagues compared the risk of

nonelective all-cause, respiratory-related, non-respiratory-related hospitalization and death after hospitalization with use of pirfenidone versus placebo over 52 weeks using data derived from three phase 3 IPF clinical trials [58]. The pooled analysis included 1247 patients (692 from the CAPACITY trials and 555 from the ASCEND trial), and the risk of hospitalization over 52 weeks was examined using standard time-to-event methods. When compared with placebo, pirfenidone treatment was associated with a lower risk of respiratory-related hospitalization at 1 year but was not associated with all-cause or non-respiratory-related hospitalization. Pirfenidone was also associated with a lower risk of death after hospitalization of any kind up to 52 weeks, but this association was no longer significant at 72 weeks. These findings strongly support the use of respiratory hospitalization (either alone or in a composite time-to-event endpoint) as an endpoint in IPF clinical trials. Respiratory hospitalization as an endpoint has several advantages: it results from a worsening of the underlying disease process; it is easier to measure and adjudicate than acute exacerbations; it is clinically meaningful and relevant to patients. Moreover, the study by Ley and co-workers suggests that the sample size required to achieve adequate power for this endpoint is not impractical. Given the 5% absolute reduction in the 1-year risk of respiratory hospitalization observed in their study (from 12% to 7%) and assuming a 2-year enrollment period and a 5% dropout in both arms, 588 participants (294 per arm) would need to be enrolled for a study to have an 80% power to detect a similar reduction in the risk of respiratory hospitalizations at 1 year [46]. Limitations to the implementation of hospitalization as an endpoint include the absence of a standard definition of respiratory hospitalization and the possibility that non-IPF-related admissions are captured in addition to other factors that can influence whether hospitalization occurs such as access to health care, social support, or regional differences in the indications for hospitalization [31]. Accordingly, consensus regarding the definition of respiratory hospitalization and the development of methods to account for regional propensities for hospital admission should be a priority of clinical trialists. Although clinically relevant, hospitalization is likely to be a secondary endpoint or a component of a composite endpoint in future trials.

Acute Exacerbations

Acute exacerbations (AEs) of IPF (AE-IPF), defined as episodes of acute respiratory worsening accompanied by the appearance of new parenchymal infiltrates on chest radiograph or high-resolution computed tomography (HRCT), are clinically relevant events associated with substantial morbidity and mortality [59]. In a recent pooled analysis of placebo-treated patients, AEs occurred in 9.1% of patients and were strongly associated (tenfold increased risk) with subsequent death [37]. AEs are more likely to manifest in patients with physiologically advanced disease and are believed to be triggered by external injury or stress to the lung [60]. Nintedanib was associated with a significantly reduced incidence of investigator-reported AEs (defined using similar criteria to the perspective published in 2007) [8, 61] in the phase 2

TOMORROW trial. AEs were also a key secondary endpoint in the phase 3 INPULSIS trials [9]. The two trials provided mixed results with time to the first AE being statistically significant in INPULSIS-2 ($p = 0.005$) but not in INPULSIS-1 ($p = 0.67$). However, a prespecified sensitivity analysis of pooled data from INPULSIS-1 and INPULSIS-2 revealed that the time to the first centrally adjudicated AE (either "confirmed" or "suspected" by an expert adjudication panel) was significantly increased in the nintedanib arm compared to placebo ($p = 0.001$). AE-IPF was a secondary endpoint in the Japanese Shionogi phase 2 (SP2) trial of pirfenidone [4]. During the 9-month study period, AE-IPF events occurred exclusively in the placebo group (5/35 vs. 0/72 in the pirfenidone group), which led to early termination of the study following an interim AE-IPF data analysis. Conversely, in the Japanese Shionogi phase 3 (SP3) trial, the incidence of AE-IPF, which was a tertiary endpoint, did not differ among the pirfenidone high-dose, pirfenidone low-dose, and placebo groups (6/106, 5.6%; 3/55, 5.5%; 5/104, 4.8%; respectively) [5]. In the CAPACITY trials, time to AE-IPF (along with death, lung transplantation, or admission to hospital for respiratory problems) was included in the worsening of IPF secondary endpoint [6]. However, no significant treatment effect was observed in either study.

Accurate identification of AE-IPF events in clinical trials is challenging, as the data required for accurate adjudication may be difficult to obtain. Indeed, as many as one-third of investigator-reported AEs may remain unclassifiable, when assessed by an adjudication committee, due to missing data [62]. However, an extended analysis of the INPULSIS dataset demonstrated no significant difference in outcomes between investigator-reported and adjudicated confirmed/suspected AEs. These data suggest that suspected AEs, which are clinically indistinguishable from definite AEs, represent clinically meaningful events, as they are associated with a similarly high risk of disease progression and short-term mortality. Whether definite and suspected AEs should be combined into a single endpoint in IPF clinical trials, however, is unclear. The recently revised definition of AE-IPF no longer requires clinical worsening to be idiopathic and also includes events triggered by infections, aspiration, or other identified factors [59]. Data from the INPULSIS trials support this concept by showing that events adjudicated as *not* AEs (according to the criteria proposed in the perspective published in 2007) [61] are associated with similar mortality rates as confirmed or suspected AE. Trialists should rethink the design of future clinical trials to capture AE-IPF events, although it is unclear whether idiopathic or triggered AEs should be used as outcome measures. Moreover, because the vast majority of patients experiencing acute deterioration are hospitalized, most AE-IPF events are also captured by respiratory-related hospitalization as an endpoint, thus resulting in largely overlapping and, to some extent, redundant information.

Mortality

Mortality-related measures (i.e., all-cause mortality, respiratory-related mortality, or IPF-related mortality) are admittedly the most robust and clinically relevant primary outcomes for phase 3 clinical trials in IPF. Specifically, all-cause mortality has

been suggested as the preferred primary endpoint, as it is the cleanest and most easily interpreted mortality-related endpoint [31], whereas respiratory-related mortality, which is commonly defined as death caused by progression of IPF, acute exacerbation, acute lung injury, pneumonia, and cor pulmonale [63], often requires external adjudication. However, death from IPF occurs too infrequently in the context of a clinical trial to serve as a realistic endpoint; this is because inclusion criteria are skewed toward patients with mild to moderate physiological impairment (i.e., FVC of 50–90%). Additionally, patients who are doing poorly are unlikely to be retained in trials until death occurs. Indeed, among 622 IPF patients randomized to placebo in the CAPACITY and INSPIRE studies, the all-cause mortality rate was 6.6% at 1 year and 13.7% at 2 years [32], which would make executing properly powered mortality trials in this population prohibitive. Mortality was a secondary endpoint in all the trials that assessed pirfenidone and nintedanib, but none of the individual studies was powered to show a significant reduction in mortality. One way to overcome this issue is to combine studies through pooling, meta-analysis, or both. In pooled analyses patient-level data are used to estimate treatment effects, whereas meta-analyses use group-level data, thus allowing for assessment of heterogeneity among studies. Nathan and colleagues performed a pooled analysis of the combined patient populations enrolled in the CAPACITY [6] and ASCEND [7] trials of pirfenidone for all-cause mortality, treatment-emergent all-cause mortality, IPF-related mortality, and treatment-emergent IPF-related mortality at weeks 52, 72, and 120 [64]. In addition, they performed a meta-analysis that included data from two Japanese trials of pirfenidone (i.e., Shionogi phase 2 (SP2) [4] and Shionogi phase 3 (SP3) [5]). At week 52 both pooled analyses and meta-analyses demonstrated that the relative risk of death for all four mortality outcomes was significantly lower in the pirfenidone group compared with placebo. Notably, the beneficial effect of pirfenidone on mortality was maintained over time irrespective of the statistical approach, although the number of patients followed up beyond 72 weeks was low, resulting in increased uncertainty around the point estimate at 120 weeks. Long study duration has the inherent drawback of difficult patient retention. Indeed, while patients may be willing to participate when they have early disease and mild symptoms, the ability to retain patients who deteriorate might be challenging. A significant dropout rate, in turn, would have deleterious consequences for the integrity and interpretation of the study results. In this regard, every effort should be made to obtain mortality/survival status for all patients withdrawing from the trial.

Potential Endpoints

Patient-Reported Outcome

As new therapies emerge, the design of clinical trials in IPF requires some radical rethinking. This includes using a more patient-centered approach to ensure that research questions address what is important, acceptable, and tolerable to an

individual patient. A patient-reported outcome (PRO) can be defined as "any report of the status of a patient's health condition that comes directly from the patient, without interpretation of the patient's response by a clinician or anyone else" [65]. Changes in physiological measures have been used as primary outcomes in most clinical trials of IPF. However, whether and to what extent changes in lung function influence patients' perceptions and HRQL is unclear. In IPF worsening breathlessness, fatigue, and/or cough limit patients' ability to perform even daily activities, thus dramatically reducing their quality of life (QoL). Therefore, the possibility to use reliable PRO measures as endpoints for clinical trials in IPF is a key issue.

The St George's Respiratory Questionnaire (SGRQ), which has been used as a secondary outcome measure in a number of recent clinical trials in IPF, was developed to assess the impact of obstructive lung diseases on quality of life, and it consists of 50 items and 3 domains that include symptoms, activity limitation, and social/emotional impact of disease [66]. In a recent study in IPF patients, Swigris and colleagues showed that the internal consistency of the SGRQ (i.e., the correlations between the different items of the questionnaire) is very good for total score, activity, and impact domains but lower for the symptoms score, which is possibly a consequence of including symptoms such as wheezing, which are infrequent in IPF [67]. Nevertheless, the SGRQ is able to differentiate IPF patients on the basis of disease severity, has a calculated MCID ranging from 8 to 9 points, and has been reported to be an independent predictor of mortality in IPF [68]. The change in the SGRQ total score from baseline over the 52-week treatment period was one of the key secondary endpoints in the INPULSIS-1 and INPULSIS-2 trials [9]. In INPULSIS-2, nintedanib treatment was associated with a significantly smaller increase in the SGRQ total score (consistent with less deterioration in QoL) compared with placebo (2.80 points in the nintedanib group vs. 5.48 points in the placebo group; $p = 0.02$), whereas in INPULSIS-1 there was no significant between-group difference in terms of QoL (4.34 points in the nintedanib group vs. 4.39 points in the placebo group; $p = 0.97$). An IPF-specific version of the SGRQ containing the most reliable items for measuring HRQL in patients with IPF has been developed (SGRQ-I) [69], but additional data are needed to evaluate its specificity. The King's Brief Interstitial Lung Disease (K-BILD) questionnaire, which consists of 15 items and 3 domains (breathlessness and activities, chest symptoms, and psychological domain), is a recently validated tool to assess health status in patients with ILD including those with IPF [70, 71]. The K-BILD questionnaire has shown good internal consistency, good test-retest accuracy, and sensitivity to change, and it has a calculated MCID of eight units. It is currently being used in several trials, and more information will therefore be forthcoming regarding its reliability in IPF alone and its potential use as a prognostic marker. The ATAQ-IPF (a tool to assess quality of life in IPF), which was specifically developed to assess HRQL in patients with IPF, displays good internal consistency. However, it still needs to be validated prospectively, and its MCID has not been defined [72]. In addition, because it has been and is currently being used predominantly in studies conducted in the USA and UK, its performance when used internationally in other countries is unknown. The University of California San Diego Shortness of Breath

Questionnaire (UCSD SOBQ) was used as a secondary outcome measure in the CAPACITY-1, CAPACITY-2, and ASCEND trials. No significant between-group differences in dyspnea were observed in either of the CAPACITY studies [6]. Similarly, analysis of UCSD SOBQ scores showed no significant treatment group differences in dyspnea at week 52 in ASCEND. Indeed, the endpoint of an increase of 20 points or more (indicating worsening) on the dyspnea score or death occurred in 81 patients (29.1%) in the pirfenidone group and in 100 patients (36.1%) in the placebo group (relative reduction, 19.3%; $p = 0.16$) [7].

The COPD Assessment Test (CAT), a short and easy to use questionnaire, consists of eight items that cover the spectrum of COPD severity (i.e., cough, phlegm, chest tightness, breathlessness, activities, confidence, sleep, and energy). Matsuda and co-workers recently evaluated the CAT in 106 patients with mild to moderate IPF [73]. The CAT displayed a high level of internal consistency, an acceptable test-retest at 3 months, and a good correlation with the SGRQ total score, whereas the correlations with the modified Medical Research Council dyspnea scale, and with 6MWT measurements, were lower for the CAT instrument compared with the SGRQ. Notably, both questionnaire scores were only weakly correlated with lung function parameters, suggesting that lung function may only capture one aspect of patients' well-being [70, 72].

Cough

Cough is one of the most disabling and troublesome symptoms for patients with IPF. In addition, it is an independent predictor of disease progression [74]. Several tools have been developed in recent years to assess different aspects of cough including both subjective (i.e., cough questionnaires or visual assessment scales) and objective (i.e., cough recorders and cough challenge tests) instruments, although experience with and validation of these tools in patients with IPF are limited [75]. Recently, van Manen and colleagues showed that pirfenidone significantly reduced objective 24-h cough as assessed by the Leicester Cough Monitor (LCM), a validated ambulatory cough monitoring system with recordings analyzed centrally with automated cough software [76]. Twenty out of 27 patients (74%) experienced an improvement in 24-h cough that was confirmed by subjective measures of cough severity and cough-related QoL. These effects were clinically meaningful to patients supporting the validity of cough as an endpoint in clinical trials of pharmacological interventions in IPF.

High-Resolution CT

HRCT is increasingly being used as an effectiveness endpoint in treatment trials of IPF to both refine the type of patients enrolled and to assess change in disease extent [77]. Progression of disease on HRCT is strongly associated with decreasing DL_{CO}

in patients with progressive systemic sclerosis [78], whereas in other studies the change in extent and nature of parenchymal abnormalities showed only a slight correlation with change in FVC and no correlation with change in DL_{CO} [79, 80]. In an open-label phase 2 trial evaluating the safety and efficacy of a monoclonal antibody specific for connective tissue growth factor (CTGF) (i.e., pamrevlumab), patients could be enrolled if they had HRCT evidence of ≥10% to <50% parenchymal fibrosis (reticulation) and <25% honeycombing within the whole lung, whereas the extent of emphysema greater than the extent of fibrosis on HRCT was an exclusion criterion [24]. Change in the extent of fibrosis – measured at baseline, 24 and 48 weeks – was one of the efficacy endpoints. For quantitative analysis of chest HRCT, a computer-aided standardized image acquisition protocol and a reconstruction algorithm were used. Specifically, the algorithm provided an overall determination of the percentage of the lung containing ground glass (GG), reticular fibrosis with architectural distortion (QLF), honeycomb fibrosis (HC), and a composite score representing a summation of QLF, GG, and HC (QILD) [81]. Not surprisingly, while reticular fibrosis increased in the majority of patients, 35% of them (n = 16) exhibited stable or improved disease regardless of the analysis method used, although the lack of a placebo arm greatly limits the interpretation of these findings. While this study identified a subset of patients in whom antifibrotic treatment may potentially reduce fibrosis, reduction of disease progression remains at present the only realistic outcome in clinical trials of IPF. Computer-aided assessment of HRCT represents a significant step forward along the way to identifying reliable imaging tools in clinical trials of pharmacological interventions in IPF, but it will probably not translate easily into clinical practice.

Biomarkers

Biomarkers are objectively measured factors (most often proteins found in blood, body fluid, or tissue but which can also be physiological measures such as FVC or imaging measures) that carry information about the health or disease state of the individual assayed [82]. Some examples of promising biomarkers that can potentially be used as endpoints in IPF clinical trials are given below.

αvβ6-integrin Saini and co-workers analyzed the expression of αvβ6-integrin, an epithelial-restricted molecule that has been implicated in multiple models of lung fibrosis, in 43 lung tissue sections of patients with IPF [83]. They observed that patients displaying the highest αvβ6-integrin expression were at increased risk of death, while those with very low expression had the lowest risk of death, suggesting that they may represent distinct disease endotypes. A phase 2 trial of STX-100, a humanized monoclonal antibody against the αvβ6-integrin, has recently been completed with results expected soon (NCT01371305).

LOXL2 Lysyl oxidase-like 2 (LOXL2) is a matrix-associated enzyme that crosslinks collagen, and serum LOXL2 levels have been associated with increased risk

for disease progression in IPF [84]. Therefore, simtuzumab, a monoclonal antibody that binds LOXL2, was a logical choice as a potentially effective pharmacological treatment for IPF. However, despite the enrollment of a large number of patients stratified by serum LOXL2 concentrations, a phase 2 study of simtuzumab in IPF (RAINIER) was terminated following an interim analysis showing lack of efficacy [26]. There are several potential explanations for the failure of simtuzumab to demonstrate efficacy in IPF patients, the most likely being the multitude and redundancy of mediators, growth factors, and signaling pathways involved in the fibrotic process [85].

Neoepitopes In a large prospective, multicenter, observational cohort study of IPF patients, Jenkins and colleagues found that several matrix metalloproteinase-degraded protein fragments (neoepitopes) reflective of collagen degradation are increased in the serum of patients with IPF compared with age-matched controls and that a change in neoepitope concentration over only 3 months may be predictive of subsequent outcome [86]. This observation may dramatically influence the duration of early-phase clinical trials.

Metaplastic epithelium signature Maher and colleagues performed an unbiased, multiplex immunoassay assessment of 123 biomarkers in 106 patients with IPF and 50 age- and sex-matched controls (discovery cohort) from the PROFILE (Prospective Observation of Fibrosis in the Lung Clinical Endpoints) study. They identified four serum biomarkers (surfactant protein D, matrix metalloproteinase 7, CA19-9, and CA-125) that were replicated in 206 patients (replication cohort). Histological assessment of CA19-9 and CA-125 suggested that these proteins were markers of epithelial damage. Baseline values of surfactant protein D and CA19-9 were significantly higher in patients with progressive disease than in patients with stable disease, and increasing concentrations of CA-125 over 3 months were associated with an increased risk of death [87].

Gene expression profiles Herazo-Maya and colleagues identified a 52-gene expression signature in peripheral blood mononuclear cells from patients with IPF and evaluated its performance in predicting transplant-free survival [88]. Based on this 52-gene signature, the same authors developed a genomic risk scoring system and tested it on 425 patients from six independent cohorts from the USA, UK, and Germany [89]. The application of the Scoring Algorithm for Molecular Subphenotypes (SAMS) to the 52-gene signature identified two groups of patients (low-risk and high-risk) with significant differences in mortality or transplant-free survival in each of the six cohorts. Moreover, temporal changes in SAMS were associated with changes in FVC in two of the cohorts. Notably, while untreated patients tended not to change their risk profiles, some high-risk patients had a reversal of their genomic risk profile after antifibrotic therapy was started. The possibility to use serial changes in biomarkers as a study endpoint is very attractive. However, at present there are no prospectively validated biomarkers that are able to track disease progression or response to treatment in IPF.

Composite Outcome Measures

Composite endpointsconsist of two or more individual outcomes combined together, and the endpoint is met whenever any component is first met. It has been recommended that composite outcomes include components that are just as likely to occur as another, similarly important to patients, and that are affected equally by the intervention [90]. In practice, however, these idealistic criteria are rarely satisfied. Death is generally incorporated as part of a composite endpoint because it occurs with such a low frequency in clinical trials of IPF that any intervention is unlikely to display a statistically significant improvement in mortality alone. While composite endpoints have several advantages (which are mainly related to the reduction in the required sample size and, therefore, the cost and duration of the trial through an increase in the event rate), such endpoints may introduce uncertainty in interpretation of the result when driven by the most frequent (but perhaps least important) of their constituents [91]. Indeed, in most trials the majority of patients who experience a composite outcome will have one of the less severe events. In such situations even statistically significant reductions in less severe outcomes may not necessarily translate into a reduced risk of serious outcomes [92].

Progression-free survival, which is commonly defined as various combinations of different measures of disease progression or death [30], is the combined outcome measure used most frequently in clinical trials of IPF. Hospital admission might be an additional component of a clinically meaningful composite endpoint. In a recent pooled cohort study of 517 IPF patients from three multicenter randomized controlled trials, Durheim and colleagues ascertained the independent and combined association of hospital admission and at least a 10% decrease in FVC with all-cause mortality [93]. To this end, they compared the incidence of nonelective hospital admission and a 10% or greater reduction in FVC across strata of baseline physiological impairment. Seven patients died before the landmark time point was reached. Of the 510 patients remaining, 38 (7%) were admitted to hospital up to the predefined time point, and 58 (11%) had a categorical decrease in FVC of at least 10%. Importantly, hospital admissions were independent of change in lung function with most patients admitted to hospital not experiencing a 10% or greater decrease in FVC (30 vs. 8). However, FVC decline and hospitalization both predicted subsequent time to death from any cause (hazard ratio for 10% or greater decline in FVC, 4.68; hazard ratio for hospital admission, 4.05). Change in FVC and change in distance walked during a 6MWT, both robust predictors of mortality, are sufficiently diverse in their ability to capture distinct pathophysiological domains of disease progression to allow them to be combined. While there is inevitably some collinearity between the two, they may also worsen independently (i.e., FVC may decline due to progression of fibrosis, while change in the 6MWT may best capture intervening pulmonary hypertension). The composite physiological index (CPI) was developed to capture disease severity regardless of the effect of emphysema on IPF by modeling pulmonary function tests to disease extent on HRCT. This index is simple to calculate (based on % predicted DL_{CO}, FVC, and forced expiratory

volume in 1 s [FEV_1]), reflects the extent of disease more accurately than single physiological indices, and is a powerful predictor of mortality [94]. However, the CPI has never been used as an endpoint in phase 3 clinical trials of IPF.

The Future of Clinical Trials in IPF

The clinical course of patients with IPF is characterized by progressive physiologic worsening, but considerable inter- and intraindividual variability exists, which makes it difficult to predict clinical behavior in individual cases. This is a crucial point as several distinct disease subsets are likely to exist within IPF, and phenotypic variability may contribute to nonuniform responses to treatment. In this scenario, even the positive effect of a given drug in a specific patient subgroup would inevitably be diluted or disappear because of its inefficacy in patients with different phenotypic characteristics. Homogenizing the study population by only including those with an a priori higher risk of progression and mortality (cohort enrichment strategy) has obvious appeal in powering the endpoint. However, while several studies have shown that baseline and longitudinal measures of disease severity and progression correlate with increased risk of subsequent mortality in patients with IPF [63], predicting short-term disease progression for individual patients is far more difficult. Indeed, Ley and co-workers [95] were unable to identify reliable baseline or 24-week longitudinal predictors of IPF worsening despite considering various combinations of endpoints in the patient population (n = 1113) enrolled in the INSPIRE (International Study of Survival Outcomes in Idiopathic Pulmonary Fibrosis with Interferon γ-1b) phase 3 trial of IFN-γ1b [21].

It has been argued that all-cause mortality should be used as the most robust and clinically relevant primary outcome for phase 3 clinical trials in IPF. Based on realistic estimates for efficacy on survival, such a study in IPF appears both unaffordable and non-executable within a reasonable time frame. Mortality might be best suited for studies tailored to individuals with advanced disease, as these patients have been shown to experience this outcome at an increased rate [96, 97]. Yet, patients with advanced disease behave quite differently from those with mild to moderate disease, often dying of pulmonary vascular complications rather than progressive fibrosis.

In IPF drug development, there is an unmet need for clinical trial design to establish robust proof of efficacy for novel agents in small phase 2 studies of brief duration [98]. Indeed, the TOMORROW (To Improve Pulmonary Fibrosis with BIBF-1120) trial of nintedanib, which can be viewed as a benchmark phase 2 study in IPF, enrolled >400 patients who were randomized to one of four escalating doses of nintedanib or placebo for 52 weeks [8]. The development of biomarkers represents a realistic approach for improving the efficiency of clinical trials in IPF. Biomarkers may identify patients at higher risk of disease progression, thereby increasing the chance of recording any positive drug effects with smaller sample size (cohort enrichment approach). They may also act as substitutes for an accepted clinical endpoint

(e.g., surrogates), or they may identify subsets of patients more likely to respond to a specific therapy while sparing other significant side effects and adverse events [99]. In this regard it has been shown that approximately 25% of patients with IPF (those carrying the *TOLLIP* rs3750920 TT genotype) may benefit from NAC therapy, whereas those with the rs3750920 CC genotype may be more susceptible to treatment-related harm [100], an observation that deserves further evaluation.

Future Perspective

A treatment trial designed to definitively determine whether a therapy is beneficial in IPF requires enormous efforts; yet the choice of the primary efficacy endpoint is one of the most critical steps in its development as an effective therapy for IPF. The clinical course of patients with mild to moderate IPF (those patients who are commonly enrolled in pharmacological studies) is characterized by minimal clinical and physiologic deterioration over time. Therefore, owing to the nature of the disease process (e.g., lung fibrosis generally progressing over many months or years), it is difficult to demonstrate large changes in functional indices such as lung function tests. Accordingly, slowing progression or stabilization of disease is probably the best that can be seen in a clinical trial. On the other hand, in patients with advanced disease in whom antifibrotic drugs are less likely to exert any beneficial effect, improving quality of life (i.e., managing and limiting dyspnea, cough, and fatigue, thus enabling patients to be as physically and socially active as possible) represents a realistic yet clinically meaningful goal for IPF clinical trials. At present there are no prospectively validated data on the value of biomarkers in the prediction of disease outcome or stratification of patients in treatment groups. It is hoped that biomarkers as well as genomic signatures will, at some point in the future, enable study enrichment with those patients at highest risk of progression and mortality. Future RCTs will need to not only use clinically relevant endpoints but also to be feasible in terms of timelines and resources. To this end, the importance of international collaborative initiatives cannot be overemphasized.

References

1. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. Lancet. 2017;389:1941–52.
2. Martinez FJ, Safrin S, Weycker D, et al. The clinical course of patients with idiopathic pulmonary fibrosis. Ann Intern Med. 2005;142:963–7.
3. Kistler KD, Nalysnyk L, Rotella P, Esser D. Lung transplantation in idiopathic pulmonary fibrosis: a systematic review of the literature. BMC Pulm Med. 2014;14:139.
4. Azuma A, Nukiwa T, Tsuboi E, et al. Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2005;171:1040–7.
5. Taniguchi H, Ebina M, Kondoh Y, et al. Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J. 2010;35:821–9.

6. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet. 2011;377:1760–9.
7. King TE Jr, Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014;370:2083–92.
8. Richeldi L, Costabel U, Selman M, et al. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med. 2011;365:1079–87.
9. Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370:2071–82.
10. Raghu G, Behr J, Brown KK. Treatment of idiopathic pulmonary fibrosis with ambrisentan: a parallel, randomized trial. Ann Intern Med. 2013;158:641–9.
11. Demedts M, Behr J, Buhl R, et al. High-dose acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med. 2005;353:2229–42.
12. Idiopathic Pulmonary Fibrosis Clinical Research Network, Martinez FJ, de Andrade JA, Anstrom KJ, King TE Jr, Raghu G. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 2012;366:1968–77.
13. King TE Jr, Behr J, Brown KK, et al. BUILD-1: a randomized placebo-controlled trial of bosentan in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2008;177:75–81.
14. King TE Jr, Brown KK, Raghu G. BUILD-3: a randomized, controlled trial of bosentan in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184:92–9.
15. Raghu G, Martinez FJ, Brown KK, et al. CC-chemokine ligand 2 inhibition in idiopathic pulmonary fibrosis: a phase 2 trial of carlumab. Eur Respir J. 2015;46:1740–50.
16. Shulgina L, Cahn AP, Chilvers ER, et al. Treating idiopathic pulmonary fibrosis with the addition of co-trimoxazole: a randomised controlled trial. Thorax. 2013;68:155–62.
17. Raghu G, Brown KK, Costabel U, et al. Treatment of idiopathic pulmonary fibrosis with etanercept: an exploratory, placebo-controlled trial. Am J Respir Crit Care Med. 2008;178:948–55.
18. Malouf MA, Hopkins P, Snell G, Glanville AR. Everolimus in IPF study investigators. An investigator-driven study of everolimus in surgical lung biopsy confirmed idiopathic pulmonary fibrosis. Respirology. 2011;16:776–83.
19. Daniels CE, Lasky JA, Limper AH, et al. Imatinib treatment for idiopathic pulmonary fibrosis: randomized placebo-controlled trial results. Am J Respir Crit Care Med. 2010;181:604–10.
20. Raghu G, Brown KK, Bradford WZ. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2004;350:125–33.
21. King TE Jr, Albera C, Bradford WZ. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. Lancet. 2009;374:222–8.
22. Raghu G, Million-Rousseau R, Morganti A, Perchenet L, Behr J, MUSIC Study Group. Macitentan for the treatment of idiopathic pulmonary fibrosis: the randomised controlled MUSIC trial. Eur Respir J. 2013;42:1622–32.
23. Idiopathic Pulmonary Fibrosis Clinical Research Network, Martinez FJ, de Andrade JA, Anstrom KJ, King TE Jr, Raghu G. Randomized trial of acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370:2093–3101.
24. Raghu G, Scholand MB, de Andrade J, et al. FG-3019 anti-connective tissue growth factor monoclonal antibody: results of an open-label clinical trial in idiopathic pulmonary fibrosis. Eur Respir J. 2016;47:1481–91.
25. Idiopathic Pulmonary Fibrosis Clinical Research Network, Zisman DA, Schwarz M, Anstrom KJ, Collard HR, Flaherty KR, Hunninghake GW. A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. N Engl J Med. 2010;363:620–8.
26. Raghu G, Brown KK, Collard HR, et al. Efficacy of simtuzumab versus placebo in patients with idiopathic pulmonary fibrosis: a randomised, double-blind, controlled, phase 2 trial. Lancet Respir Med. 2017;5:22–32.
27. Parker JM, Glaspole IN, Lancaster LH, et al. A phase 2 randomized controlled study of Tralokinumab in subjects with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2018;197:94–103.

28. Noth I, Anstrom KJ, Calvert SB, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186:88–95.
29. Karimi-Shah BA, Chowdhury BA. Forced vital capacity in idiopathic pulmonary fibrosis – FDA review of pirfenidone and nintedanib. N Engl J Med. 2015;372:1189–91.
30. Nathan SD, Meyer KC. IPF clinical trial design and endpoints. Curr Opin Pulm Med. 2014;20:463–71.
31. Raghu G, Collard HR, Anstrom KJ, et al. Idiopathic pulmonary fibrosis: clinically meaningful primary endpoints in phase 3 clinical trials. Am J Respir Crit Care Med. 2012;185:1044–8.
32. King TE Jr, Albera C, Bradford WZ, et al. All-cause mortality rate in patients with idiopathic pulmonary fibrosis. Implications for the design and execution of clinical trials. Am J Respir Crit Care Med. 2014;189:825–31.
33. du Bois RM, Weycker D, Albera C, et al. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;184:459–66.
34. Flaherty KR, Mumford JA, Murray S, et al. Prognostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. Am J Respir Crit Care Med. 2003;168:543–8.
35. Richeldi L, Ryerson CJ, Lee JS, et al. Relative versus absolute change in forced vital capacity in idiopathic pulmonary fibrosis. Thorax. 2012;67:407–11.
36. Zappala CJ, Latsi PI, Nicholson AG, et al. Marginal decline in forced vital capacity is associated with a poor outcome in idiopathic pulmonary fibrosis. Eur Respir J. 2010;35:830–6.
37. Paterniti MO, Bi Y, Rekić D, et al. Acute exacerbation and decline in forced vital capacity are associated with increased mortality in idiopathic pulmonary fibrosis. Ann Am Thorac Soc. 2017;14:1395–402.
38. Latsi PI, du Bois RM, Nicholson AG, et al. Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. Am J Respir Crit Care Med. 2003;168:531–7.
39. Collard HR, King TE Jr, Bartelson BB, et al. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2003;168:538–42.
40. King TE Jr, Safrin S, Starko KM, et al. Analyses of efficacy end points in a controlled trial of interferon-gamma1b for idiopathic pulmonary fibrosis. Chest. 2005;127:171–7.
41. Jegal Y, Kim DS, Shim TS, et al. Physiology is a stronger predictor of survival than pathology in fibrotic interstitial pneumonia. Am J Respir Crit Care Med. 2005;171:639–44.
42. Peelen L, Wells AU, Prijs M, et al. Fibrotic idiopathic interstitial pneumonias: mortality is linked to a decline in gas transfer. Respirology. 2010;15:1233–43.
43. Corte TJ, Wort SJ, Macdonald PS, et al. Pulmonary function vascular index predicts prognosis in idiopathic interstitial pneumonia. Respirology. 2012;17:674–80.
44. Schmidt SL, Nambiar AM, Tayob N, et al. Pulmonary function measures predict mortality differently in idiopathic pulmonary fibrosis versus combined pulmonary fibrosis and emphysema. Eur Respir J. 2011;38:176–83.
45. Ley B. Clarity on endpoints for clinical trials in idiopathic pulmonary fibrosis. Ann Am Thorac Soc. 2017;14:1383–4.
46. Podolanczuk AJ, Lederer DJ. Patient-centered outcomes in idiopathic pulmonary fibrosis clinical trials. Am J Respir Crit Care Med. 2017;196:674–5.
47. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166:111–7.
48. Caminati A, Bianchi A, Cassandro R, et al. Walking distance on 6-MWT is a prognostic factor in idiopathic pulmonary fibrosis. Respir Med. 2009;103:117–23.
49. Chetta A, Aiello M, Foresi A, et al. Relationship between outcome measures of six-minute walk test and baseline lung function in patients with interstitial lung disease. Sarcoidosis Vasc Diffuse Lung Dis. 2001;18:170–5.
50. Eaton T, Young P, Milne D, et al. Six-minute walk, maximal exercise tests: reproducibility in fibrotic interstitial pneumonia. Am J Respir Crit Care Med. 2005;171:1150–7.
51. Lederer DJ, Arcasoy SM, Wilt JS, et al. Six-minute-walk distance predicts waiting list survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006;174:659–64.

52. du Bois RM, Weycker D, Albera C, et al. Six-minute-walk test in idiopathic pulmonary fibrosis: test validation and minimal clinically important difference. Am J Respir Crit Care Med. 2011;183:1231–7.
53. Nathan SD, du Bois RM, Albera C, et al. Validation of test performance characteristics and minimal clinically important difference of the 6-minute walk test in patients with idiopathic pulmonary fibrosis. Respir Med. 2015;109:914–22.
54. Lama VN, Flaherty KR, Toews GB, et al. Prognostic value of desaturation during a 6-minute walk test in idiopathic interstitial pneumonia. Am J Respir Crit Care Med. 2003;168:1084–90.
55. Swigris JJ, Swick J, Wamboldt FS, et al. Heart rate recovery after 6-min walk test predicts survival in patients with idiopathic pulmonary fibrosis. Chest. 2009;136:841–8.
56. Brown AW, Nathan SD. The value and application of the 6-minute-walk test in idiopathic pulmonary fibrosis. Ann Am Thorac Soc. 2018;15:3–10.
57. Heresi GA, Dweik RA. Strengths and limitations of the six-minute-walk test: a model biomarker study in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;183:1122–4.
58. Ley B, Swigris J, Day BM, et al. Pirfenidone reduces respiratory-related hospitalizations in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2017;196:756–61.
59. Collard HR, Ryerson CJ, Corte TJ, et al. Acute exacerbation of idiopathic pulmonary fibrosis. An international working group report. Am J Respir Crit Care Med. 2016;194:265–75.
60. Collard HR, Richeldi L, Kim DS, et al. Acute exacerbations in the INPULSIS trials of nintedanib in idiopathic pulmonary fibrosis. Eur Respir J. 2017;49(5). pii: 1601339. https://doi.org/10.1183/13993003.01339-2016.
61. Collard HR, Moore BB, Flaherty KR, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2007;176:636–43.
62. de Andrade J, Schwarz M, Collard HR, et al. The idiopathic pulmonary fibrosis clinical research network (IPFnet) diagnostic and adjudication processes. Chest. 2015;148:1034–42.
63. Ley B, Collard HR, King TE Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011;183:431–40.
64. Nathan SD, Albera C, Bradford WZ, et al. Effect of pirfenidone on mortality: pooled analyses and meta-analyses of clinical trials in idiopathic pulmonary fibrosis. Lancet Respir Med. 2017;5:33–41.
65. Deshpande PR, Rajan S, Sudeepthi BL, Abdul Nazir CP. Patient-reported outcomes: a new era in clinical research. Perspect Clin Res. 2011;2:137–44.
66. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The St. George's respiratory questionnaire. Am Rev Respir Dis. 1992;145:1321–7.
67. Swigris JJ, Esser D, Wilson H, et al. Psychometric properties of the St George's respiratory questionnaire in patients with idiopathic pulmonary fibrosis. Eur Respir J. 2017;49(1). pii: 1601788. https://doi.org/10.1183/13993003.01788-2016.
68. Furukawa T, Taniguchi H, Ando M, et al. The St. George's respiratory questionnaire as a prognostic factor in IPF. Respir Res. 2017;18:18.
69. Yorke J, Jones PW, Swigris JJ. Development and validity testing of an IPF-specific version of the St George's respiratory questionnaire. Thorax. 2010;65:921–6.
70. Patel AS, Siegert RJ, Brignall K, et al. The development and validation of the King's brief interstitial lung disease (K-BILD) health status questionnaire. Thorax. 2012;67:804–10.
71. Wapenaar M, Patel AS, Birring SS, et al. Translation and validation of the King's brief interstitial lung disease (K-BILD) questionnaire in French, Italian, Swedish, and Dutch. Chron Respir Dis. 2017;14:140–50.
72. Swigris JJ, Wilson SR, Green KE, et al. Development of the ATAQ-IPF: a tool to assess quality of life in IPF. Health Qual Life Outcomes. 2010;8:77.
73. Matsuda T, Taniguchi H, Ando M, et al. COPD assessment test for measurement of health status in patients with idiopathic pulmonary fibrosis: a cross-sectional study. Respirology. 2017;22:721–7.

74. Ryerson CJ, Abbritti M, Ley B, et al. Cough predicts prognosis in idiopathic pulmonary fibrosis. Respirology. 2011;16:969–75.
75. van Manen MJ, Birring SS, Vancheri C, et al. Cough in idiopathic pulmonary fibrosis. Eur Respir Rev. 2016;25:278–86.
76. van Manen MJG, Birring SS, Vancheri C, et al. Effect of pirfenidone on cough in patients with idiopathic pulmonary fibrosis. Eur Respir J. 2017;50:1701157. https://doi.org/10.1183/13993003.01157-2017.
77. Hansell DM, Goldin JG, King TE Jr, et al. CT staging and monitoring of fibrotic interstitial lung diseases in clinical practice and treatment trials: a position paper from the Fleischner society. Lancet Respir Med. 2015;3:483–96.
78. Kim EA, Johkoh T, Lee KS, et al. Interstitial pneumonia in progressive systemic sclerosis: serial high-resolution CT findings with functional correlation. J Comput Assist Tomogr. 2001;25:757–63.
79. Jeong YJ, Lee KS, Müller NL, et al. Usual interstitial pneumonia and non-specific interstitial pneumonia: serial thin-section CT findings correlated with pulmonary function. Korean J Radiol. 2005;6:143–52.
80. Iwasawa T, Ogura T, Sakai F, et al. CT analysis of the effect of pirfenidone in patients with idiopathic pulmonary fibrosis. Eur J Radiol. 2014;83:32–8.
81. Kim HJ, Li G, Gjertson D, et al. Classification of parenchymal abnormality in scleroderma lung using a novel approach to denoise images collected via a multicenter study. Acad Radiol. 2008;15:1004–16.
82. Zhang Y, Kaminski N. Biomarkers in idiopathic pulmonary fibrosis. Curr Opin Pulm Med. 2012;18:441–6.
83. Saini G, Porte J, Weinreb PH, et al. αvβ6 integrin may be a potential prognostic biomarker in interstitial lung disease. Eur Respir J. 2015;46:486–94.
84. Chien JW, Richards TJ, Gibson KF, et al. Serum lysyl oxidase-like 2 levels and idiopathic pulmonary fibrosis disease progression. Eur Respir J. 2014;43:1430–8.
85. Spagnolo P, Rossi G, Cavazza A. Pathogenesis of idiopathic pulmonary fibrosis and its clinical implications. Expert Rev Clin Immunol. 2014;10:1005–17.
86. Jenkins RG, Simpson JK, Saini G, et al. Longitudinal change in collagen degradation biomarkers in idiopathic pulmonary fibrosis: an analysis from the prospective, multicentre PROFILE study. Lancet Respir Med. 2015;3:462–72.
87. Maher TM, Oballa E, Simpson JK, et al. An epithelial biomarker signature for idiopathic pulmonary fibrosis: an analysis from the multicentre PROFILE cohort study. Lancet Respir Med. 2017;5:946–55.
88. Herazo-Maya JD, Noth I, Duncan SR, et al. Peripheral blood mononuclear cell gene expression profiles predict poor outcome in idiopathic pulmonary fibrosis. Sci Transl Med. 2013;5:205ra136.
89. Herazo-Maya JD, Sun J, Molyneaux PL, 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;5:857–68.
90. Ferreira-González I, Permanyer-Miralda G, Busse JW, et al. Methodologic discussions for using and interpreting composite endpoints are limited, but still identify major concerns. J Clin Epidemiol. 2007;60:651–7.
91. Freemantle N, Calvert M, Wood J, Eastaugh J, Griffin C. Composite outcomes in randomized trials: greater precision but with greater uncertainty? JAMA. 2003;289:2554–9.
92. Ferreira-González I, Busse JW, Heels-Ansdell D, et al. Problems with use of composite end points in cardiovascular trials: systematic review of randomised controlled trials. BMJ. 2007;334:786.
93. Durheim MT, Collard HR, Roberts RS, et al. Association of hospital admission and forced vital capacity endpoints with survival in patients with idiopathic pulmonary fibrosis: analysis of a pooled cohort from three clinical trials. Lancet Respir Med. 2015;3:388–96.

94. Wells AU, Desai SR, Rubens MB, et al. Idiopathic pulmonary fibrosis: a composite physiologic index derived from disease extent observed by computed tomography. Am J Respir Crit Care Med. 2003;167:962–9.
95. Ley B, Bradford WZ, Vittinghoff E, et al. Predictors of mortality poorly predict common measures of disease progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2016;194:711–8.
96. Collard HR, Brown KK, Martinez FJ, et al. Study design implications of death and hospitalization as endpoints in idiopathic pulmonary fibrosis. Chest. 2014;146:1256–62.
97. Ley B, Bradford WZ, Weycker D, et al. Unified baseline and longitudinal mortality prediction in idiopathic pulmonary fibrosis. Eur Respir J. 2015;45:1374–81.
98. O'Riordan TG, Smith V, Raghu G. Development of novel agents for idiopathic pulmonary fibrosis: progress in target selection and clinical trial design. Chest. 2015;148:1083–92.
99. Spagnolo P, Tzouvelekis A, Maher TM. Personalized medicine in idiopathic pulmonary fibrosis: facts and promises. Curr Opin Pulm Med. 2015;21:470–8.
100. Oldham JM, Ma SF, Martinez FJ, et al. TOLLIP, MUC5B, and the response to N-acetylcysteine among individuals with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2015;192:1475–82.

Chapter 20
Future Directions for IPF Research

J. Matt Craig, Neil R. Aggarwal, and James P. Kiley

Introduction

With evidence of increasing incidence and a median survival time of less than 3 years following diagnosis, resulting in approximately 40,000 annual deaths in the United States alone, idiopathic pulmonary fibrosis (IPF) is a growing global public health concern [1]. Occurring most often between the ages of 50 and 70, IPF is a progressive disease for which the prevalence is higher among men and increases with age [2]. Additional risk factors appear to include genetic predisposition, cigarette smoking, prolonged exposure to occupational or environmental irritants, infection, and gastroesophageal reflux disease (GERD) [3]. From these associations, mechanisms of excessive alveolar epithelial injury and repair, including processes related to myofibroblast differentiation and extracellular matrix remodeling, have come to the forefront of basic IPF research. Although study of aberrant repair pathways in the context of fibrosis has shed some light on potential cellular and molecular disease mechanisms, the translation of this work into effective therapies for IPF patients continues to lag, suggesting that much remains to be learned about the pathogenesis of this disease.

From a clinical perspective, IPF is a chronic, progressive interstitial lung disease with diffuse parenchymal involvement characterized by usual interstitial pneumonia (UIP) [4]. Diagnostic criteria for UIP in IPF include the presence of heterogenous subpleural and basal septal thickening and honeycombing cysts with or without traction bronchiectasis that manifest as abnormal reticular opacities on high-resolution computed tomography (HRCT) and can be confirmed by surgical lung

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biopsy (SLB) [5]. Temporal heterogeneity among biopsy specimens is a disease-defining characteristic, as fibrotic zones are interspersed with normal lung architecture and often contain regions of dense collagen deposition and proliferating myofibroblasts referred to as fibroblastic foci [6]. Although useful for assessing the severity of disease and monitoring progression, the recognition of these characteristic features using existing imaging modalities would benefit from novel predictive biomarkers that coalesce patients displaying pathological heterogeneity and aid in disease prevention strategies.

Current recommended therapy for the treatment of IPF includes the use of nintedanib or pirfenidone that aims to slow the decline in lung function in patients with mild to moderate disease, followed by single or bilateral lung transplantation for patients with advanced-stage disease [7]. Unfortunately, despite modest preservation of forced vital capacity (FVC) and survival prolongation, these medical therapies are not curative, and median graft survival following lung transplantation is less than 5 years [8, 9]. Thus, the need for additional therapeutic interventions to reduce mortality associated with IPF remains real and unmet. With the limitations in our current understanding of the pathogenesis, diagnostic capabilities, and treatment options as they relate to IPF, this chapter aims to highlight high-priority research areas where considerable scientific progress may further ameliorate the effects of this debilitating disease.

Alveolar Epithelial Cells

The initiating events in IPF are thought to include repetitive or unresolving damage to the pulmonary epithelium, for which a predilection to injury may serve as an important risk factor [10]. Evidence suggests that reprogramming of alveolar epithelial cells (AECs) via aberrant gene expression or environmental exposures including infection can increase endoplasmic reticulum (ER) stress [11]. In turn, activation of the unfolded protein response (UPR) may push AECs toward a dysfunctional, pro-fibrotic phenotype that exhibit increased apoptosis and an altered epithelial-to-mesenchymal transition (EMT). Moreover, epithelial stem cells or other progenitor cell populations may contribute to the generation and maintenance of these dysfunctional AEC phenotypes [12]. Further study of epithelial-mesenchymal interactions and the role that mesenchymal changes acquired by AECs or their progenitors play in fibrogenesis is needed and may pave the way for improved cell-based regenerative medicine approaches. This could include the molecular characterization of AEC and epithelial progenitor cell subsets from the lungs of healthy and IPF patients or those isolated from animal models of fibrosis, as well as the study of the interactions between these cells in three-dimensional cell culture systems. Such characterization will require the identification of better phenotypic markers for epithelial, progenitor, and mesenchymal cells in the lung. To this end, a recent study performed the first single-cell RNA sequencing analysis of epithelial cells in IPF [13]. Ultimately these types of studies may yield new

biomarkers of alveolar injury that indicate susceptibility to, or the early onset of, fibrotic lung disease and thus will aid in prevention of disease and in the development of novel therapeutic targets to arrest fibrosis progression.

Myofibroblasts and the Extracellular Matrix

Myofibroblasts that secrete excessive extracellular matrix components are generally thought of as the primary pathogenic cell in IPF [14]. However, the origin and dynamics of myofibroblasts acquiring a pathologic signature distinct from that of normal wound healing is less well defined. Experimental evidence has implicated a range of cell types that can differentiate into myofibroblasts, including recruited circulating fibrocytes, resident lung fibroblasts, and AECs via the EMT [15]. Further studies, for example, those that combine temporal lineage tracing and phenotyping during the various stages of IPF progression, are needed to elucidate the contribution of these cells to pathogenic myofibroblast differentiation. In addition, work to identify specific signals derived from hematopoietic and non-hematopoietic cells in lung that direct myofibroblast activation will be critical for uncovering novel therapies that modulate the activity of these cells.

Another emerging area in myofibroblast research involves the determinants of a senescent, apoptosis-resistant phenotype. Senescence enables age-related cell cycle arrest that is associated with cellular events implicated in IPF, including telomere shortening, oxidative stress, and DNA damage [16]. In fact, a new study found a critical role for the secretome of senescent fibroblasts in the development of experimental IPF [17]. Furthermore, other work highlights the significance of myofibroblasts acquiring an invasive, senescent phenotype in the formation of fibroblastic foci [18, 19]. While some of the molecular factors that enable IPF fibroblasts to acquire these pro-survival and invasive phenotypes have been suggested, additional mechanistic studies demonstrating a causal link between these pathways and the development and/or progression of IPF are needed. Particularly informative would be studies investigating how altered cellular metabolism can promote long-lived IPF phenotypes. Along these lines, recent work has shown that decreased expression of PTEN-induced putative kinase 1 (PINK1) in mice results in a heightened susceptibility to lung fibrosis that is associated with increased mitochondrial dysfunction, AEC apoptosis, and myofibroblast transformation [20].

Having been firmly entrenched as a central feature of IPF for some time, extracellular matrix (ECM) deposition is now understood to be a dynamic process responsive to biomechanical stress [21]. Following deposition, the ECM can signal surrounding tissues through mechanotransduction and the release of bioactive soluble mediators. Several studies have demonstrated that increased matrix stiffness and the proteolytic release of ECM components can influence myofibroblast differentiation, findings which suggest that excess ECM may promote, rather than simply be a consequence of, pathogenic fibroblast activation [22, 23]. Characterization of the numerous glycoproteins, proteoglycans, and fibrous proteins that comprise the lung

matrisome highlights the complexity of the ECM and the need to further understand the differences between normal and pathologic ECM composition, organization, and function in IPF [24, 25].

Immune Activation

The role of inflammation and immunity in IPF remains controversial, with many in the field viewing immune-mediated inflammation as a marginable contributor to the disease process. This notion is supported by the low level of inflammation typically observed in UIP, as well as the failure of immunosuppressants to stem disease progression clinically [26]. However, tissue repair is inherently an inflammatory process, and cross talk between immune cells and the mesenchyme in the lung appears inevitable [27]. Therefore, revisiting the role that leukocytes play in regulating fibrotic repair processes holds merit for future research. Although scarce in terms of clear mechanistic connections, a few studies have identified associations with disease progression or severity and leukocytes, notably T cells and macrophages [28]. Given what we now know about the existence of immune cell subsets and improved surface marker phenotyping, a better characterization of the role and activation status of these populations at various stages of IPF is warranted. For example, assessing the distinct roles of recruited blood monocytes, interstitial macrophages, and alveolar macrophages to disease progression would be informative, but to date, collecting this information has been limited by the absence of experimental tools to effectively deplete specific macrophage populations. As a result, macrophages have often been labeled as pro-fibrotic with few mechanistic studies to definitively show that they act in such a manner. As a sign of progress in this area, a recently published study demonstrated a critical role for monocyte-derived lung macrophages in promoting and sustaining experimental lung fibrosis [29]. Extending these types of studies to include the identification of specific molecules released by macrophages or other immune cells that contribute explicitly to fibrogenesis would signify a crucial step forward.

Beyond macrophages, other immune cell types remain understudied in IPF. For example, both innate lymphoid cells (ILCs) and mast cells, which are clearly associated with epithelial wound repair in other mucosal diseases, have also been implicated in IPF, but their exact roles have yet to be fully elucidated [30, 31]. Furthermore, the study of effector and memory T-cell populations in IPF, including related aspects of immune tolerance and autoantibody production, is still in its infancy [32, 33]. While broad-spectrum immunosuppressants confer only a marginal benefit to IPF patients, targeted immunotherapy may prove to be a more personalized and efficacious approach. The potential for harnessing the immunomodulatory effects of endogenous mediators such as cytokines, soluble inhibitory receptors, and regulatory cells which have been shown to suppress fibrotic inflammation has yet to be extensively evaluated in the context of IPF [34]. Given the predominance of lymphocytic aggregates proximal to fibroblastic foci in IPF lungs and the observation

that IPF shares common pathological features with some autoimmune-associated lung diseases, these lines of immunological investigation may prove to be informative [35].

Genes and the Environment

One of the more successful areas of IPF research has included the use of genetic analyses such as genome-wide association studies to identify disease susceptibility loci in both familial and sporadic pulmonary fibrosis. Identified genes include surfactant protein C (*SFTPC*) and A2 (*SFTPA2*), telomerase RNA component (*TERC*) and reverse transcriptase (*TERT*), dyskerin (*DKC1*), mucin 5B (*MUC5B)*, and toll-interacting protein (*TOLLIP*) [36]. Investigation to determine whether these discoveries can be leveraged for precision or personalized medicine approaches to identify the subset of individuals who are likely to respond to a given IPF therapy is indicated. One study supportive of this notion suggested that IPF patients receiving *N*-acetylcysteine (NAC) therapy stratified in terms of responsiveness based on the specific *TOLLIP* alleles they expressed [37]. Beyond traditional genomic analyses, the next phase of genetic IPF research has materialized, with recent studies characterizing the lung epigenome, microbiome, long noncoding RNAs (lncRNAs), and microRNAs (miRNAs) [38–45]. Importantly, such data is now being collated by organizations such as NHLBI's TransOmics for Precision Medicine (TOPMed) program, the Global IPF Network, and the Lung Genomics Research Consortium (LGRC) and is being made available to the broader research community. The next step is to begin validating these newly discovered genetic associations with mechanistic studies in preclinical models and to connect observed epigenetic changes with environmental exposures that increase IPF risk.

Given that aberrant fibrogenesis at the cellular level shows similarities across different organ systems, we see added value in the integration of omics data from IPF with findings from fibrotic diseases in the heart, kidney, liver, skin, and bone marrow [46]. Moreover, diseases such as systemic sclerosis or radiation fibrosis syndrome often result in fibrotic changes to more than one organ system. Thus, collaborative projects that seek to identify overlap through comparative studies may uncover common risk variants, pathogenic mechanisms, and therapeutic targets that can be used to treat fibrosis in multiple organs.

Preclinical Models

The lack of a preclinical model system that effectively reproduces the pathologic features of human disease is arguably the most significant barrier to the realization of clinical treatments stemming from basic IPF research. Investigators have traditionally relied on single-dose intratracheal administration of bleomycin to model pulmonary fibrosis [47]. Although using this model has led to many important mechanistic

findings in the field, it does not induce progressive disease that manifests with the histologic UIP pattern observed in human IPF. Other exposures that model IPF, including the administration of asbestos, silica, and fluorescein isothiocyanate (FITC), similarly reproduce some but not all disease features. Genetically modified mice, such as those induced to overexpress TGF-β, TGF-α, IL-13, IL-1β, or TNFα, have also yielded important mechanistic insights into the pathogenesis of IPF but are likely not physiologically relevant models. Using mutations associated with familial interstitial pneumonia as a guide, mice deficient in *SFTPC*, *SFTPA2*, *TERC*, or *TERT* have also been used to model fibrosis. Yet, mice with these genetic manipulations do not develop spontaneous fibrosis, potentially limiting their value to that of understanding susceptibility to IPF in association with environmental exposures.

Given the age-dependent onset of IPF, further research is needed to continue the development of age-related models of fibrosis. Receptor of the advanced glycation end products (RAGE) and relaxin knockout mice, as well as mice infected with γ-herpesvirus-68, all develop age-related fibrosis [47]. Extending these models to further understand the role of cellular senescence in IPF may be a valuable scientific venture. Additionally, studying age-related effects in the context of humanized mouse models of IPF may increase translatability, although current models are limited by issues associated with xenoreactivity and thus require the use of an immunodeficient background. Further refinement of humanized models to include immune reconstitution may yield additional insights into the role that both structural and hematopoietic cells play in IPF. Fully appreciating the inherent difficulty in designing models for a disease which is idiopathic in nature, we would encourage the research community to consider studies that utilize multiple existing models of fibrosis to identify areas of mechanistic overlap and to continue working toward the development of new and better in vivo model systems for the study of human pulmonary fibrosis.

In addition to animal models, the use of in vitro culture systems still holds merit for the preclinical study of IPF. Notably, a recent study reported the generation of 3D pulmospheres, multicellular aggregates of alveolar epithelial cells, endothelial cells, macrophages, smooth muscle cells, and myofibroblasts surrounded by ECM components including collagen and fibronectin that were derived from human IPF lung biopsies [48]. Using such culture systems derived from specific individuals to test experimental therapies could facilitate antifibrotic drug screening and personalized medicine approaches that predict patient responsiveness to a given therapy.

Diagnostic Criteria, Biomarker Validation, and Cohort Establishment

The heterogeneous nature of patient presentation and histopathological features in IPF continues to pose serious challenges for clinical diagnosis and treatment. Consequently, there remains the need to better phenotype patient populations

using a combination of imaging data, pulmonary function testing, and biomarker monitoring. A 2011 update to the IPF diagnosis guidelines by a joint American Thoracic Society (ATS), European Respiratory Society (ERS), Japanese Respiratory Society (JRS), and Latin American Thoracic Association (ALAT) task force marked a major step forward in this regard [4]. Through the establishment of uniform benchmarks for definite, probable, and possible IPF diagnoses, most notably by outlining the criteria required to verify presence of a UIP pattern by HRCT or single lung biopsy (SLB), these guidelines have served to lessen variability among treatment centers. However, some patients lack characteristic honeycombing on HRCT and are unable to undergo SLB due to the presence of contraindications, which can result in mis- or underdiagnoses [49]. Furthermore, a definite IPF diagnosis does little to predict the ensuing disease course in terms of severity and rate of progression, leaving clinicians with limited information from which to formulate tailored treatment regimens. As such, development of physiological and molecular biomarkers that accurately predict disease susceptibility, onset, and progression would go a long way toward improving the diagnosis and management of IPF.

In terms of quantitative measures of lung function, FVC and diffusing capacity for carbon monoxide (DL_{CO}) decline can be useful predictive measures, especially when combined with traditional risk factors like age, gender, body mass, pulmonary hypertension, and smoking status [50]. From this, several multiparameter lung function models have been proposed to aid in IPF diagnosis and the assessment of mortality risk. These include the composite physiologic index (CPI) and gender-age-physiology (GAP) models that quantify IPF risk scores weighted by FVC, DL_{CO}, and/or FEV1 values [51]. These scoring systems become even more accurate when incorporating longitudinal assessments or CT imaging. However, while useful for forecasting outcomes, these measures do not provide information regarding disease pathogenesis or the probability of a beneficial response to interventions. Instead what is needed are additional biomarkers that can be used to stratify distinct IPF patient populations and enable more personalized patient care approaches.

Potential biomarkers including circulating fibrocyte number, MMP-7, CCL-18, periostin, LOXL2, S100A9, fibulin-1, and TLR-9 correlate with forced vital capacity (FVC) decline and can in some cases predict slow versus rapid IPF progressors [51]. To further determine their reliability and potential role in IPF pathogenesis, longitudinal assessment in large cohorts and follow-up mechanistic studies in preclinical models may be required. By extending the use of standardized, comprehensive phenotyping and novel biomarker monitoring into larger longitudinal cohorts of IPF patients, improved validation of biological data derived from basic research and superior clinical trial design to assess the safety and efficacy of novel therapies would be possible. A coordinated effort will be needed to support large patient cohorts with the potential promise of increased translation of IPF research from bench to bedside.

Clinical Trials and Improved Outcome Measures

A growing number of phase I, II, and III trials assessing the safety and efficacy of IPF therapies have been completed in recent years, leading the ATS/ERS/JRS/ALAT committee to update the clinical practice guidelines for the treatment of IPF in 2015 [7]. Notable updates to the 2015 guidelines include recommendations against the use of anticoagulants such as warfarin, the fibroblast-specific tyrosine kinase inhibitor imatinib, the endothelin receptor antagonists macitentan and bosentan, the phosphodiesterase-5 inhibitor sildenafil, as well as *N*-acetylcysteine monotherapy or in combination with prednisone and azathioprine for the treatment of IPF. These agents join corticosteroid monotherapy, cyclophosphamide, everolimus, interferon-λ, etanercept, and more recently the anti-IL-13 antibody tralokinumab and the anti-lysyl oxidase-like 2 (LOXL2) antibody simtuzumab on the list of potential IPF therapies that have failed to demonstrate sufficient clinical benefit to merit FDA approval for primary use in IPF [34, 52]. However, the FDA's approval of pirfenidone and nintedanib to treat IPF in 2014, combined with some promising ongoing trials, leaves reason for optimism and provides rationale for the investigation of implementation strategies to assess and improve adherence to proven beneficial therapies.

A recently completed open-label phase II trial of FG-3019, a humanized monoclonal antibody that targets connective tissue growth factor (CTGF), demonstrated improved pulmonary function and a reduction in radiographically assessed fibrosis in a subset of IPF patients [53]. Given that GF-3019 also appeared to be safe and well tolerated, a follow-up randomized, placebo-controlled study may be warranted. Several smaller trials are also building the case for the use of mesenchymal stem cells derived from bone marrow, placenta, or adipose-derived stromal cells as regenerative therapy in IPF [54, 55]. In addition, findings from recently completed trials of BG00011 (formerly STX-100), a monoclonal antibody that inhibits αvβ6 and blocks TGF-β1 activation, as well as low-dose inhaled carbon monoxide, have not yet been reported (NCT01371305, NCT01214187).

Ongoing NIH-supported clinical trials include the CleanUP-IPF study, a randomized, unblinded, multicenter phase III study stemming from the NHLBI Pulmonary Trials Cooperative that seeks to evaluate whether oral antimicrobial therapy with either co-trimoxazole or doxycycline reduces the risk of respiratory hospitalization and death in IPF patients (NCT02759120). Given the strong association with gastroesophageal reflux disease (GERD) and the relative success of antacid therapy with proton pump inhibitors (PPIs) or histamine-2 blocker receptor antagonists (H2Ras) in IPF patients, the current WRAP-IPF trial is addressing the potential for laparoscopic anti-reflux surgery to ameliorate disease (NCT01982968). In addition, the ART-IPF study is currently enrolling participants to assess the efficacy of rituximab in reducing autoantibody production and IPF disease progression through B-cell depletion (NCT01969409).

A critical question that remains for IPF clinical trials is whether current studies are utilizing optimal primary endpoints to allow for proper downstream evaluation of success or failure. Studies commonly utilize the ATS/ERS/JRS/ALAT guidelines for diagnosis and FVC as the primary physiologic endpoint. However, there is

debate as to whether all-cause mortality and non-elective hospitalizations might be more suitable endpoints versus FVC [56–59]. One limitation for using mortality and hospitalizations involves enrolling patients with mild to moderate IPF severity that typically do not have measurable disease progression within the timeframe of a standard clinical trial and therefore require the use of large cohorts to adequately power studies for observable treatment effects. In addition, the success of studies like ASCEND with pirfenidone supports the use of FVC as a surrogate endpoint for mortality [60]. The development of a validated composite endpoint of FVC, 6-min walk test, serum or imaging biomarkers, hospitalization, and mortality may improve the value of IPF trials [61]. Furthermore, the creation of IPF-specific patient-reported outcome tools such as health surveys that include quality of life measures may be useful for extending the reach of clinical trial data collection by promoting community-based care and reducing health disparities.

Summary

In summary, the future for advancing our understanding of IPF pathobiology and rapidly translating this knowledge into improved interventions with the goal of curing this disease is potentially on the horizon. To realize this goal, a number of critical challenges and high-priority research opportunities have been identified in this chapter that range from fundamental discovery-based studies to clinical and implementation science. These include the establishment of more appropriate animal models to probe mechanisms that can be readily translated to clinical studies, improved characterization of patient populations to enable precision medicine interventions, the continued pursuit of lung regeneration and other cell-based therapies, and the utilization of a systems medicine approach to integrate whole genome DNA sequencing with other omics, biomarkers, and factors such as the microbiome. These advancements hinge on our ability to better understand the molecular mechanisms underlying the dysregulation of lung homeostasis in IPF, how pathogenic mechanisms vary from individual to individual, and the organ specificity of the disease in the absence of apparent stimuli. Given the progress made to date across the biomedical spectrum in understanding the mechanisms underlying IPF and the potential to build new collaborative research programs to develop and test novel therapies, we are optimistic that new breakthroughs will lead to better outcomes for the many patients suffering from this disease.

References

1. Olson AL, Swigris JJ, Lezotte DC, Norris JM, Wilson CG, Brown KK. Mortality from pulmonary fibrosis increased in the United States from 1992 to 2003. Am J Respir Crit Care Med. 2007;176(3):277–84. https://doi.org/10.1164/rccm.200701-044OC.
2. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006;174(7):810–6. https://doi.org/10.1164/rccm.200602-163OC.

3. Wolters PJ, Collard HR, Jones KD. Pathogenesis of idiopathic pulmonary fibrosis. Annu Rev Pathol. 2014;9:157–79. https://doi.org/10.1146/annurev-pathol-012513-104706.
4. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE Jr, Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schunemann HJ, Fibrosis AEJACoIP. 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. https://doi.org/10.1164/rccm.2009-040GL.
5. Misumi S, Lynch DA. Idiopathic pulmonary fibrosis/usual interstitial pneumonia: imaging diagnosis, spectrum of abnormalities, and temporal progression. Proc Am Thorac Soc. 2006;3(4):307–14. https://doi.org/10.1513/pats.200602-018TK.
6. Smith M, Dalurzo M, Panse P, Parish J, Leslie K. Usual interstitial pneumonia-pattern fibrosis in surgical lung biopsies. Clinical, radiological and histopathological clues to aetiology. J Clin Pathol. 2013;66(10):896–903. https://doi.org/10.1136/jclinpath-2013-201442.
7. Raghu G, Rochwerg B, Zhang Y, Garcia CA, Azuma A, Behr J, Brozek JL, Collard HR, Cunningham W, Homma S, Johkoh T, Martinez FJ, Myers J, Protzko SL, Richeldi L, Rind D, Selman M, Theodore A, Wells AU, Hoogsteden H, Schunemann HJ, American Thoracic S, European Respiratory S, Japanese Respiratory S, Latin American Thoracic A. An official ATS/ERS/JRS/ALAT clinical practice guideline: treatment of idiopathic pulmonary fibrosis. An update of the 2011 clinical practice guideline. Am J Respir Crit Care Med. 2015;192(2):e3–19. https://doi.org/10.1164/rccm.201506-1063ST.
8. Mason DP, Brizzio ME, Alster JM, McNeill AM, Murthy SC, Budev MM, Mehta AC, Minai OA, Pettersson GB, Blackstone EH. Lung transplantation for idiopathic pulmonary fibrosis. Ann Thorac Surg. 2007;84(4):1121–8. https://doi.org/10.1016/j.athoracsur.2007.04.096.
9. Karimi-Shah BA, Chowdhury BA. Forced vital capacity in idiopathic pulmonary fibrosis – FDA review of pirfenidone and nintedanib. N Engl J Med. 2015;372(13):1189–91. https://doi.org/10.1056/NEJMp1500526.
10. Chambers RC, Mercer PF. Mechanisms of alveolar epithelial injury, repair, and fibrosis. Ann Am Thorac Soc. 2015;12(Suppl 1):S16–20. https://doi.org/10.1513/AnnalsATS.201410-448MG.
11. Tanjore H, Blackwell TS, Lawson WE. Emerging evidence for endoplasmic reticulum stress in the pathogenesis of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2012;302(8):L721–9. https://doi.org/10.1152/ajplung.00410.2011.
12. Rock JR, Hogan BL. Epithelial progenitor cells in lung development, maintenance, repair, and disease. Annu Rev Cell Dev Biol. 2011;27:493–512. https://doi.org/10.1146/annurev-cellbio-100109-104040.
13. Xu Y, Mizuno T, Sridharan A, Du Y, Guo M, Tang J, Wikenheiser-Brokamp KA, Perl AT, Funari VA, Gokey JJ, Stripp BR, Whitsett JA. Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis. JCI Insight. 2016;1(20):e90558. https://doi.org/10.1172/jci.insight.90558.
14. Moore MW, Herzog EL. Regulation and relevance of myofibroblast responses in idiopathic pulmonary fibrosis. Curr Pathobiol Rep. 2013;1(3):199–208. https://doi.org/10.1007/s40139-013-0017-8.
15. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. Am J Pathol. 2007;170(6):1807–16. https://doi.org/10.2353/ajpath.2007.070112.
16. Munoz-Espin D, Serrano M. Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol. 2014;15(7):482–96. https://doi.org/10.1038/nrm3823.
17. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, Oberg AL, Birch J, Salmonowicz H, Zhu Y, Mazula DL, Brooks RW, Fuhrmann-Stroissnigg H, Pirtskhalava T, Prakash YS, Tchkonia T, Robbins PD, Aubry MC, Passos JF, Kirkland JL, Tschumperlin DJ, Kita H, LeBrasseur NK. Cellular senescence mediates fibrotic pulmonary disease. Nat Commun. 2017;8:14532. https://doi.org/10.1038/ncomms14532.

18. Li Y, Jiang D, Liang J, Meltzer EB, Gray A, Miura R, Wogensen L, Yamaguchi Y, Noble PW. Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. J Exp Med. 2011;208(7):1459–71. https://doi.org/10.1084/jem.20102510.
19. Li Y, Liang J, Yang T, Monterrosa Mena J, Huan C, Xie T, Kurkciyan A, Liu N, Jiang D, Noble PW. Hyaluronan synthase 2 regulates fibroblast senescence in pulmonary fibrosis. Matrix Biol. 2016;55:35–48. https://doi.org/10.1016/j.matbio.2016.03.004.
20. Bueno M, Lai YC, Romero Y, Brands J, St Croix CM, Kamga C, Corey C, Herazo-Maya JD, Sembrat J, Lee JS, Duncan SR, Rojas M, Shiva S, Chu CT, Mora AL. PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. J Clin Invest. 2015;125(2):521–38. https://doi.org/10.1172/JCI74942.
21. Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. Nat Rev Mol Cell Biol. 2014;15(12):802–12. https://doi.org/10.1038/nrm3896.
22. Klingberg F, Hinz B, White ES. The myofibroblast matrix: implications for tissue repair and fibrosis. J Pathol. 2013;229(2):298–309. https://doi.org/10.1002/path.4104.
23. White ES. Lung extracellular matrix and fibroblast function. Ann Am Thorac Soc. 2015;12(Suppl 1):S30–3. https://doi.org/10.1513/AnnalsATS.201406-240MG.
24. Hynes RO, Naba A. Overview of the matrisome – an inventory of extracellular matrix constituents and functions. Cold Spring Harb Perspect Biol. 2012;4(1):a004903. https://doi.org/10.1101/cshperspect.a004903.
25. Naba A, Clauser KR, Hoersch S, Liu H, Carr SA, Hynes RO. The matrisome: in silico definition and in vivo characterization by proteomics of normal and tumor extracellular matrices. Mol Cell Proteomics. 2012;11(4):M111 014647. https://doi.org/10.1074/mcp.M111.014647.
26. Bringardner BD, Baran CP, Eubank TD, Marsh CB. The role of inflammation in the pathogenesis of idiopathic pulmonary fibrosis. Antioxid Redox Signal. 2008;10(2):287–301. https://doi.org/10.1089/ars.2007.1897.
27. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med. 2012;18(7):1028–40. https://doi.org/10.1038/nm.2807.
28. Kolahian S, Fernandez IE, Eickelberg O, Hartl D. Immune mechanisms in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2016;55(3):309–22. https://doi.org/10.1165/rcmb.2016-0121TR.
29. Misharin AV, Morales-Nebreda L, Reyfman PA, Cuda CM, Walter JM, McQuattie-Pimentel AC, Chen CI, Anekalla KR, Joshi N, Williams KJN, Abdala-Valencia H, Yacoub TJ, Chi M, Chiu S, Gonzalez-Gonzalez FJ, Gates K, Lam AP, Nicholson TT, Homan PJ, Soberanes S, Dominguez S, Morgan VK, Saber R, Shaffer A, Hinchcliff M, Marshall SA, Bharat A, Berdnikovs S, Bhorade SM, Bartom ET, Morimoto RI, Balch WE, Sznajder JI, Chandel NS, Mutlu GM, Jain M, Gottardi CJ, Singer BD, Ridge KM, Bagheri N, Shilatifard A, Budinger GRS, Perlman H. Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span. J Exp Med. 2017. https://doi.org/10.1084/jem.20162152.
30. Hams E, Armstrong ME, Barlow JL, Saunders SP, Schwartz C, Cooke G, Fahy RJ, Crotty TB, Hirani N, Flynn RJ, Voehringer D, McKenzie AN, Donnelly SC, Fallon PG. IL-25 and type 2 innate lymphoid cells induce pulmonary fibrosis. Proc Natl Acad Sci U S A. 2014;111(1):367–72. https://doi.org/10.1073/pnas.1315854111.
31. Veerappan A, O'Connor NJ, Brazin J, Reid AC, Jung A, McGee D, Summers B, Branch-Elliman D, Stiles B, Worgall S, Kaner RJ, Silver RB. Mast cells: a pivotal role in pulmonary fibrosis. DNA Cell Biol. 2013;32(4):206–18. https://doi.org/10.1089/dna.2013.2005.
32. Feghali-Bostwick CA, Wilkes DS. Autoimmunity in idiopathic pulmonary fibrosis: are circulating autoantibodies pathogenic or epiphenomena? Am J Respir Crit Care Med. 2011;183(6):692–3. https://doi.org/10.1164/rccm.201010-1727ED.
33. Donahoe M, Valentine VG, Chien N, Gibson KF, Raval JS, Saul M, Xue J, Zhang Y, Duncan SR. Autoantibody-targeted treatments for acute exacerbations of idiopathic pulmonary fibrosis. PLoS One. 2015;10(6):e0127771. https://doi.org/10.1371/journal.pone.0127771.
34. Rafii R, Juarez MM, Albertson TE, Chan AL. A review of current and novel therapies for idiopathic pulmonary fibrosis. J Thorac Dis. 2013;5(1):48–73. https://doi.org/10.3978/j.issn.2072-1439.2012.12.07.

35. Hoyne GF, Elliott H, Mutsaers SE, Prele CM. Idiopathic pulmonary fibrosis and a role for autoimmunity. Immunol Cell Biol. 2017. https://doi.org/10.1038/icb.2017.22.
36. Kropski JA, Blackwell TS, Loyd JE. The genetic basis of idiopathic pulmonary fibrosis. Eur Respir J. 2015;45(6):1717–27. https://doi.org/10.1183/09031936.00163814.
37. Oldham JM, Ma SF, Martinez FJ, Anstrom KJ, Raghu G, Schwartz DA, Valenzi E, Witt L, Lee C, Vij R, Huang Y, Strek ME, Noth I, Investigators IP. TOLLIP, MUC5B, and the response to N-acetylcysteine among individuals with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2015;192(12):1475–82. https://doi.org/10.1164/rccm.201505-1010OC.
38. Tzouvelekis A, Kaminski N. Epigenetics in idiopathic pulmonary fibrosis. Biochem Cell Biol. 2015;93(2):159–70. https://doi.org/10.1139/bcb-2014-0126.
39. Huang C, Yang Y, Liu L. Interaction of long noncoding RNAs and microRNAs in the pathogenesis of idiopathic pulmonary fibrosis. Physiol Genomics. 2015;47(10):463–9. https://doi.org/10.1152/physiolgenomics.00064.2015.
40. Molyneaux PL, Willis-Owen SAG, Cox MJ, James P, Cowman S, Loebinger M, Blanchard A, Edwards LM, Stock C, Daccord C, Renzoni EA, Wells AU, Moffatt MF, Cookson WOC, Maher TM. Host-microbial interactions in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2017;195(12):1640–50. https://doi.org/10.1164/rccm.201607-1408OC.
41. Han MK, Zhou Y, Murray S, Tayob N, Noth I, Lama VN, Moore BB, White ES, Flaherty KR, Huffnagle GB, Martinez FJ, Investigators C. Lung microbiome and disease progression in idiopathic pulmonary fibrosis: an analysis of the COMET study. Lancet Respir Med. 2014;2(7):548–56. https://doi.org/10.1016/S2213-2600(14)70069-4.
42. Molyneaux PL, Cox MJ, Wells AU, Kim HC, Ji W, Cookson WO, Moffatt MF, Kim DS, Maher TM. Changes in the respiratory microbiome during acute exacerbations of idiopathic pulmonary fibrosis. Respir Res. 2017;18(1):29. https://doi.org/10.1186/s12931-017-0511-3.
43. Pandit KV, Milosevic J. MicroRNA regulatory networks in idiopathic pulmonary fibrosis. Biochem Cell Biol. 2015;93(2):129–37. https://doi.org/10.1139/bcb-2014-0101.
44. Mizuno K, Mataki H, Seki N, Kumamoto T, Kamikawaji K, Inoue H. MicroRNAs in non-small cell lung cancer and idiopathic pulmonary fibrosis. J Hum Genet. 2017;62(1):57–65. https://doi.org/10.1038/jhg.2016.98.
45. Yang IV, Schwartz DA. Epigenetics of idiopathic pulmonary fibrosis. Transl Res. 2015;165(1):48–60. https://doi.org/10.1016/j.trsl.2014.03.011.
46. Wenzke KE, Cantemir-Stone C, Zhang J, Marsh CB, Huang K. Identifying common genes and networks in multi-organ fibrosis. AMIA Jt Summits Transl Sci Proc. 2012;2012:106–15.
47. Moore BB, Lawson WE, Oury TD, Sisson TH, Raghavendran K, Hogaboam CM. Animal models of fibrotic lung disease. Am J Respir Cell Mol Biol. 2013;49(2):167–79. https://doi.org/10.1165/rcmb.2013-0094TR.
48. Surolia R, Li FJ, Wang Z, Li H, Liu G, Zhou Y, Luckhardt T, Bae S, Liu RM, Rangarajan S, de Andrade J, Thannickal VJ, Antony VB. 3D pulmospheres serve as a personalized and predictive multicellular model for assessment of antifibrotic drugs. JCI Insight. 2017;2(8). https://doi.org/10.1172/jci.insight.94088.
49. Wells AU. The revised ATS/ERS/JRS/ALAT diagnostic criteria for idiopathic pulmonary fibrosis (IPF) – practical implications. Respir Res. 2013;14(Suppl 1):S2. https://doi.org/10.1186/1465-9921-14-S1-S2.
50. 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. https://doi.org/10.1164/rccm.201006-0894CI.
51. Daccord C, Maher TM. Recent advances in understanding idiopathic pulmonary fibrosis. F1000Res. 2016;5. https://doi.org/10.12688/f1000research.8209.1.
52. Raghu G, Brown KK, Collard HR, Cottin V, Gibson KF, Kaner RJ, Lederer DJ, Martinez FJ, Noble PW, Song JW, Wells AU, Whelan TP, Wuyts W, Moreau E, Patterson SD, Smith V, Bayly S, Chien JW, Gong Q, Zhang JJ, O'Riordan TG. Efficacy of simtuzumab versus placebo in patients with idiopathic pulmonary fibrosis: a randomised, double-blind, controlled, phase 2 trial. Lancet Respir Med. 2017;5(1):22–32. https://doi.org/10.1016/S2213-2600(16)30421-0.

53. Raghu G, Scholand MB, de Andrade J, Lancaster L, Mageto Y, Goldin J, Brown KK, Flaherty KR, Wencel M, Wanger J, Neff T, Valone F, Stauffer J, Porter S. FG-3019 anti-connective tissue growth factor monoclonal antibody: results of an open-label clinical trial in idiopathic pulmonary fibrosis. Eur Respir J. 2016;47(5):1481–91. https://doi.org/10.1183/13993003.01030-2015.
54. Chambers DC, Enever D, Ilic N, Sparks L, Whitelaw K, Ayres J, Yerkovich ST, Khalil D, Atkinson KM, Hopkins PM. A phase 1b study of placenta-derived mesenchymal stromal cells in patients with idiopathic pulmonary fibrosis. Respirology. 2014;19(7):1013–8. https://doi.org/10.1111/resp.12343.
55. Glassberg MK, Minkiewicz J, Toonkel RL, Simonet ES, Rubio GA, DiFede D, Shafazand S, Khan A, Pujol MV, LaRussa VF, Lancaster LH, Rosen GD, Fishman J, Mageto YN, Mendizabal A, Hare JM. Allogeneic human mesenchymal stem cells in patients with idiopathic pulmonary fibrosis via intravenous delivery (AETHER): a phase I safety clinical trial. Chest. 2017;151(5):971–81. https://doi.org/10.1016/j.chest.2016.10.061.
56. Raghu G, Collard HR, Anstrom KJ, Flaherty KR, Fleming TR, King TE Jr, Martinez FJ, Brown KK. Idiopathic pulmonary fibrosis: clinically meaningful primary endpoints in phase 3 clinical trials. Am J Respir Crit Care Med. 2012;185(10):1044–8. https://doi.org/10.1164/rccm.201201-0006PP.
57. Wells AU. Forced vital capacity as a primary end point in idiopathic pulmonary fibrosis treatment trials: making a silk purse from a sow's ear. Thorax. 2013;68(4):309–10. https://doi.org/10.1136/thoraxjnl-2012-202640.
58. du Bois RM, Nathan SD, Richeldi L, Schwarz MI, Noble PW. Idiopathic pulmonary fibrosis: lung function is a clinically meaningful endpoint for phase III trials. Am J Respir Crit Care Med. 2012;186(8):712–5. https://doi.org/10.1164/rccm.201206-1010PP.
59. Olson AL, Swigris JJ, Brown KK. Clinical trials and tribulations – lessons from pulmonary fibrosis. QJM. 2012;105(11):1043–7. https://doi.org/10.1093/qjmed/hcs066.
60. King TE Jr, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, Gorina E, Hopkins PM, Kardatzke D, Lancaster L, Lederer DJ, Nathan SD, Pereira CA, Sahn SA, Sussman R, Swigris JJ, Noble PW, Group AS. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2083–92. https://doi.org/10.1056/NEJMoa1402582.
61. Nathan SD, Meyer KC. IPF clinical trial design and endpoints. Curr Opin Pulm Med. 2014;20(5):463–71. https://doi.org/10.1097/MCP.0000000000000091.

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