HISTOPATHOLOGICAL FEATURES IN IDIOPATHIC PULMONARY FIBROSIS

INTEROBSERVER VARIATION, INORGANIC PARTICULATE MATTER AND PROGNOSTIC FACTORS DETECTED BY ARTIFICIAL INTELLIGENCE

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public examination online on 11th of June 2021, at 13 o'clock.

Helsinki, Finland 2021

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ISBN 978-951-51-7270-9 (pbk.)
ISBN 978-951-51-7271-6 (PDF)
Unigrafia Oy
Helsinki 2021

ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial lung disease of a dismal prognosis. While IPF is a rare disease, it is still the most common of idiopathic interstitial pneumonias. The radiological and histopathological manifestation of the disease is the usual interstitial pneumonia (UIP) pattern. The etiology of IPF is unknown, but inorganic dust is one of the known risk factors for IPF. The diagnosis of IPF is usually based on clinical and radiological data, but a surgical lung biopsy is required for a minority of patients. Confirming the diagnosis can be challenging as many interstitial lung diseases share similar features, and interobserver variation between radiologists and pathologists is significant. Separating IPF from other interstitial lung diseases is crucial due to differences in treatment and prognosis.

In this doctoral thesis, we hypothesized that histopathological features in IPF lung tissue would be associated with survival and lung function. In addition, we aimed at investigating interobserver agreement among pathologists, inorganic particulate matter (PM) in the lung tissue of patients with IPF, and the use of artificial intelligence (AI) in analyzing lung tissue samples of IPF patients. Our study cohort originated from a prospective, multicenter registry study, namely the FinnishIPF registry. We searched for patients with available histological lung tissue samples and compared the histopathological features to the registry data.

In Study I, four pathologists experienced in pulmonary pathology re-evaluated 60 lung tissue samples using the 2011 diagnostic criteria of IPF. They also recorded atypical histopathological features for IPF. Most of the samples were re-evaluated as definite UIP (38/60, 63%). The most common atypical feature for IPF was abundant inflammation (15/60, 25%). Using Cohen's κ coefficient, the interobserver agreement varied from slight to substantial (κ=0.04-0.78); the variation might be partly causative of differences in the interpretation of the presence of giant cells. Radiologically definite UIP associated with a poor survival. However, the histopathological UIP pattern or atypical features for IPF were not associated with survival.

In Study II, we focused on inorganic PM in 73 IPF lung tissue samples. We developed a semiquantitative scoring method (0-5) for coal dust pigment and inorganic PM using polarizing light microscopy. PM scores were compared to clinical, population density, and air quality data. An energy dispersive spectrometry with a field emission scanning electron microscope was used to analyze the elemental compositions of six IPF lung tissue samples. There were high scores of inorganic PM in the samples from southern Finnish university hospital districts compared to the samples from northern districts (31/50, 62% vs. 7/23, 30%, p=0.02). The highest scores of 4 and 5 were connected to an exposure to inorganic dust (n=15, p=0.004). Aluminum, silicon, and potassium were found in all six samples.

In Study III, we tested AI in the analysis of histopathological features in IPF samples. With 20 different IPF samples, we developed an AI model using a convolutional neural network in Aiforia® platform. The AI model was taught to recognize alveolar parenchyma from the lung tissue, fibroblast foci (FF), interstitial mononuclear inflammation, and intra-alveolar macrophages. The samples of 71 IPF patients were analyzed with the model. The high area of FF was associated with a poor survival ($p=0.01$), and we found that high amounts of interstitial mononuclear inflammation and intra-alveolar macrophages were associated with a prolonged survival ($p=0.01$ and $p=0.01$, respectively). FF and intra-alveolar macrophages also had a link with lung function. High numbers of FF were associated with a low diffusing capacity for carbon monoxide ($p=0.03$), whereas a high intra-alveolar macrophage density was associated with a high forced vital capacity of predicted ($p=0.03$).

In conclusion, FF seem to be the most potent single histological prognostic markers of survival in IPF. Of the other markers, inflammatory cells appeared to predict a prolonged survival. The interobserver agreement on the histopathological features of IPF varied, and especially the interpretation of giant cells seemed to cause a discrepancy. Inorganic PM in the lung tissue of IPF patients was not associated with the survival. Instead, the histological PM could reflect the level of exposure to air pollution. In the prognostic evaluation of the histopathological features in IPF lung tissue samples, AI could function as a future tool.

TIIVISTELMÄ (FINNISH ABSTRACT)

Idiopaattinen keuhkofibroosi (idiopathic pulmonary fibrosis, IPF) on huonoennusteinen krooninen keuhkoparenkyymisairaus. Vaikka se on harvinainen sairaus, se on silti yleisin idiopaattinen interstitiaalinen pneumonia. IPF:n ilmentymä radiologiassa ja histopatologiassa on usual intestitial pneumonia (UIP). Taudin alkuperä on tuntematon, mutta epäorgaaninen pöly on yksi tunnetuista riskitekijöistä. IPF diagnosoidaan yleensä kliinis-radiologisesti, mutta pieneltä osalta potilaista tarvitaan kirurginen keuhkokoepala. IPF:n diagnosointi voi olla vaikeaa, sillä monet keuhkoparenkyymisairaudet muistuttavat toisiaan. Radiologien ja patologien välinen yksimielisyys (interobserver agreement) vaihtelee. IPF:n erottaminen muista keuhkoparenkyymisairauksista on tärkeää, sillä tautien hoidot ja ennusteet ovat erilaisia.

Väitöskirjatutkimukseni hypoteesina oli, että IPF-potilaiden keuhkokudosnäytteiden histopatologiset piirteet ovat yhteydessä ennusteeseen. Lisäksi tutkimme patologien välistä yksimielisyyttä, epäorgaanista pölyä IPF-potilaiden keuhkokudosnäytteissä ja tekoälyn käyttöä IPF-potilaiden keuhkokudosnäytteiden arvioinnissa. Tutkimusaineiston potilaat kerättiin suomalaisesta IPF-rekisteristä, joka on prospektiivinen monikeskusrekisteritutkimus. Potilailta oli otettu keuhkokudosnäytteet, joiden histopatologisia piirteitä verrattiin potilaiden rekisteritietoihin.

Ensimmäisessä osatyössä neljä keuhkopatologiaan perehtynyttä patologia arvioi 60 näytettä uudelleen vuoden 2011 IPF:n diagnostisten kriteereiden mukaisesti. Lisäksi he tarkastelivat histopatologisia piirteitä, jotka ovat IPF:lle epätyypillisiä. Suurin osa näytteistä edusti tyypillistä UIP:tä (38/60, 63%). Yleisin IPF:lle epätyypillinen piirre oli runsas tulehdus (15/60, 25%). Patologien välinen yhtenevyys vaihteli lievästä merkittävään (κ=0.04-0.78), ja vaihtelu saattoi osittain johtua erilaisista tavoista tulkita näytteiden jättisoluja. Radiologinen UIP oli huonon ennusteen merkki. Sen sijaan histopatologinen UIP tai IPF:lle epätyypilliset histopatologiset piirteet eivät olleet yhteydessä ennusteeseen.

Toisessa osatyössä tutkittiin polarisoivalla valomikroskoopilla epäorgaanisten hiukkasten esiintymistä 73 IPF-potilaan keuhkokudosnäytteessä. Näytteiden hiilipigmenttiä ja epäorgaanisia hiukkasia arvioitiin polarisoivaa valomikroskooppia hyödyntäen tutkimusta varten kehitetyllä asteikolla (0-5). Hiukkaspitoisuuksia verrattiin kliinisiin, väestöntiheys- ja ilmanlaatutietoihin. Lisäksi energiadispersiivisellä spektrometrillä ja pyyhkäisyelektronimikroskoopilla tarkasteltiin kuuden näytteen hiukkasten alkuainekoostumusta. Eteläisten yliopistosairaaloiden erityisvastuualueilta peräisin olevista näytteissä oli korkeat hiukkasarvot verrattuna näytteisiin pohjoisilta erityisvastuualueilta (31/50, 62% vs. 7/23, 30%, p=0.02).

Korkeimmat pitoisuudet 4 ja 5 olivat yhteydessä epäorgaaniselle pölylle altistumiseen (n=15, p=0.004). Kaikista kuudesta näytteestä löytyi alumiinia, piitä ja kaliumia.

Kolmannessa osatyössä testattiin tekoälyä IPF-näytteiden histopatologisessa arvioinnissa. Aiforia®-alustan konvoluutioneuroverkolla kehitettiin 20 IPF-näytteellä tekoälymalli. Se opetettiin tunnistamaan keuhkokudoksesta ilmatilat, fibroblastifokukset, interstitiaalinen mononukleaarinen tulehdus ja intra-alveolaariset makrofagit. Mallilla analysoitiin 71 IPF-potilaan näytteet. Fibroblastifokukset olivat yhteydessä huonon ennusteeseen (p=0.01), kun taas interstitiaalinen mononukleaarinen tulehdus (p=0.01) ja intra-alveolaariset makrofagit olivat yhteydessä parempaan ennusteeseen (p=0.01). Fibroblastifokuksilla ja intra-alveolaarisilla makrofageilla oli myös yhteys keuhkojen toimintaan. Fibroblastifokukset liittyivät matalaan diffuusiokapasiteettiin (p=0.03), kun taas intra-alveolaariset makrofagit olivat yhteydessä korkeaan nopeaan vitaalikapasiteettiin (p=0.03).

Idiopaattisessa keuhkofibroosissa fibroblastifokukset ovat vahvasti yhteydessä huonoon ennusteeseen. Tulehdussolut saattavat olla merkki hyvästä ennusteesta. Patologien välinen yksimielisyys vaihteli, mikä saattoi johtua jättisolujen merkityksen erilaisista tulkinnoista. Kudoksessa havaittavat epäorgaaniset hiukkaset eivät olleet yhteydessä ennusteeseen, mutta ne saattavat kuvastaa altistumista ilmansaasteille. Tekoäly on mahdollinen tulevaisuuden työkalu arvioidessa ennustetta IPF-keuhkokudosnäytteistä.

ACKNOWLEDGEMENTS

This study was carried out at the Faculty of Medicine, University of Helsinki and Heart and Lung Center, Helsinki University Hospital. Personal financial support from the Foundation of the Finnish Anti-Tuberculosis Association, the Research Foundation of the Pulmonary Diseases, the Väinö and Laina Kivi Foundation, the University of Helsinki, the Biomedicum Helsinki Foundation, the Finnish Medical Society, and HUS Heart and Lung Center made this thesis possible.

I owe my sincerest gratitude to the supervisors of this thesis, Professor Marjukka Myllärniemi and Professor Riitta Kaarteenaho. When I was a medical student, Marjukka's lectures inspired me to embark on the field of research. I am thankful to Marjukka for this opportunity and all the guidance. To Riitta, who has taught me a great deal of pulmonary pathology, I am grateful for all the invaluable advice.

I am grateful to Professor Timo Paavonen and Adjunct professor Maritta Kilpeläinen, the reviewers of this thesis, for their time and thorough comments. My gratitude goes also to my steering committee, to Adjunct professor Mikko Mäyränpää and to PhD Hanna-Riikka Kreivi for their support. Also, I would like to thank Adjunct professor Mari Ainola for excellent comments on this thesis. I owe my gratitude to all of the people who have contributed to this work. Especially I want to thank Adjunct professor Mikko Mäyränpää, PhD Hanna-Kaisa Sihvo, and the staff of Aiforia. For the statistical help, I am grateful to Risto Bloigu, Anna But, and Paula Bergman. I also wish to thank our whole research group Lung Factor.

The support from friends has been invaluable during the thesis project, and I owe my sincerest gratitude to all of you. Seojong Joo and Tuuli Rautajoki, I am deeply grateful for our newfound friendship in the Haartman Institute. Anna Hartikainen, you are one of the dearest friends to me and I don't have the right words for describing how precious our friendship has been especially during this thesis project. Thank you for always being ready to listen, which is one of the finest skills that one can have. I also wish to thank Elena Reinistö, Hanna Savioja, and Heli Siitonen for the support.

To the Karvonen family, thank you for all the love and support. Especially, I owe my gratitude to my sister-in-law Lotta for proof-reading this thesis. My parents Anu and Vesa and my brother Tommi, I am indebted to you for the never-ending love and support. Tassu, Sissi, Jesse, Ansa, and Oiva, you have been the greatest mentors in life teaching me many lessons on the art of prioritizing. To my husband Konsta, I express all my love and gratitude for your support in this work and in life as well.

Vantaa, May 2021

Kati Mäkelä

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which will be referred to in the text by their Roman numerals:

I **Mäkelä K**, Hodgson U, Piilonen A, Kelloniemi K, Bloigu R, Sutinen E, Salmenkivi K, Rönty M, Lappi-Blanco E, Myllärniemi M and Kaarteenaho R. Analysis of the histologic features associated with interobserver variation in idiopathic pulmonary fibrosis. *Am. J. Surg. Pathol*. 2018;42:672-678.

II **Mäkelä K**, Ollila H, Sutinen E, Vuorinen V, Peltola E, Kaarteenaho R and Myllärniemi M. Inorganic particulate matter in the lung tissue of idiopathic pulmonary fibrosis patients reflects population density and fine particle levels. *Ann. Diagn. Pathol*. 2019;40:136-142.

III **Mäkelä K**, Mäyränpää M, Sihvo H-K, Bergman P, Sutinen E, Ollila H, Kaarteenaho R and Myllärniemi M. Artificial intelligence identifies inflammation and confirms fibroblast foci as a prognostic tissue biomarker in idiopathic pulmonary fibrosis. *Hum. Pathol.* 2021;107:58-68.

ABBREVIATIONS

α-SMA	alpha smooth muscle actin
AE	acute exacerbation
AI	artificial intelligence
BAL	bronchoalveolar lavage
CHP	chronic hypersensitivity pneumonitis
COP	cryptogenic organizing pneumonia
CNN	convolutional neural network
CTD-ILD	connective tissue disease-related ILD
DAD	diffuse alveolar damage
DIP	desquamative interstitial pneumonia
DLCO	diffusing capacity of the lung for carbon monoxide
ECM	extracellular matrix
FF	fibroblastic focus/foci
FVC	forced vital capacity
HE	hematoxylin and eosin
HP	hypersensitivity pneumonitis
HRCT	high-resolution computed tomography
IIP	idiopathic interstitial pneumonia
ILD	interstitial lung disease
IPF	idiopathic pulmonary fibrosis
MDD	multidisciplinary discussion
NSIP	non-specific interstitial pneumonia
OP	organizing pneumonia
PM	particulate matter
$PM_{2.5}$	particulate matter with aerodynamic diameter less than 2.5 μm
PM_{10}	particulate matter with aerodynamic diameter less than 10 μm
SEM	scanning electron microscope
SiO_2	silicon dioxide
SLB	surgical lung biopsy
TBLC	transbronchial cryobiopsy
TGF-β	transforming growth factor beta
Th	T helper
Treg	regulatory T cell
UIP	usual interstitial pneumonia
VATS	video-assisted thoracoscopic surgery

1 INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a rare, chronic lung disease in which connective tissue replaces the normal gas-exchanging lung tissue (Raghu et al. 2018a). The etiology of the disease remains elusive. Patients with IPF manifest often with dyspnea and dry cough. Without lung transplantation, IPF leads ultimately to respiratory failure and death. IPF is the most common of idiopathic interstitial pneumonias (IIP), which are interstitial lung diseases (ILD) without a known underlying cause. Compared to other ILDs, the prognosis of IPF is dismal: after the diagnosis, death is expected in only a few years (Bjoraker et al. 1998, King et al. 2001). However, the disease trajectory is heterogeneous and often unpredictable (Ley, Collard and King 2011). For the correct timing of antifibrotic treatment and lung transplantation for suitable patients, more information on prognostic markers of the disease is needed.

The diagnosis of IPF can be challenging as other ILDs might manifest similarly. The current diagnostic criteria for IPF were published in 2018 by the American Thoracic Society (ATS), the European Respiratory Society (ERS), the Japanese Respiratory Society (JRS), and the Latin American Thoracic Association (ALAT) (Raghu et al. 2018a). Characteristic for IPF is a patient aged over 60 years, with auscultation findings of bibasilar inspiratory crackles, and a radiological manifestation of bilateral pulmonary fibrosis (Raghu et al. 2018a). On a high-resolution computed tomography (HRCT), a usual interstitial pneumonia (UIP) pattern is seen; however, also other ILDs can manifest with UIP (Raghu et al. 2018a). In clinically and radiologically uncertain cases, a surgical lung biopsy (SLB) is recommended, and histopathological UIP supports the diagnosis of IPF (Raghu et al. 2018a). Still, a significant interobserver variation exists both in radiological and histopathological diagnosis (Hashisako et al. 2016, Walsh et al. 2016a). Separating IPF from other ILDs is essential as treatment strategies differ (Travis et al. 2013). In addition, expensive antifibrotic medication is indicated for IPF (Raghu et al. 2018a). Recent evidence suggests that also other progressive fibrosing ILDs benefit from antifibrotic medication (Distler et al. 2019, Flaherty et al. 2019, Maher et al. 2020, Wells et al. 2020). On the other hand, immunosuppressive treatment, which is mainly harmful to IPF patients (Raghu et al. 2012) but even curative in many other ILDs (Travis et al. 2013), should be avoided (Raghu et al. 2018a). A confident diagnosis of IPF will also predict a dismal disease trajectory. That is why an early evaluation of lung transplantation is more relevant than many other ILDs (Raghu et al. 2018a). Hence, it is necessary to improve the diagnostic criteria of IPF.

Despite active research, the full etiology and pathogenesis of IPF remain unsolved. One of the known risk factors for IPF is inorganic dust (Baumgartner et al. 2000). Still, efforts exist to expand the epidemiological evidence on the adverse effects of air pollution on IPF patients (Johannson 2018a). Occupational dust exposure causes the early onset of IPF and predicts a dismal prognosis for a patient with IPF (Lee et al.

2015). The histopathological evidence, however, on particulate matter in the lung tissue of IPF patients is scarce.

An increased number of fibroblast foci (FF) has been the only histopathological feature of IPF marking a poor prognosis that has been confirmed in multiple studies (King et al. 2001, Nicholson et al. 2002, Enomoto et al. 2006, Tiitto et al. 2006, Lee et al. 2011, Harada et al. 2013). Inflammatory cells are seen in the lungs of patients with IPF (Daniil et al. 2005, El-Zammar, Rosenbaum and Katzenstein 2009, Rabeyrin et al. 2015), yet their clinical significance and role in pathobiology are not clear (Heukels et al. 2019). Different histopathological features in tissue samples can be quantitated manually, but it is time-consuming and prone to intra- and interobserver variation. The development of artificial intelligence (AI) in image analysis offers a possibility for gathering quantitative information on the histopathological features. Developing automated image analysis methods in the analysis of IPF samples could ultimately work as a tool for pathologists. Meanwhile, it could also possibly help in decreasing the interobserver variation.

In this doctoral thesis, we analyzed a unique, national cohort of lung tissue samples of IPF patients whose diagnosis had been re-evaluated. Our goal was to recognize histopathological markers that could be utilized in the evaluation of disease progression. Using patient data from the FinnishIPF registry, we analyzed the histopathological features and compared them to the registry data and survival. In addition, we analyzed interobserver variation in the histopathological diagnosis of IPF. For the observation of inorganic dust in the lungs, we developed a novel scoring method with a polarizing light microscope. We also tested AI in the image analysis of the lung tissue samples of IPF patients.

2 REVIEW OF THE LITERATURE

2.1 INTERSTITIAL LUNG DISEASES

ILDs, or diffuse parenchymal lung diseases, are a heterogeneous disease group affecting the interstitium of the lung (Ryerson and Collard 2013). In the lungs, the interstitium is defined as the tissue adjacent to the parenchyma, i.e., alveolar spaces, including the alveolar epithelium, pulmonary capillaries, basement membranes, perivascular and perilymphatic tissue (Mukhopadhyay 2016). ILDs can be mainly categorized by known and unknown etiologies (Figure 1). A major group of ILDs with unknown etiology are known as idiopathic interstitial pneumonia (IIP) (Figure 2). Approximately one-tenth of ILD patients represent unclassifiable pulmonary fibrosis (Ryerson et al. 2013, Wijsenbeek and Cottin 2020). Some ILDs respond well to anti-inflammatory treatment, even leading to full recovery. In contrast, many progressive fibrosing ILDs inevitably lead to death without lung transplantation, and the prognosis is poorer than in many malignancies (George et al. 2020, Nasser et al. 2021). For years, IPF was the only disease for which antifibrotic medication was indicated (Raghu et al. 2018a). Other progressive fibrosing ILDs seem to benefit from the antifibrotic medication, as well (Distler et al. 2019, Flaherty et al. 2019, Maher et al. 2020, Wells et al. 2020).

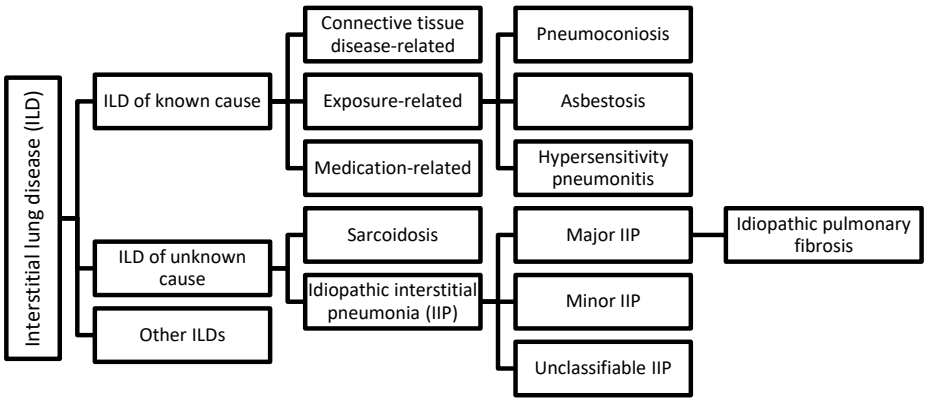


Figure 1 A flowchart representing the categorizations of interstitial lung diseases with an emphasis on idiopathic pulmonary fibrosis adapted from Ryerson and Collard 2013 and Kebbe and Abdo 2017.

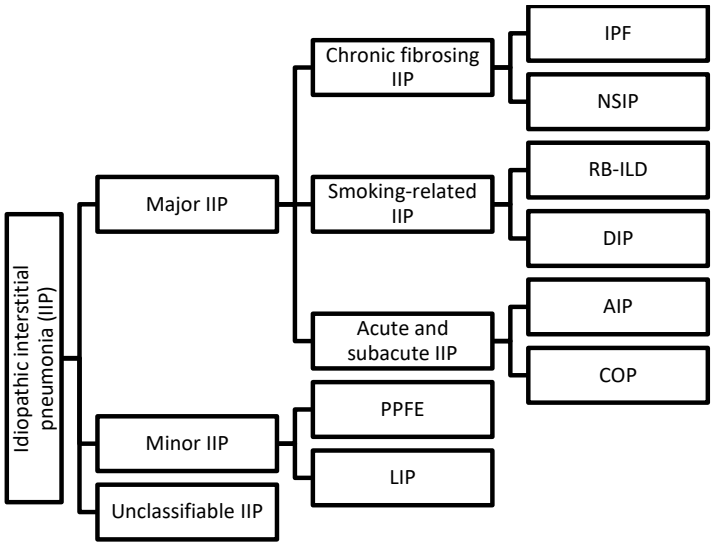


Figure 2 A flowchart representing the categorizations of idiopathic interstitial pneumonias adapted from Ryerson and Collard 2013 and Kebbe and Abdo 2017. IIP, idiopathic interstitial pneumonia; IPF, idiopathic pulmonary fibrosis; NSIP, non-specific interstitial pneumonia; RB-ILD, respiratory bronchiolitis-associated interstitial lung disease; DIP, desquamative interstitial pneumonia; AIP, acute interstitial pneumonia; COP, cryptogenic organizing pneumonia; PPFE, pleuroparenchymal fibroelastosis; LIP, lymphocytic interstitial pneumonia.

The most common fibrosing ILDs are IPF, connective tissue disease-related ILDs (CTD-ILD), and sarcoidosis (Ryerson et al. 2013, Wijsenbeek and Cottin 2020). The estimated prevalence for patients with ILDs is 76.0 per 100 000 people in Europe (Wijsenbeek and Cottin 2020). IPF is the archetype of progressive fibrosing ILDs, and of patients with other ILDs, only from 13% to 40% manifest with a progressive fibrosing disease (Wijsenbeek and Cottin 2020). Out of ILDs, idiopathic non-specific interstitial pneumonia (NSIP) and fibrotic hypersensitivity pneumonitis (HP)/chronic hypersensitivity pneumonitis (CHP) are the most significant differential diagnoses for IPF (Travis et al. 2013). In addition, out of pneumoconioses, asbestosis is a noteworthy differential diagnosis (Cullinan and Reid 2013). Out of CTD-ILDs, systemic sclerosis (SSc) is the most common disease behind the progressive pulmonary fibrosis (Wijsenbeek and Cottin 2020).

2.2 IDIOPATHIC PULMONARY FIBROSIS

2.2.1 DEFINITION AND CLINICAL PICTURE

IPF is a chronic and progressive IIP with the histopathological manifestation of the UIP pattern (Raghu et al. 2011). By definition, the etiology of the fibrosis is unknown

(Raghu et al. 2011). IPF affects only the lungs (Raghu et al. 2011) and the disease manifests typically in adults who are over 60 years old (Richeldi et al. 2014a, Behr et al. 2015, Kaunisto et al. 2019). Patients are more often men than women, and many have a history of smoking (Baumgartner et al. 2000, Richeldi et al. 2014a, Behr et al. 2015, Kaunisto et al. 2019).

The leading symptoms are similar to other pulmonary fibrosis: slowly progressing breathlessness, dyspnea, and desaturation, especially on exertion, and cough (Behr et al. 2015). Typically, the initial symptoms of the disease manifest approximately two years before the diagnosis of IPF (Lamas et al. 2011). In rare cases however, the first manifestation of the disease can be a quickly (in a matter of weeks) worsening dyspnea combined with typical radiological findings, i.e., the acute exacerbation of IPF (Collard et al. 2016). In clinical investigation, bibasilar inspiratory crackles can be heard, and many patients have clubbed fingers (Baughman et al. 1991, Behr et al. 2015). As the pulmonary fibrosis progresses, the disease leads to increasing symptoms and a decline in pulmonary function, with the inevitable end being respiratory failure and death if no lung transplantation is introduced (Kim, Perlman and Tomic 2015). However, the disease trajectory of IPF is heterogeneous (Kim, Perlman and Tomic 2015).

2.2.2 PREVALENCE AND INCIDENCE

The prevalence and incidence of IPF vary among studies. One explanation is that the uniform international diagnostic guidelines were only published quite recently, in 2011 (Raghu et al. 2011). In addition, some of the variation is explained by the differences in methodologies of re-evaluating IPF diagnoses. IPF shares ICD-10 diagnosis codes of J84.1 and J84.9 with other pulmonary fibrosis, and only 20-30% of the real-life patients with those codes have IPF (Kaunisto et al. 2015).

IPF is the most common IIP as 68% of IIP patients are diagnosed with IPF (Duchemann et al. 2017). From 12% to 21% of all ILD patients have IPF (Ryerson et al. 2013, Wijsenbeek and Cottin 2020). In Finland, the prevalence of IPF has been estimated to be 8.6 cases per 100 000 (Kaunisto et al. 2015). There is a great variation of the prevalence of IPF, namely the prevalence estimates of IPF have ranged from 1.25 to 63 per 100 000 in the general population (Nalysnyk et al. 2012). From 2000 onwards, the incidence of IPF has been estimated to be from three to nine cases per 100 000 in Europe and North America and from 0.4 to four cases per 100 000 in East Asia and South America (Hutchinson et al. 2015).

2.2.3 PROGNOSTIC FACTORS

IPF is a disease with a very dismal prognosis: the median survival is approximately four years (Bjoraker et al. 1998, King et al. 2001, Nathan et al. 2011). In Finland, the five-year survival rate is 45% and the annualized mortality rate is 13.1% (Kaunisto et al. 2019). The disease trajectory, however, can be very heterogeneous (Ley, Collard

and King 2011). From 20% to 30% of IPF patients have a stable disease course, and they live longer than five years without lung transplantation (Nathan et al. 2011, Kärkkäinen et al. 2019). On the other hand, one-third of the patients experience a very rapid progression of the disease, the survival without lung transplantation being less than two years (Nathan et al. 2011, Kärkkäinen et al. 2019). Approximately 20% of patients with IPF manifest with acute worsening periods, i.e., exacerbations (Song et al. 2011). It is hard to predict what kind of a disease trajectory an IPF patient will have since information on prognostic markers is limited.

2.2.3.1 Acute exacerbation

The acute exacerbation (AE) of IPF is defined as an acute respiratory event excluding cardiac failure and pulmonary edema that typically develops within 30 days and manifests with new bilateral ground-glass opacification and/or consolidation superimposed on the UIP pattern on HRCT (Collard et al. 2016). The AE can be triggered, e.g., by infection, a procedure subjected to airways or aspiration (Collard et al. 2016). Furthermore, it can be idiopathic when no known trigger is recognized (Collard et al. 2016). During the disease course of IPF, acute respiratory deterioration can occur at any stage of the disease, even as its first manifestation (Parambil et al. 2007, Song et al. 2011). High mortality rate is associated with the AE of IPF; the median survival is 2.6 months after hospitalization due to AE (Salonen et al. 2020b). The exact pathogenesis of AE remains obscure. Known risk factors for AE are low lung function values, procedures such as BAL, SLB or other surgery, mechanical ventilation, and secondary pulmonary hypertension (Song et al. 2011, Kondoh et al. 2015, Qiu, Chen and Ye 2018).

Histopathologically, AE of IPF is characterized by a diffuse alveolar damage (DAD) pattern that is an unspecific acute lung injury due to various reasons (Parambil, Myers and Ryu 2005, Kim et al. 2006, Kaarteenaho and Kinnula 2011). In addition to IPF, DAD can be seen superimposed on other ILD patterns (Churg et al. 2007). Severe damage of the alveolar epithelium and alveolar septal capillary endothelium triggers the acute, exudative phase of DAD (Mukhopadhyay 2016). If the acute phase of DAD does not lead to death, hyaline membranes will eventually be replaced by proliferation of fibroblasts and myofibroblasts, and alveolar septae thicken diffusely (Mukhopadhyay 2016). This next phase is known as the organizing, fibroproliferative phase of DAD. It can sometimes be hard to differentiate from UIP, but the critical element of DAD is the diffuse nature of its histopathological features (Mukhopadhyay 2016). OP can be seen superimposed on UIP in the samples of IPF patients experiencing AE (Parambil, Myers and Ryu 2005, Kim et al. 2006, Churg et al. 2007, Oda et al. 2014). In some samples of IPF patients with AE, UIP with extensive FF have been noted (Churg et al. 2007).

Histopathological features predictive of AE are not fully known. Previously, FF have been associated with a poor survival, but their count seem not to predict AE (Tiitto et al. 2006). In the study of 33 clinically stable IPF patients, small foci of alveolar

epithelium damage, cell debris, and intra-alveolar exudates were defined as minute lesions of alveolar damage (MLAD), precursors of DAD (Emura et al. 2015). Macrophages and neutrophils were adjacent to MLAD, and MLAD were often located near nodular granulation tissue and FF (Emura et al. 2015). The amount of MLAD correlated with AE and mortality (Emura et al. 2015). Recently, the presence of FF in SLB was shown to predict AE (Kishaba et al. 2020). Understanding the histopathological features preceding AE could elucidate the disease progression in IPF.

2.2.3.2 Clinicoradiological prognostic factors

Out of clinical factors, the predictors of rapid disease progression are low baseline values and fast decline in forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide (DLCO), older age, male gender, a long smoking history, the presence of comorbidities, a low body mass index, six-minute walk test values, the use of supplemental oxygen, the experience of dyspnea, and diagnostic delay (Collard et al. 2003, Lamas et al. 2011, Ley, Collard and King 2011, Kim, Perlman and Tomic 2015, Kärkkäinen et al. 2019).

In radiology, the definite UIP pattern, honeycombing, and traction bronchiectasis on HRCT have been shown to be markers of a poor prognosis (Sumikawa et al. 2014, Romei et al. 2015, Salisbury et al. 2017). However, there are also contradicting results on the prognostic effect of radiological honeycombing (Yamauchi et al. 2016, Kärkkäinen et al. 2019).

A validated serum biomarker specific for IPF disease progression is yet to be discovered. Promising results of several prognostic serum markers do already exist, especially on alveolar epithelial markers, such as Krebs von den Lungen-6 antigen (KL-6), surfactant protein A and D (SP-A and SP-D), and matrix metalloproteinase (MMP) (Inoue et al. 2020). Still, none of the serum biomarkers are specific for IPF (Inoue et al. 2020).

2.2.3.3 Histopathological prognostic factors

Compared to other histopathological ILD patterns, UIP is the strongest predictor of a poor prognosis (Bjoraker et al. 1998, Nicholson et al. 2000, Flaherty et al. 2003b). Sometimes the NSIP pattern can be seen in some of the biopsies of IPF patients (Monaghan et al. 2004). These patients' survival is equally poor than of the patients with only a UIP pattern in their biopsies (Monaghan et al. 2004). The UIP pattern associated with IPF marks a worse prognosis than the UIP pattern associated with CTD (Park et al. 2007, Moua et al. 2014, Strand et al. 2014). Besides the results by Hashisako et al. (2016) and our results presented in Study I, the prognostic effect of the histological UIP pattern by the 2011 (Raghu et al. 2011) or by the 2018 diagnostic UIP categories (Raghu et al. 2018a) is, to my knowledge, unknown.

The only morphological prognostic markers in the biopsies of IPF patients, that have been confirmed in several studies, are abundant FF. This is discussed in detail in section 2.4.2. Furthermore, the evidence on the prognostic role of interstitial mononuclear inflammation and intra-alveolar macrophages is discussed in depth in section 2.4.3. The prognostic effect of histopathological honeycombing has not been widely studied even though it is considered as a marker of end-stage pulmonary fibrosis. In SLBs, the amount of honeycombing had no association with the outcome of an IPF patient (Kim et al. 2020). On the other hand, honeycombing did not affect the prognosis of IPF patients in cryobiopsies either (Ravaglia et al. 2019b). Using the Elastica Van Gieson staining method, the amount of digitally annotated elastic fibers had an association with a poor survival, and an inverse correlation with FVC (Enomoto et al. 2013). However, a recent study presented high numbers of germinal centers to predict prolonged survival (Kim et al. 2020).

Some studies have been conducted on the prognostic immunohistochemistry markers in IPF. Tenascin-C is an extracellular matrix (ECM) protein associated with fibrotic disorders, and it has been associated with a dismal prognosis in IPF (Kaarteenaho-Wiik et al. 1996). Many markers of a poor prognosis are expressed in the lung epithelium, such as SP-A, protease-activated receptor 2 (PAR-2), mammalian target of rapamycin (mTOR), zinc finger E-box-binding homeobox 1 (ZEB1), and growth differentiation factor 15 (GDF15). The two latter can also be associated with the transforming growth factor beta (TGF-ß) pathway (Nagata et al. 2011, Park et al. 2013, 2014, Zhang et al. 2019a). A high expression of gremlin, which is an antagonist for a bone morphogenetic protein (BMP) that also participates in the TGF-ß pathway, correlates negatively with FVC (Myllärniemi et al. 2008). High alpha smooth muscle actin (α-SMA) expression and interleukin 4 (IL-4) in dense fibrosis areas have been linked with a poor survival (Waisberg et al. 2012). The high density of mast cells that are part of IgE mediated inflammation response has been shown to correlate with a slow decline in FVC (Cha et al. 2012).

2.2.4 TREATMENT

The treatment of IPF has evolved radically during the past decade. Even though antifibrotic medication has given hope for the patients with IPF, there is no curative treatment, apart from lung transplantation. IPF is one of the most common diseases leading to lung transplantation (Kistler et al. 2014). In the years 1990-2016, 108 of 241 (44%) Finnish lung transplant patients have had a pretransplant diagnosis of ILD (Halme 2017).

Before the era of antifibrotic medication, the target in treating IPF was to dampen the inflammation that was understood to drive the disease pathogenesis. In the 2000 consensus statement of IPF, a combination of corticosteroid and azathioprine or cyclophosphamide was recommended for selected patients (Idiopathic Pulmonary Fibrosis: Diagnosis and Treatment 2000). The combination of N-acetylcysteine (NAC)

to prednisone and azathioprine as a triple-therapy seemed to decrease the decline of FVC and DLCO than prednisone and azathioprine alone (Demedts et al. 2005). In the clinical trial PANTHER-IPF, the triple-therapy was compared to NAC alone and placebo (Raghu et al. 2012). The combination therapy group had a higher mortality rate, increased hospitalization, and more adverse effects than the placebo groups, without evidence of benefits (Raghu et al. 2012).

Currently, there are two antifibrotic medications indicated for IPF: pirfenidone and nintedanib. Neither of them is curative or superior to one other, and their main effect is slowing down the decline of FVC. Pirfenidone is a synthetic molecule of which antifibrotic and anti-inflammatory properties were shown in bleomycin-induced pulmonary fibrosis hamster models in the 1990s (Iyer et al. 1995, Iyer, Hyde and Giri 2000). In 2012, pirfenidone was approved in the European Union (EU) as it was shown to slow down the decline of FVC (Taniguchi et al. 2010, Noble et al. 2011). Nintedanib was first time introduced in 2009 as a novel angiogenesis inhibitor (Roth et al. 2009). Nintedanib inhibits three types of tyrosine kinase receptors: platelet-derived growth factor (PDGF) receptors, vascular endothelial growth factor (VEGF) receptors, and fibroblast growth factor (FGF) receptors (Roth et al. 2009, Wollin et al. 2014). In 2014, nintedanib was accepted in the EU, as it also was shown to slow down the decline of FVC (Richeldi et al. 2011, Richeldi et al. 2014b).

The exact effect of the antifibrotic medication in the lungs of IPF patients is not entirely understood. Both pirfenidone and nintedanib inhibit the proliferation of fibroblasts and myofibroblasts (Lehtonen et al. 2016) and reduce fibroblasts' transformation towards myofibroblasts (Epstein Shochet, Wollin and Shitrit 2018). In explant samples of IPF patients who have had prior antifibrotic treatment, less interstitial lymphocytic inflammation, DAD, and OP have been seen. However, no difference in the amount of fibrosis, FF, or the expression of senescence markers has been noted (Zhang et al. 2019b). Recently, SLB findings were compared between the responders and non-responders to nintedanib, and edematous changes in the interlobular septum were more frequently seen in biopsies of patients with a progressive disease (Nemoto et al. 2021). More understanding of the disease's pathobiology could be gained from further studies on the histopathological features associated with antifibrotic medication in the lungs of IPF patients.

2.2.5 ETIOLOGY AND RISK FACTORS

2.2.5.1 Intrinsic risk factors

Despite active research, the etiology of IPF remains elusive. In the light of current evidence, no single etiologic factor probably exists for IPF. The susceptibility for IPF increases significantly after the age of 60. Hence, it is likely that biological changes, which are a part of normal aging, are required for the clinical onset of IPF: cumulating DNA mutations, increasing oxidative and cell stress, dysfunction of mitochondria,

dysregulated apoptosis, telomere length dysfunction, loss of proteostasis, alveolar epithelial cell senescence, immune senescence, changes in epigenetics, stem cell exhaustion, and impaired communication between cells (Kapetanaki, Mora and Rojas 2013, Selman and Pardo 2014). Having a family history in pulmonary fibrosis is a major risk factor for the development of IPF (Steele et al. 2005, García-Sancho et al. 2011). Genetics play a significant, yet partly an obscure role in the risk for both familial and sporadic IPF. Several rare and common sequence variants have been associated with IPF (Adegunsoye, Vij and Noth 2019). A single-nucleotide polymorphism in a common sequence variant of glycoprotein, mucin 5B (MUC5B) has been strongly associated with IPF (Seibold et al. 2011, Fingerlin et al. 2013, Noth et al. 2013). Rare gene variants of TERT, TERC, PARN, RTEL1, and DKC1, that cause telomere shortening, have been associated with familial IPF (Armanios et al. 2007, Tsakiri et al. 2007, Kropski et al. 2014, Stuart et al. 2015). Also, gastroesophageal reflux disease (GERD) has been suggested to increase the risk for IPF (Johannson et al. 2017). IPF patients have higher exposure to gastroesophageal reflux and more frequent reflux episodes than controls (Savarino et al. 2013). In the biopsies of IPF patients, high numbers of airway-centered FF have been associated with airway-centered acute inflammation, peribronchiolar granulomas, and hiatal hernias, but not with GERD (Bois et al. 2016).

2.2.5.2 Extrinsic risk factors

In addition to intrinsic risk factors, extrinsic risk factors that trigger the disease are considered necessary for the development of IPF. Having a history of smoking has been shown to be a risk factor for IPF (Taskar 2006, Baumgartner et al. 2000, Abramson et al. 2020). The exact pathogenetic effect of smoking in IPF is elusive. One possible mechanism is that PM and free radicals in cigarette smoke increase profibrotic growth factors in the lung tissue, such as TGF-β, and shorten the telomeres (Church and Pryor 1985, Churg et al. 2006, Morlá et al. 2006). Herpesviruses, especially Epstein-Barr (EBV), herpes simplex 1, 6, 7, and 8 (HSV), and cytomegalovirus (CMV), adenovirus, hepatitis C virus (HCV), and Torque-Teno virus have been associated with IPF (Moore and Moore 2015). Out of bacteria, Streptococcus, Haemophilus, Neisseria, and Veillonella spp. have been more abundant in BAL fluids of IPF patients compared to controls (Molyneaux et al. 2014). Staphylococcus and Streptococcus bacteria, increased bacterial burden in BAL fluid, and host-microbiome interactions (Han et al. 2014, Molyneaux et al. 2014, Huang et al. 2017) have been associated with accelerated disease progression of patients with IPF (Han et al. 2014, Molyneaux et al. 2014, Huang et al. 2017).

2.2.5.3 Domestic and occupational dust

Despite the fact that the diagnosis of IPF is based on excluding known causes of ILDs, such as domestic, occupational, and environmental exposures, environmental dust is an acknowledged risk factor for IPF (Raghu et al. 2011). Especially metal dust

has been shown to increase the risk for IPF (Scott, Johnston and Britton 1990, Hubbard et al. 1996, Baumgartner et al. 2000, Koo et al. 2017, Paolocci et al. 2018). In addition, many other occupational risk factors for IPF exist, namely wood dust, sand dust, stone cutting or polishing, working in the metallurgical or steel industry, agricultural exposures, hairdressing, chemical fumes, and military exposures (Baumgartner et al. 2000, Taskar 2006, García-Sancho et al. 2011, Koo et al. 2017, Paolocci et al. 2018). Evidence for dose-dependent effects of occupational exposures has also been reported (Hubbard et al. 1996, Paolocci et al. 2018). In a large Korean study, any occupational exposure of an IPF patient seemed to cause an earlier onset of the disease and worsen the prognosis (Lee et al. 2015). Considering the incidence of IPF, metal dust has been confirmed as a risk factor in a recent multiple logistic regression analysis (Koo et al. 2017). IPF patients with known exposure to bird dust or mold have been reported to have better survival than IPF patients without those exposures, the overall survival being still worse than for CHP patients (Sadeleer et al. 2018). In a recent Australian IPF registry study, respirable dust and asbestos were associated with an increased risk for IPF (Abramson et al. 2020).

In a recent statement given by ATS and ERS, the occupational population attributable fraction (PAF), i.e., the proportional reduction that would occur if the occupational exposures were eliminated, was 26% for IPF patients (Blanc et al. 2019). In comparison, PAF values for asthma and chronic obstructive pulmonary disease were 16% and 14%, respectively (Blanc et al. 2019). For IPF, the odds ratios for exposures to vapors, gas, dust, fumes, metal dust, wood dust, and silica ranged from 1.7 to 2.0 (Blanc et al. 2019). However, agricultural exposure did not reach statistical significance (Blanc et al. 2019). The studies included in the statistical analysis were mainly before the 2011 diagnostic guidelines (Raghu et al. 2011). The accuracy of IPF diagnoses is uncertain: other ILDs and pneumoconioses could form a significant part of study populations. Furthermore, smoking, asbestos exposure, and gender are major confounding factors when analyzing dust exposure retrospectively. However, in a recent study with a study population of IPF patients with re-evaluated diagnoses, 8.4% of cases with IPF were estimated to be reduced by avoidance of respirable dust and asbestos (Abramson et al. 2020).

2.2.5.4 Air pollution

Particulate matter (PM) has been studied the most out of air pollutants, and it has been used as an indicator of overall exposure to air pollution (World Health Organization 2006). PM has different properties and sources depending on its size. Respirable, coarse particulate matter (PM_{10}) has a diameter below 10 µm; bigger particles are filtered in the upper airways (Mussalo-Rauhamaa et al. 2020). PM_{10} is the most widely reported air quality measure (World Health Organization 2006). The coarse PM is defined by a diameter between 2.5 µm and 10 µm. It originates mainly from mechanical activities such as construction, road dust re-suspension, and wind (Churg and Brauer 2000). Smaller particles are called fine particulate matter ($PM_{2.5}$); their diameter is below 2.5 µm, and $PM_{2.5}$ can reach the level of alveoli (Mussalo-

Rauhamaa et al. 2020). Fine particles are mainly produced in combustion sources (Mussalo-Rauhamaa et al. 2020). $PM_{2.5}$ and $PM_{0.1}$ are included in PM_{10} measurements (World Health Organization 2006). The acceptable levels of annual mean and 24-hour mean for PM_{10} are 20 µg/m^3 and 50 µg/m^3, and for $PM_{2.5}$, 10 µg/m^3 and 25 µg/m^3, respectively (World Health Organization 2006). However, no minimum threshold has been reported where no adverse health effects would exist, and adverse health effects have also been reported below these guidelines (World Health Organization 2006). Toxic elements such as cadmium, lead, or arsenic, and allergens can potentiate the detrimental effects of PM (Schraufnagel et al. 2019).

The underlying mechanisms seen behind the harmful effects of PM seem to be inflammation and endothelial dysfunction (Tamagawa et al. 2008). In BAL fluids of healthy subjects, exposure to $PM_{2.5}$ causes an influx of monocytes, oxidant radical generation, and an increase in an interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Schaumann et al. 2004). In a murine model of acute respiratory distress syndrome, exposure to $PM_{2.5}$ causes increased macrophages in BAL, interleukins 1β and 6, septal thickening, and decreased alveolar air space volume (de Souza Xavier Costa et al. 2020). However, the resolution of the induced acute lung injury was not by fibrosis as in the control group (de Souza Xavier Costa et al. 2020). There might be an inability to clear PM from the lungs, genetic changes, particularly in genes regulating the glutathione pathway and inflammatory mediators, and damage to epigenetics (Schraufnagel et al. 2019). Exposure to ambient elemental carbon increases the risk both for the development of interstitial lung abnormalities on CT, and also for the progression of the abnormalities (Rice et al. 2019).

There have been several epidemiological studies on the detrimental effects of air pollution on IPF. The reported evidence has been summarized in Table 1. Mortality has been investigated in two studies (Sesé et al. 2018, Winterbottom et al. 2018). The study conducted in France reported the association between PM and mortality (Sesé et al. 2018). In contrast, the American study did not report the association between PM and mortality (Winterbottom et al. 2018). The difference in results could partly be explained by the lower PM levels in Pennsylvania compared to France; a threshold level of PM exposure might exist. In addition, Winterbottom et al. (2018) showed an association between the rate of FVC% decline and PM_{10} but not with $PM_{2.5}$. Johannson et al. (2018b) reported a connection only to low FVC% but not with FVC% decline, the latter being probably explained by a small number of patients (N=25).

In the lung tissue, inorganic PM can be inspected with polarizing light microscopy due to its birefringence. With scanning electron microscopy (SEM) combined with electron dispersive spectrometry, the size, structure, and elemental composition can be analyzed. The evidence for PM in the fibrotic lungs is limited, while histopathological studies on PM in healthy lungs are scarce.
PM concentration seems to correlate inversely with the size of aiways; the concentration of PM is highest in small airways (Churg and Brauer 2000). UIP

pathology is also the most prominent in the peripheral parts (Raghu et al. 2018a). In forensic autopsy samples of patients without a respiratory disease, inorganic PM was noted mainly adjacent to fibrotic lesions (Pinkerton et al. 2000, Schenker et al. 2009). Dust deposits can be seen relatively often in IPF samples (Rabeyrin et al. 2015). IPF lung tissue has been observed to contain more inorganic PM than control samples (Tsuchiya et al. 2007). Silicon (Si) and aluminum (Al) have been reported to exist in high amounts in IPF lung tissue (Monso et al. 1990, Tsuchiya et al. 2007) and in pulmonary lymph nodes (Kitamura et al. 2007). Mediastinal lymph node enlargement on HRCT has been associated with a dismal prognosis in IPF, and the enlargement of lymph nodes might reflect an increased immunological response to environmental exposures (Sin et al. 2018).

Table 1 The reported associations between different air pollutants and idiopathic pulmonary fibrosis.

Pollutant	Effect on IPF patients
NOx	Acute exacerbations (Johannson et al. 2014, Sesé et al. 2018)
NO_2	Chronic exposure leads to increase in IPF incidence (Conti et al. 2018)
	Lower FVC (Johannson et al. 2018b)
	Increased hospitalization (Dales, Blanco-Vidal and Cakmak 2020)
O_3	Acute exacerbations (Johannson et al. 2014, Sesé et al. 2018)
PM_{10}	Increased IPF mortality (Sesé et al. 2018)
	Increased rate of FVC decline (Winterbottom et al. 2018)
	Lower FVC (Johannson et al. 2018b)
	Increased hospitalization (Dales, Blanco-Vidal and Cakmak 2020)
$PM_{2.5}$	Increased IPF mortality (Sesé et al. 2018)
	Increase in the use of supplemental oxygen in 6MWT (Winterbottom et al. 2018)
	Lower FVC (Johannson et al. 2018b)

IPF, idiopathic pulmonary fibrosis; NOx, nitrogen oxides; NO_2, nitrogen dioxide; O_3, ozone; $PM_{10,}$ particulate matter of diameter below 10 μm; $PM_{2.5}$, particulate matter of diameter below 2.5 μm; FVC, forced vital capacity; 6MWT, six-minute walk test.

2.3 DIAGNOSIS OF IDIOPATHIC PULMONARY FIBROSIS

2.3.1.1 The evolution of the diagnostic criteria

First in 1838, a rare disease called “cirrhosis of the lung” was introduced in the medical literature (Corrigan 1838). In 1933, Hamman and Rich (1944) described “acute diffuse interstitial fibrosis of the lungs” with clinicopathological features resembling acute respiratory distress syndrome or DAD. Some patients probably represented AIP instead of chronic pulmonary fibrosis (Hamman and Rich 1944). Liebow and Carrington introduced the UIP pattern, which got its name for being simply

more common than the other histopathological patterns (Liebow and Carrington 1969). Terms of UIP, IPF, and cryptogenic fibrosing alveolitis were intermingled and used for many other IIPs. In the beginning of the 1990s, the development of HRCT had provided a much more straightforward way to analyze ILD patients radiologically (Tung et al. 1993, Orens et al. 1995). Katzenstein and Myers (1998) formed the basis of IPF diagnosis today, as only the UIP pattern was stated to be the manifestation of IPF.

The first international consensus statement on the diagnosis and treatment of IPF was published in 2000 (Idiopathic Pulmonary Fibrosis: Diagnosis and Treatment 2000). SLB was recommended to be taken from all patients with suspected IPF who had any atypical features for IPF (Idiopathic Pulmonary Fibrosis: Diagnosis and Treatment 2000). A couple of years later, the differentiation of other IIPs from IPF was emphasized by the publication of the ATS/ERS consensus statement on IIP (American Thoracic Society/European Respiratory Society 2002). In 2011, the first evidence-based guidelines for diagnosing and managing IPF were made in collaboration with ATS, ERS, JRS, and ALAT (Raghu et al. 2011). The categorizations by the level of confidence on UIP are shown in Table 3 and 4. In 2017, the Fleischner Society published a systematic review on the updated diagnostic criteria of IPF, of which histopathological and radiological categorizations are shown in Table 3 and 4 (Lynch et al. 2018). A crucial update was the statement that a patient with clinical IPF characteristics could have a definite IPF diagnosis with the probable UIP pattern on HRCT without a SLB. In 2018, ATS/ERS/JRS/ALAT published the currently used update on diagnostic guidelines of IPF shown in Table 3 and 4 (Raghu et al. 2018a). The histopathological and radiological categorization were very similar to the Fleischner Society guidelines (Table 3 and 4). Only minor disagreement exists between the two recent updates. In the criteria by the Fleischner Society, there is a stronger statement against taking a SLB when honeycombing is missing on HRCT (probable UIP) (Lynch et al. 2018).

2.3.1.2 The current diagnostic criteria

When an over 60-year-old patient has unexplained radiological bilateral pulmonary fibrosis, that is either symptomatic or asymptomatic and bibasilar inspiratory crackles on auscultation, IPF should be suspected (Raghu et al. 2018a). For the diagnosis of IPF, two of three requirements need to be fulfilled:

1) Excluding known causes of ILD, including domestic and occupational exposures, CTD, and pneumotoxic medication

AND

2) The UIP pattern on HRCT

OR when a lung biopsy is performed

3) An appropriate combination of histopathological and HRCT patterns of UIP (Raghu et al. 2018a).

A detailed medication history is necessary with a focus on pneumotoxic drugs, such as nitrofurantoin, methotrexate, cyclophosphamide, and chemotherapeutic drugs (Raghu et al. 2018a). Systematic questionnaires are recommended to evaluate environmental exposures (Raghu et al. 2018a). Serological testing, including C-reactive protein, erythrocyte sedimentation rate, antinuclear antibodies, rheumatoid factor, myositis panel, and anti-cyclic citrullinated peptide, is recommended by the majority of the panelists of the current guidelines (Raghu et al. 2018a). As lung disease might be the first or dominating feature of CTD, symptoms associated with CTD should be taken into account during the follow-up (Raghu et al. 2018a). Cellular analysis of bronchoalveolar lavage (BAL) fluid is recommended for patients that do not have the definite UIP pattern on HRCT, mainly due to the impact of differential diagnosis of eosinophilic pneumonia, cryptogenic organizing pneumonia (COP), and sarcoidosis (Raghu et al. 2018a). Even though BAL analysis cannot differentiate IPF from other ILDs, a BAL fluid of an IPF patient often has neutrophilia and eosinophilia and lacks lymphocytosis (Ryu et al. 2007, Raghu et al. 2018a).

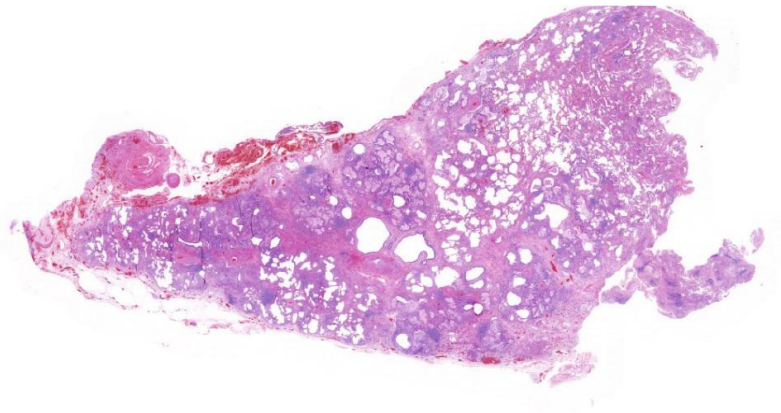


Figure 3 A representative biopsy of the usual interstitial pneumonia pattern.

The radiological and histopathological criteria are shown in Table 3 and 4. A combination of HRCT and histopathological patterns are added into the diagnostic algorithm that is shown in Table 2. Multidisciplinary discussion (MDD) is the gold standard in the diagnosis of IPF: IPF can be diagnosed or excluded by the combination of HRCT and histopathological patterns (Raghu et al. 2018a). Interobserver agreement on IPF diagnosis between multidisciplinary teams has been reported to range from fair to moderate (Walsh et al. 2016b).

In histopathology, UIP is characterized by a dense fibrosis of spatial heterogeneity (Figure 3); fibrotic tissue is intermingled with less affected or normal lung parenchyma (Smith et al. 2013, Raghu et al. 2018a). Fibrosis distorts the lung architecture (Smith et al. 2013, Raghu et al. 2018a). Temporal heterogeneity is also seen; FF, that are foci of active, “new,” and loose fibrosis, co-exist with eosinophilic, dense, and “old”

fibrosis (Smith et al. 2013, Raghu et al. 2018a). The UIP pathology is seen in the lungs' subpleural and paraseptal parts (Smith et al. 2013, Raghu et al. 2018a). The lung's remodeling leads into honeycomb cysts that are not a requirement for the definite UIP pattern (Smith et al. 2013, Raghu et al. 2018a). Honeycomb changes are fibrotic, cystic airspaces filled with mucus and inflammatory cells, often lined by bronchiolar epithelium (Smith et al. 2013, Raghu et al. 2018a). Honeycombing is not specific to UIP (Mukhopadhyay 2016). Smooth muscle metaplasia often localizes in fibrotic areas and adjacent to honeycombing (Smith et al. 2013, Raghu et al. 2018a). Similar to honeycombing, peribronchiolar metaplasia has the lining of bronchiolar epithelium without forming cysts (Smith et al. 2013, Raghu et al. 2018a). Marked interstitial inflammation and lymphoid hyperplasia suggests CTD-UIP, while centrilobular and bridging fibrosis, peribronchiolar metaplasia, granulomas, and giant cells indicate CHP (Smith et al. 2013, Raghu et al. 2018a). However, the specificity of the features is low, and MDD is needed to differentiate the UIP pattern secondary to IPF from other ILDs (Smith et al. 2013, Raghu et al. 2018a). The categorizations presented in the most recent guidelines do not overrule the traditional histopathological diagnosis (Smith et al. 2020). The current guidelines are designed to be used in the clinical suspicion of IPF, not in the suspicion of other ILDs (Lynch et al. 2018, Raghu et al. 2018a).

Table 2 The diagnostic algorithm for idiopathic pulmonary fibrosis taking into account the high-resolution computed tomography and histopathology pattern, adapted and modified from Raghu et al. 2018a.

	HRCT pattern				
Histopathological pattern		**UIP**	**Probable UIP**	**Indeterminate for UIP**	**Alternative diagnosis**
	UIP	IPF	IPF	IPF	Likely IPF*/non-IPF
	Probable UIP	IPF	IPF	Likely IPF*	Non-IPF
	Indeterminate for UIP	IPF	Likely IPF*	Indeterminate§	Non-IPF
	Alternative diagnosis	Non-IPF	Non-IPF	Non-IPF	Non-IPF

*Likely idiopathic pulmonary fibrosis (IPF), when at least one of the features are seen: marked traction bronchiectasis/bronchiolectasis in a man aged over 50 years or in a woman aged over 60 years, extensive reticulation on high-resolution computed tomography an over 70-year-old patient, increased neutrophils or absence of lymphocytosis in bronchoalveolar lavage fluid, multidisciplinary discussion (MDD) leads to confident IPF diagnosis.
§Indeterminate, unlikely to be IPF without a biopsy, and a biopsy might suggest a more specific diagnosis after MDD.

Table 3 The histopathological 2011 criteria by the American Thoracic Society (ATS), the European Respiratory Society (ERS), the Japanese Respiratory Society (JRS), and the Latin American Thoracic Association (ALAT), the 2018 criteria by the Fleischner Society, and the 2018 criteria by ATS/ERS/JRS/ALAT.

Raghu et al. 2011	Lynch et al. 2018	Raghu et al. 2018
UIP • Fibrosis/architectural distortion with or without honeycombing mainly subpleurally/ paraseptally • Patchiness of fibrosis • Fibroblast foci • No features suggesting a different diagnosis	**Definite UIP-IPF** • Architectural distortion caused by dense fibrosis with honeycombing subpleurally/paraseptally • Patchiness of fibrosis • Fibroblast foci adjacent to dense scars • No features suggesting a different diagnosis	**UIP** • Architectural distortion and dense fibrosis with or without honeycombing mainly subpleurally/ paraseptally • Patchiness of fibrosis • Fibroblast foci • No features suggesting a different diagnosis
Probable UIP • Fibrosis/architectural distortion with or without honeycombing • Either fibroblast foci or patchiness of fibrosis missing • No features suggesting a different diagnosis OR • Only honeycombing	**Probable UIP-IPF** • Fibrosis/architectural distortion with honeycombing • Patchiness of fibrosis • Fibroblast foci might be missing • No features suggesting a different diagnosis OR • Only honeycombing	**Probable UIP** • Features from "UIP" but not all • No features suggesting a different diagnosis OR • Only honeycombing
Possible UIP • All three criteria: patchy or diffuse fibrosis with or without interstitial inflammation, UIP pattern features are absent, features favoring non-UIP pattern are absent	**Indeterminate for UIP-IPF** • Presence of fibrosis with features favoring more non-UIP pattern or UIP pattern associated with other ILD than IPF • Features most consistent with an alternative diagnosis are not prominent	**Indeterminate for UIP** • Presence of fibrosis with or without architectural distortion, features favoring non-UIP pattern or UIP pattern associated with other ILD than IPF • Definite UIP but with overlapping features suggesting an alternative diagnosis
Not UIP Pattern • Any of the features seen prominently: • Hyaline membranes without acute exacerbation • Organizing pneumonia, mild changes are allowed • Granulomas, single and rare are allowed • Interstitial inflammation away from honeycombing • Airway centered changes, mild changes are allowed • Other features strongly associated with other ILDs	**Features most consistent with an alternative diagnosis** • UIP pattern and features strongly associated with an alternative diagnosis or non-UIP pattern including features from other ILDs	**Alternative Diagnosis** • Features associated with other ILDs

Table 4 The radiological 2011 criteria by the American Thoracic Society (ATS), the European Respiratory Society (ERS), the Japanese Respiratory Society (JRS), and the Latin American Thoracic Association (ALAT), the 2018 criteria by the Fleischner Society, and the 2018 criteria by ATS/ERS/JRS/ALAT.

Raghu et al. 2011	Lynch et al. 2018	Raghu et al. 2018
UIP • Subpleural and basal predominance • Reticularity • Honeycombing with or without traction bronchiectasis • Absence of features inconsistent with the UIP pattern	**Typical UIP** • Subpleural and basal predominance of fibrosis • Often heterogeneous distribution • Honeycombing with or without traction bronchiectasis in periphery or bronchiolectasis, possibly superimposed on ground-glass opacities • Absence of features suggestive an alternative diagnosis	**UIP** • Subpleural and basal predominance of fibrosis • Often a heterogeneous distribution • Honeycombing with or without traction bronchiectasis in periphery or bronchiolectasis
Possible UIP • Subpleural and basal predominance • Reticularity • No honeycombing • Absence of features inconsistent with the UIP pattern	**Probable UIP** • Features from "typical UIP" without honeycombing • Reticular pattern, possibly superimposed on ground glass opacities •	**Probable UIP** • Features from "UIP" without honeycombing • Reticular pattern • Mild ground-glass opacities possible
	Indeterminate for UIP • Variable or diffuse distribution of fibrotic changes • Some mild features associated with non-UIP pattern	**Indeterminate for UIP** • Subpleural and basal predominance • "Early UIP pattern": mild reticulation with mild ground-glass opacities or distortion • Features or distribution of fibrosis that are indeterminate for etiology
Not UIP Pattern • Upper- or mid-lung predominance • Peribronchovascular predominance • Extensive ground-glass opacities • Extensive micronodules • Cysts away from honeycombing areas • Marked mosaic attenuation • Predominant consolidations	**Features most consistent with non-IPF diagnosis** • Upper- or mid-lung predominance • Predominant consolidations • Extensive ground-glass opacities without acute exacerbation • Marked mosaic attenuation and marked lobular air-trapping • Diffuse nodules or cysts	**Alternative Diagnosis** • Features associated with other ILDs

2.3.1.3 Lung biopsies

For IPF suspected patients, SLB is conditionally recommended for confirming the diagnosis when the HRCT pattern is non-definite for UIP (Raghu et al. 2018a). The diagnosis of IPF has been based on SLB in 25% of cases and on transbronchial cryobiopsy (TBLC) in 11% of cases (Pannu et al. 2019). Video-assisted thoracoscopic surgery (VATS) is the preferred technique in performing SLB (Raghu et al. 2018a). Regarding other types of histopathological confirmation, the guideline panel made no recommendations of taking transbronchial lung biopsies (TBB) or TBLCs from suspected IPF patients with a non-definite UIP pattern due to very low quality of evidence (Raghu et al. 2018a). TBB can be more suitable for suspected ILD patients whose lungs have a pathology affecting central parts of the lungs, such as sarcoidosis (Raj et al. 2017).

The diagnostic yield of SLB is high, ranging from 75% to 99% (Sigurdsson et al. 2009, Kayatta et al. 2013, Fibla et al. 2015, Ravaglia et al. 2016, Tomassetti et al. 2016). The benefits and risks of SLB for an IPF patient are shown in Table 5. The procedure-related mortality ranges from 0% to 2.7% (Qureshi et al. 2002, Ravaglia et al. 2016). In a recent large study, the overall 30-day mortality associated with SLB was 7.1% (Fisher et al. 2019). The highest mortality of 20.2% was associated with non-elective SLBs, whereas the mortality was only 1.9% associated with elective SLBs (Fisher et al. 2019). Other risk factors for mortality after SLB are AE, mechanical ventilation, low DLCO, old age, male sex, supplemental oxygen, immunosuppression, OLB, and the low yearly SLB volume at the hospital (Park et al. 2007, Fibla et al. 2012, Kayatta et al. 2013, Fisher et al. 2019). In another recent study, no mortality or AEs were reported within 90 days of SLB, which is partly explained by the avoidance of the above-mentioned risk factors (Nagano, Miyamoto and Kikunaga 2021).

Table 5 The benefits and risks of a surgical lung biopsy for patients having idiopathic pulmonary fibrosis (Raj et al. 2017, Raghu et al. 2018a).

Benefits	Risks
Definite diagnosis	Over-all mortality (3.5%)
Antifibrotic medication indicated	SLB-related mortality (1.7%)
Avoidance of immunosuppression	Acute exacerbation (6.1%)
Accurate estimation of prognosis	Respiratory infection (6.5%)
Early evaluation of the possibility to lung transplantation	Pneumothorax (5.9%)
	Bleeding (0.8%)
Cessation of additional diagnostic testing	Neuropathic pain (4.5%)
Integrating early palliative treatment	Delayed wound healing (3.3%)

As SLB bears fatal risks, TBLC offers a tempting option for a less invasive histopathological confirmation. The diagnostic yield ranges from 74% to 98% (Fruchter et al. 2014, Pajares et al. 2014, Ravaglia et al. 2016, Tomassetti et al. 2016, Ravaglia et al. 2019a). The overall mortality after TBLC seems to be smaller than in SLB (Ravaglia et al. 2016, Tomassetti et al. 2016, Ravaglia et al. 2019a).

Histopathological findings of TBLCs and SLBs have been compared from the same patient. In one study, the interobserver agreement on the findings of TBLC and SLBs was fair, and 38% of the diagnoses between TBLC and SLB were fully concordant (Romagnoli et al. 2019). In another larger study, TBLC led to a high or definite diagnostic confidence in 60% of cases, and the agreement between TBLC and SLB was 71% (Troy et al. 2020). UIP features except for honeycombing were mainly found both TBLC and SLB (Zaizen et al. 2019). Diffuse lesions might be missed in TBLC compared to SLB (Zaizen et al. 2019). SLB after TBLC seems to be beneficial when the diagnosis after TBLC is still indeterminate, or the NSIP pattern is suspected (Bondue et al. 2020).

2.3.1.4 Interobserver variation

In the diagnosis of IPF, interobserver variation both among radiologists and pathologists is an acknowledged issue. In research, the interobserver agreement is commonly measured with Cohen's κ coefficient (Landis and Koch 1977). Its values are categorized as follows: <0.00, poor, below the agreement that would be expected "by chance"; 0.00 to 0.20, slight; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; and 0.81 to 1.00, excellent, the value of 1 representing perfect agreement. Moderate agreement between observers has been suggested as the minimum for clinical use of a diagnostic test (McHugh 2012).

The clinicoradiological data alone is sufficient for the diagnosis of most IPF patients. Hence, HRCT interpretation should be as repeatable as possible. In studies preceding the 2011 guidelines (Raghu et al. 2011), the interobserver agreement among thoracic radiologists has been reported to vary from fair to substantial (Aziz et al. 2004, Lynch et al. 2005, Thomeer et al. 2008). In a multicenter study of 112 radiologists by the 2011 diagnostic guidelines (Raghu et al. 2011), the interobserver agreement for UIP on HRCT was moderate (Walsh et al. 2016a). The experience of radiologists or using a binary categorization of the UIP pattern ("UIP" vs. "possible or inconsistent with UIP") did not affect the agreement (Walsh et al. 2016a). Also, in another multicenter study conducted by the 2011 diagnostic guidelines (Raghu et al. 2011), the interobserver agreement among radiologists on IPF/UIP was moderate (Walsh et al. 2016b). However, the agreement was high compared to the agreement regarding HRCTs of NSIP, CTD-ILD, or HP (Walsh et al. 2016b). The limited repeatability of radiological evaluation should be kept in mind when considering performing SLB.

SLBs are taken from patients whose diagnoses remain uncertain with only clinicoradiological information. Multiple studies have tested the interobserver agreement among pathologists on the presence of the UIP pattern. Only a few studies have been conducted by the 2011 diagnostic guidelines (Raghu et al. 2011), and to my knowledge, only one study has presented any results of the interobserver agreement between pathologists by the current 2018 guidelines (Raghu et al. 2018a). Studies on the interobserver agreement on the UIP pattern are listed in Table 6. In

studies conducted before the 2011 guidelines, the interobserver agreement on UIP has varied from slight to substantial (Hunninghake et al. 2001, Nicholson et al. 2004, Collard et al. 2007, Thomeer et al. 2008, Leslie et al. 2012). In studies where more than two pathologists have evaluated the cases, interobserver agreement has not improved after the implementation of the 2011 guidelines (Hunninghake et al. 2001, Nicholson et al. 2004, Leslie et al. 2012, Hashisako et al. 2016, Walsh et al. 2016b, Mäkelä et al. 2018). After the 2011 guidelines, it seems possible to reach substantial agreement only when the presence of the UIP pattern has been evaluated in a binary manner either as “UIP” or “not UIP” (Tomassetti et al. 2012, Casoni et al. 2014, Tomassetti et al. 2016). The pathologists have been the same in the above-mentioned studies, highlighting the effect of previous collaboration on the agreement. In a recent study using the 2018 guidelines (Raghu et al. 2018a), a substantial agreement on the UIP pattern in SLBs was presented between three blinded pathologists, and only moderate agreement for TBLCs taken from the same patients was shown (Troy et al. 2020).

In studies in which every κ coefficient value has been measured between multiple pathologists, instead of only one overall κ value, the level of agreement seems to be highly variable, ranging from poor to substantial (Leslie et al. 2012, Hashisako et al. 2016, Mäkelä et al. 2018). Categorizing the four diagnostic categories of the 2011 guidelines (Raghu et al. 2011) into binary scores, such as “UIP and probable UIP” vs. “indeterminate for UIP and not UIP,” seems to increase the interobserver agreement a little but not to have a more significant effect (Hashisako et al. 2016, Mäkelä et al. 2018). A weighted κ coefficient can also be used to measure interobserver agreement, and it takes account of the varying levels of disagreement between categories (Tang 2015). In the study of Walsh et al. (2016b), the unweighted κ coefficient of 0.46 on the interobserver agreement on IPF increased to 0.58 after weighting. Other factors that increase the interobserver agreement among pathologists are biopsies from multiple lobes, and taking into account the level of confidence of first-choice ILD diagnosis (Nicholson et al. 2004). Nearly all of the studies in Table 6 have been conducted in a blinded setting or only with limited clinical data available, such as age, sex, and smoking status; this does not reflect the real-life situation where pathologists have the multidisciplinary data available and are able to consult each other. In studies in which some or all clinical information were offered for pathologists, the interobserver agreement has not been remarkably higher than in studies with a blinded setting (Nicholson et al. 2004, Walsh et al. 2016b, Jo et al. 2019).

The interobserver agreement on specific histopathological features has been less studied. Neither has the impact of the specific features on the interobserver agreement been examined in many studies. In a study of Cherniack et al. (1991b) conducted before the 2000 consensus statement on IPF (Idiopathic Pulmonary Fibrosis: Diagnosis and Treatment 2000), the interobserver agreement on specific histopathological features, such as interstitial fibrosis, honeycombing, alveolar space cellularity, and mural inflammation, ranged from poor to fair, while the percentage of

absolute agreement on scoring the features from 0 to 5 being approximately 50-60% (Cherniack et al. 1991b). In the study of Leslie et al. (2012), the agreement on specific features varied from poor to substantial. The agreement on FF was substantial between all pathologists. Contrarily, the agreement varied from poor to moderate on honeycombing and subpleural, peripheral accentuation of fibrosis, and was poor or slight on diffuse alveolar septal fibrosis, diffuse chronic inflammation, and smooth muscle hyperplasia. In the study of Yagihashi et al. (2016), several histopathological features of 100 cases were evaluated to find an explanation for the radiological-pathological discordance. The agreement ranged from poor (κ of -0.04 for granulomas) to fair (κ of 0.34 for peribronchial metaplasia). The agreement on giant cells was fair (κ =0.29). In TBLCs, interobserver agreement on honeycombing, FF, and patchy fibrosis were fair or moderate (Casoni et al. 2014). Recently, only slight agreement was reported on honeycombing and FF, while agreement ranged from slight to fair on hyaline membranes, OP, granulomas, airway inflammation, and airway fibrosis (Jo et al. 2019). The agreement on the degree of inflammation in IPF samples seems to be low in several studies (Cherniack et al. 1991b, Leslie et al. 2012, Yagihashi et al. 2016). Altogether, the agreement on specific histological features seems to be more varied than on the level of confidence of the UIP pattern, which possibly explains the low agreement to some extent.

MDD is important when diagnosing IPF. When the interobserver agreement on UIP histopathology is high, it might be able to predict prognosis (Hashisako et al. 2016). In a large multicentre study, clinicians, radiologists, and pathologists made independent first-choice diagnoses of 70 patients using the 2013 IIP diagnostic criteria (Travis et al. 2013, Walsh et al. 2016b). Seven pathologists knew only the age, sex, and smoking history of the patient (Walsh et al. 2016b). The overall κ value between pathologists for 22 IPF patients was 0.46, moderate, being higher than for other ILD diagnoses NSIP, CTD-ILD, and HP, with κ values of 0.23, 0.22, and 0.20, respectively (Walsh et al. 2016b). The confidence for the first-choice diagnosis of IPF was 0.59 for clinicians and 0.46 for radiologists; the agreement on the multidisciplinary team diagnosis on IPF was 0.60 (Walsh et al. 2016b). On non-biopsied IPF patients, the agreements between seven multidisciplinary teams were slightly better than for biopsied patients (κ values of 0.70 and 0.60, respectively). The result reflects that only diagnostically challenging patients are biopsied, which decreases the interobserver agreement between clinicians, radiologists, and pathologists. In addition, the interobserver agreement seems to be better on IPF than on other IIPs, resulting from the well-defined diagnostic criteria of IPF in comparison to other ILDs.

Table 6 Study results on interobserver agreement among pathologists on the usual interstitial pneumonia pattern.

Study	Samples	Multicenter study	Blinded	Pathologists	Diagnostic criteria	κ value
Hunninghake 2001	91 SLBs (ILD)	8 centers	Yes	3	Katzenstein 1998	0.68
Nicholson 2004	83 SLBs (ILD)	No	Age, sex, site of biopsy known	10	2002 IIP criteria	0.42
Collard 2007	56 SLBs (IPF)	No	Yes	2	2000 IPF criteria	0.37
Thomeer 2008	82 SLBs (IPF)	European trial (IFIGENIA)	Yes	2	2000 IPF criteria	0.30 (weighted)
Leslie 2012	29 SLBs, 1 autopsy sample (familial IIP)	Three centers	Yes	3	2002 IIP	0.19-0.37
Tomassetti 2012, Casoni 2014	64 SLBs & 63 TBLCs (IPF)	No	Yes	2 (same pathologists)	2011 IPF	0.82 & 0.83
Hashisako 2016	20 SLBs (fibrosing IIP)	Pathologists from three countries	Yes	IIP: 9, IPF: 11	2002 IIP & 2011 IPF	IIP: overall 0.23 (-0.04-0.51), IPF: overall 0.19 (-0.07-0.66)
Tomassetti 2016	59 SLB, 58 TBLC (fibrotic ILD)	No	Yes	3	Not reported	0.86 (SLB) & 0.59 (TBLC)
Walsh 2016b	22 SLB (ILD)	Yes	Age, sex, smoking history known	7	2013 IIP	0.46 (unweighted), 0.58 (weighted)
Mäkelä 2018	50 SLBs, 6 explant samples, 4 autopsy samples (IPF)	Yes	Yes	4, 3 observations	2011 IPF	0.04-0.59
Jo 2019	63 SLBs (IPF)	No	No	3	2011 IPF	Overall 0.44 (weighted)
Troy 2020	65 SLBs, 65 TBLCs	Yes	Yes	3	2018 IPF	0.64 (SLB) & 0.53 (TBLC)

SLB, surgical lung biopsy; ILD, interstitial lung disease; IIP, idiopathic interstitial pneumonia; IPF, idiopathic pulmonary fibrosis, TBLC, transbronchial cryobiopsy.

2.4 HISTOPATHOLOGICAL FEATURES OF IDIOPATHIC PULMONARY FIBROSIS

2.4.1 PATHOGENESIS

Despite active research, the pathogenesis of IPF remains elusive. Previously, IPF was considered as “alveolitis,” that had pulmonary fibrosis as its end product Crystal et al. 1976). In 2001, Selman, King and Pardo represented the concept of IPF pathogenesis being an abnormal wound healing against microscopic repetitive alveolar injury. In healthy lungs, alveolar injury against type I pneumocytes leads to the proliferation of type II pneumocytes that can act as adult-type stem cells in the lungs and repair the alveolar epithelium (Barkauskas et al. 2013). In IPF lungs, type II pneumocytes have been observed to express shortened telomeres (Naikawadi et al. 2016). The combination of repetitive alveolar micro-injuries and epithelial cell alterations causes alveolar epithelial cells to lose epithelial cell markers and start to express mesenchymal markers (Willis et al. 2005, Marmai et al. 2011). The phenomenon of an epithelial cell acquiring mesenchymal cell features is called epithelial-to-mesenchymal transition (EMT). The overexpression of integrin αvβ6 activates the TGF-ß1 pathway that is currently deemed to be the most critical profibrotic cell signaling pathway of IPF (Horan et al. 2008). In addition to TGF-ß1, an increase in other profibrotic mediators, such as platelet-derived growth factor (PDGF) (Gurujeyalakshmi, Hollinger and Giri 1999), and also in the activation of developmental pathways such Wnt signaling pathway (Königshoff et al. 2008) are seen. Inflammatory cells are seen to accommodate the phases of pulmonary fibrosis, but the actual role in the pathogenesis remains unclear (Heukels et al. 2019). In normal wound repair, fibroblasts eventually activate apoptosis, but fibroblasts are desensitized to apoptotic signals in the IPF lung tissue (Maher et al. 2010). The hypothesis of aberrant epithelial-mesenchymal crosstalk is presumed to be the vicious circle driving pulmonary fibrosis.

2.4.2 FIBROBLAST FOCI

FF (Figure 4) is the hallmark of the UIP pattern (Raghu et al. 2018a) and is currently considered as the hotspot for the pathogenesis of IPF (Sgalla et al. 2018). In the interstitium, FF can be seen as dome-shaped, myxoid, and lightly staining small aggregates of spindle-shaped fibroblasts and myofibroblasts (Katzenstein and Myers 1998). Type II pneumocytes or alveolar epithelium affected by squamous metaplasia often separate FF from airspaces (Myers and Katzenstein 1988, Katzenstein and Myers 1998). The structure of FF is usually easy to identify subepithelially adjacent to airspaces in hematoxylin and eosin (HE) staining. FF are often co-localized with inflammatory cells, and few inflammatory cells can exist within FF (Mukhopadhyay 2016). FF can occur in the areas of prominent fibrosis and honeycombing, but also the less affected areas (Katzenstein and Myers 1998). Their presence represents temporal heterogeneity that is essential for typical UIP, since FF are markers of active fibrosis in comparison with dense, eosinophilic, “end-stage” fibrosis (Katzenstein and

Myers 1998). FF can be seen in other ILD patterns such as NSIP and CHP, but it is the high number of FF that defines typical UIP (Mukhopadhyay 2016).

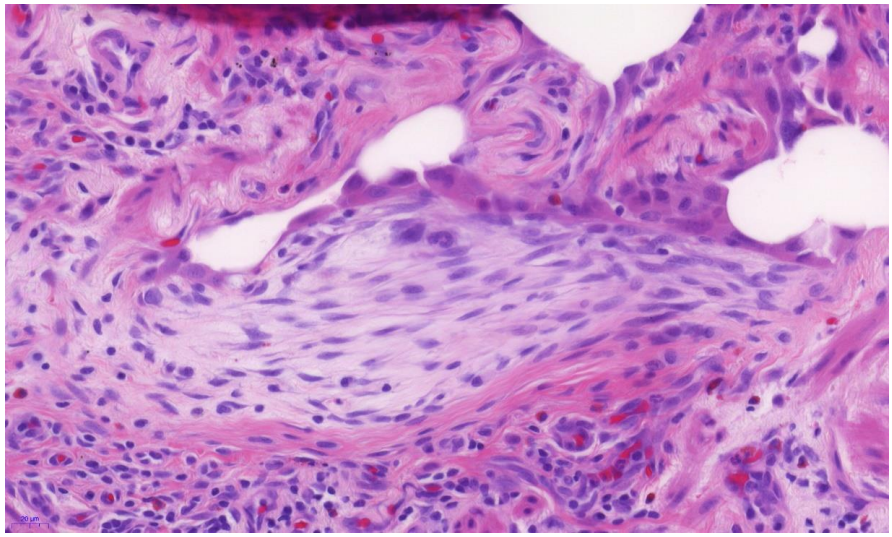


Figure 4 A fibroblast focus in a hematoxylin and eosin stained biopsy at 400x magnification.

The precise composition of FF is not fully understood. Myofibroblasts produce dense but poorly organized ECM more than fibroblasts and have an ability to contract similarly to smooth muscle cells due to α-SMA stress fibers (Scotton and Chambers 2007). The myofibroblast cores of FF express collagens I, III, IV, V, VI, fibronectin, and versican (Herrera et al. 2019). Uniquely to other ECM components, hyaluronan is seen both inside FF but also in areas of early lesions where FF are forming (Herrera et al. 2019). Collagen triple helix repeat containing 1 (CTHRC1) -expressing fibroblasts are concentrated within FF; they produce high levels of collagen (Tsukui et al. 2020). Fibrinogen, a marker of active tissue injury, can be found adjacent to the damaged alveolar epithelium and the myofibroblast core of FF (Herrera et al. 2019). Markers of severe endothelial reticulum stress and apoptosis have been reported in type II pneumocytes of IPF lungs adjoining dense fibrosis and FF (Korfei et al. 2008). EMT is possibly one source of myofibroblasts within FF, while epithelial cells adjacent to FF can express mesenchymal markers, and epithelial markers can be seen within FF (Harada et al. 2010, Lomas et al. 2012, Fabro et al. 2014, Yamaguchi et al. 2017). In addition to EMT, dormant pulmonary fibroblasts, both bone-marrow-derived, blood-circulating, and pulmonary fibrocytes, are possible progenitors of myofibroblasts (Zhang et al. 1994, Andersson-Sjöland et al. 2008). TGF-β is highly expressed in type II pneumocytes and also in FF in varying amounts (Lomas et al. 2012). Proliferative activity, which is measured by Ki-67 positive cells, is low in fibroblasts and the overlying epithelium of FF (El-Zammar, Rosenbaum and Katzenstein 2009, Lomas et al. 2012). With an integrated micro-CT and histopathological method, FF were noted to vary in shape and size, and not to be connected with each other as “fibroblast

reticulum" like previously was suggested (Cool et al. 2006, Jones et al. 2016). An association between FF and traction bronchiectasis on HRCT exists (Walsh et al. 2015). Airway-centered FF are associated with hiatal hernias, inflammation, and granulomas, suggesting a connection to microaspiration (Bois et al. 2016).

In the IPF pathogenesis, intraluminal or intra-alveolar fibrosis was previously thought to be an essential component (Basset et al. 1986). It was hypothesized that an alveolar injury would lead to the migration of fibroblasts or myofibroblasts into the intra-alveolar spaces in IPF and many other ILDs (Basset et al. 1986). In the case of limited initial injury, intraluminal fibrosis would develop into an intraluminal bud, a precursor name for OP, often seen in HP and rarely in IPF (Basset et al. 1986). A severe injury would lead to collapse and obliteration of alveolar lumens similar to DAD (Basset et al. 1986). In IPF, it was hypothesized that intraluminal fibrosis would form a fibrous tissue mass covered by re-epithelialization of the remnants of damaged alveolar epithelium or bronchiolar cells (Basset et al. 1986). Other studies also confirmed the finding that FF seemed to arise from alveoli and that they were very similar to Masson bodies that are part of OP (Kuhn and McDonald 1991, Fukuda et al. 1995). Intraluminal FF or OP were thought to represent "normal wound healing," whereas FF are part of "abnormal wound healing" that is essential in the pathogenesis of IPF (Selman, King and Pardo 2001). In studies using electron microscopy, the lack of basal lamina beneath the epithelium covering FF, the necrosis of epithelial cells, and the collapse of alveoli were noted, and it was suggested that FF might be the organizing processes of alveolar exudates caused by alveolar injury (Myers and Katzenstein 1988, Kuhn and McDonald 1991). Abnormal fibroblasts in FF could induce the apoptosis of alveolar epithelial cells, as combined data from electron microscopy and picrosirius red technique showed both the proliferation of alveolar epithelium and epithelial cell death adjacent to FF (Uhal et al. 1998). The basal lamina of the alveolar epithelium that is in direct contact with FF has indeed been observed to have small breaks, allowing direct contact between the epithelium and the mesenchyma (Fabro et al. 2014). Histopathologically, FF have been associated with minute lesions of alveolar damage (Emura et al. 2015). One possible pathogenetic mechanism of FF is that they might destroy alveolar capillary vessels, which eventually leads to the collapse of alveolar septae and perhaps is the origin of honeycombing (Yamaguchi et al. 2017). Using informatics-based analysis of the gene expression, TSC2/RHEB was recently identified in FF as a critical signaling pathway that mediates the TGF-β and collagen gene expression (Guillotin et al. 2021).

FF have morphological similarities with OP that obliterates the airways. Differentiating FF from OP can sometimes be a challenge (Smith et al. 2020). The outcomes of IPF and COP are, however, completely different as FF are driving the irreversible fibrosis, and COP has a reversible disease course (Cottin and Cordier 2012). Both proliferative and apoptotic activity are higher in OP than in UIP (Lappi-Blanco, Soini and Pääkkö 1999, El-Zammar, Rosenbaum and Katzenstein 2009). The neovascularization is decreased in FF compared to OP (Lappi-Blanco et al. 1999). Marker of EMT, β-catenin, is expressed in FF and not in OP (Chilosi et al. 2003). The

good response to anti-inflammatory therapy in COP might be causative of the microenvironment that is abundant of inflammatory cells, whereas FF are associated with minimal inflammation (Jonigk et al. 2019). However, OP can also be seen in IPF samples (Collard et al. 2007, Takemura et al. 2012). OP in IPF lung tissue has been associated with a decline in FVC% (Collard et al. 2007). Microscopic foci of OP are seen in macroscopically normal lung areas of IPF tissue (Todd et al. 2016). Despite the irreversible nature of OP lesions, OP in IPF lung tissue has been associated with worsening pulmonary function, similarly to FF (Collard et al. 2007). Pirfenidone and nintedanib seem to decrease OP but not FF in IPF tissue (Zhang et al. 2019b). To some extent, OP might play a role in the pathogenesis of IPF.

As discussed in the section 2.2.3.1, “Acute exacerbations,” FF have been noted to have a connection with minute lesions of alveolar damage that were associated with acute exacerbations and mortality (Emura et al. 2015). Contradicting the hypothesis of FF driving disease progression, increased amounts of FF have not been associated with acute exacerbations (Tiitto et al. 2006). No significant difference in the FF density has not been noted in SLBs of IPF patients having either rapid or slow disease course (Selman et al. 2007). The amounts of FF have not been increased in explant samples than in previously taken SLBs from the same IPF patients (Todd et al. 2013).

FF are the only strong histopathological prognostic markers in IPF (King et al. 2001, Nicholson et al. 2002, Enomoto et al. 2006, Tiitto et al. 2006, Lee et al. 2011, Harada et al. 2013), although it has not been verified in many studies (Flaherty et al. 2003a, Collard et al. 2007, Hanak et al. 2008, Nagata et al. 2011, Triantafillidou et al. 2011). Studies on the association between FF and IPF prognosis are represented in Table 7.

In conclusion, the exact origin of FF, their composition, and association with disease progression are elusive; better understanding could shed light on IPF pathogenesis. The amount, frequency, and location of FF that are definitive for IPF are also unresolved (Smith et al. 2020). The relationship of OP, that morphologically resembles FF, to IPF is neither fully understood. Studies on FF originate mainly from the era before evidence based diagnostic IPF criteria (Raghu et al. 2011, Raghu et al. 2018a). Also, the definition of FF has specified during years. The development of AI might provide a solution for the quantitation of FF in a patient cohort with re-evaluated IPF diagnoses.

Table 7 Studies on the associations between high amounts of fibroblastic foci and idiopathic pulmonary fibrosis survival and lung function.

Study	Samples	Methods	Results
King 2001	87 SLBs (IPF)	Semiquantitative scoring 0-5 and derivation of factor scores	High granulation/connective tissue score predicted poor survival
Nicholson 2002	83 SLBs (IPF)	Counting of selected areas and semiquantitative Brompton scoring 0-6	High FF score associated with mortality and greater declines in FVC and DLCO
Flaherty et al. 2003a	108 SLBs (99 IPF, 9 CTD)	0-3 semiquantitative Michigan scale	No relationship between IPF survival and FF; with CTD patients included, FF associated with survival
Tiitto 2006	76 SLBs (64 IPF, 12 CTD)	Quantitative whole-slide analysis	Patients with more than 50 FF/cm^2 had shorter survival
Enomoto 2006	31 SLBs (16 IPF, 15 CTD-UIP)	Quantitative, at least 10 fields with image analysis software	FF area score associated with poor survival (both for IPF and also with CTD)
Collard 2007	56 SLBs (IPF)	Semiquantitative (0-2)	No relationship between IPF survival and FF or OP; high FF and OP predict decline in FVC
Hanak 2008	43 SLBs (stable IPF)	Quantitative point-counting technique, five randomly selected fields	No relationship between profusion of FF and survival
Lee 2011	86 SLBs (IPF)	Semiquantitative "Brompton" scoring 0-6	High FF frequency associated with poor survival
Nagata 2011	43 SLBs (19 UIP)	Counting of selected areas, semiquantitative scoring 0-3	No association between FF and survival (p=0.1), score higher for patients with an unstable disease course
Triantafillidou 2011	24 SLBs (IPF)	Semiaquantitative scaling, both Brompton (0-6) and Michigan (0-3)	No association between FF and survival or lung function
Harada 2013	24 scanned SLBs (19 IPF, 5 CTD)	Quantitative whole-slide analysis with image analysis software	High FF area and density in the samples that had deceased

SLB, surgical lung biopsy; IPF, idiopathic pulmonary fibrosis; FF, fibroblast foci; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide; CTD, connective tissue disease; UIP, usual interstitial pneumonia; OP, organizing pneumonia.

2.4.3 INFLAMMATION

As previously discussed, the hypothesis of “pneumonitis” as a driving force in the pathogenesis of IPF was in the lead for an extended period of time before the theory of aberrant alveolar epithelial injury repair emerged. Anti-inflammatory treatments have not been successful in IPF, and probably that is why the role of inflammatory cells has not been the main focus in IPF research. However, inflammatory cells are often seen to accommodate the phases of IPF pathogenesis, but their role and the extent of their impact is unclear (Heukels et al. 2019).

2.4.3.1 Interstitial mononuclear inflammation

Interstitial mononuclear inflammatory cells are lymphocytes and plasma cells that are responsible for the immune response of adaptive immunity. According to the 2018 diagnostic criteria, inflammation is usually mild in IPF lung tissue samples (Raghu et al. 2018a). Mononuclear interstitial inflammation can exist as a patchy interstitial infiltrate adjacent to hyperplastic type 2 pneumocytes and bronchiolar epithelium (Raghu et al. 2018a). Features that are suggestive for other ILD are inflammatory infiltrates away from honeycombing or fibrotic areas and substantial lymphoid hyperplasia, especially with secondary germinal centers (Raghu et al. 2018a).

Even after applying the 2011 diagnostic criteria (Raghu et al. 2011), marked interstitial mononuclear inflammation is relatively often seen in IPF lung tissue (Takemura et al. 2012, Rabeyrin et al. 2015, Yagihashi et al. 2016). Inflammatory cells in the interstitium of IPF/UIP samples are mainly mononuclear (Daniil et al. 2005, Parra et al. 2007). Lymphoid aggregates in IPF samples stain positively for CD3 and CD20 that mark for T and B lymphocytes, respectively (Daniil et al. 2005, Todd et al. 2013). Only a few macrophages are seen (Daniil et al. 2005, Todd et al. 2013). CD4 cells representing T helper (Th) cells have also been seen inside lymphoid aggregates or adjacent to them (Daniil et al. 2005). CD8 cells that mark cytotoxic T cells locate mainly diffusely in the parenchyma, but also within the alveolar wall around FF and areas with alveolar thickening (Daniil et al. 2005). In explant samples of patients with IPF, increased numbers of B cells, CD4 and CD8 lymphocytes, and macrophages are observed (Tanabe et al. 2020, Verleden et al. 2020), both in the areas of minimal and marked fibrosis (Verleden et al. 2020).

According to the previous hypothesis, T lymphocytes were considered essential cells driving the chronic inflammation in IPF (Cherniack et al. 1991a). T cells were thought to affect the pathogenesis, first, by directing B cells to producing antibodies against exogenous particles or the lung parenchyma, or second, by cytotoxicity via CD8 cells against lung cells (Cherniack et al. 1991a). The mice that lacked T cells were also seen to have reduced ECM formation and fibroblast proliferation after exposure to bleomycin (Schrier, Phan and McGarry 1983). According to the current perception, lymphocytes participate in pulmonary fibrosis, but their exact role on pulmonary fibrosis is unclear and seems to vary depending on the subtype of a lymphocyte (Kolahian et al. 2016). Interleukin 4 and 13 that are produced by Th2 cells are

connected to increased fibrogenic response (Saito et al. 2003). In the mice with bleomycin-induced pulmonary fibrosis, interferon gamma (IFN-γ) and interleukin 2, produced by Th1 cells, have the opposite effect (Keane et al. 2001). Profibrotic $CD28^{null}$ cytotoxic CD8 memory T cells are increased in IPF explant lung tissue (Habiel et al. 2019). $CD28^{null}$ T cells seem to be resistant to dexamethasone, but immune checkpoint proteins cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) inhibit their action (Habiel et al. 2019). In the mice models lacking lymphocytes and having an acute lung injury by intratracheal lipopolysaccharides, administration of regulatory T cells (Treg) caused collagen deposition at first (Garibaldi et al. 2013). However, in a later stage of fibrosis, Tregs attenuated fibroproliferation. Same cells having both pro- and antifibrotic effects reflect the complexity of immunology associated with fibrosis.

Clinical trials on treatments targeting T cells and cytokine production have failed to reach positive results (Raghu et al. 2004, Parker et al. 2018, Raghu et al. 2018b). These include IFN-γ, a cytokine produced by Th1 cells (Raghu et al. 2004), tralokinumab and lebrikizumab, which are antibodies against anti-inflammatory interleukin 13 (IL-13) secreted by CD4 T helper cells (Parker et al. 2018), and also SAR156597, which is an antibody against both interleukin 4 (a stimulator of T cell proliferation) and IL-13 (Raghu et al. 2018b). By using the anti-IL-13 treatment, some positive effects on acute exacerbation rates were noted (Raghu et al. 2018b). However, in addition to antifibrotic properties, pirfenidone inhibits the proliferation of T cells and the production of cytokines without having an effect on Tregs (Visner et al. 2009). Both antifibrotic medications have been shown to decrease the number of interstitial mononuclear inflammatory cells (Zhang et al. 2019b).

Before properly defining the diagnostic criteria of IPF, cellular histopathology, i.e., more inflammatory cells, was associated with a good prognosis in IPF (Stack and Heard 1972, Turner-Warwick, Burrows and Johnson 1980, Tukiainen et al. 1983). These results were probably an outcome of the inclusion of other ILDs like NSIP and HP, under the diagnosis of IPF. More recent evidence suggests that abundant inflammation is a marker of poor prognosis in IPF. High amounts of interstitial mononuclear inflammation have been associated with a decline in pulmonary function (Nicholson et al. 2002, Daniil et al. 2005, Parra et al. 2007). A trend for poor survival has been noted in a cohort of 20 IPF patients with elevated lymphocytes in SLB (Parra et al. 2007). In a cohort of 16 IPF patients having undergone SLB and also lung transplantation, more lymphoid aggregates were found in the explant samples compared to SLBs (Todd et al. 2013). The cells in the lymphoid aggregates of explant samples did not, however, show high proliferative activity (Todd et al. 2013). High concentrations of circulating chemokine C-X-C motif ligand 13 (CXCL13), a B-cell mediator, have been associated with a poor survival and declining pulmonary function values (Vuga et al. 2014). High amounts of subtypes of circulating activated T cells also seem to predict a dismal prognosis (Moore et al. 2014). Nevertheless, the lymphocyte counts were not elevated in all of the measurements during the follow-up. Balestro et al. (2016) examined 41 explanted IPF lungs and showed that in rapidly

progressing IPF, both acute and chronic inflammatory cell numbers were higher in the lungs compared to slowly progressing IPF. Furthermore, slowly progressing IPF patients with acute exacerbations had larger total leucocyte numbers in the lungs than patients without exacerbations (Balestro et al. 2016). For patients that did not have acute exacerbations, leucocyte amounts correlated with the yearly decline of FVC (Balestro et al. 2016). High amounts of mononuclear inflammation seem to be involved in accelerated disease progression of IPF, possibly mainly via acute exacerbations.

2.4.3.2 Intra-alveolar macrophages

Intra-alveolar macrophages (Figure 5) are innate immune cells that are primarily derived from the yolk sac and are highly regulated by the granulocyte-macrophage colony-stimulating factor (Allard, Panariti and Martin 2018). They are essential in responding to infectious agents and epithelial damage (Allard, Panariti and Martin 2018). If the alveolar injury is severe, monocytes from the peripheral blood are recruited in the lung tissue, where they differentiate into intra-alveolar macrophages (Morales-Nebreda et al. 2015). In murine models, monocyte-derived macrophages in the fibrotic lungs seem to have upregulation of profibrotic genes, whereas tissue-derived intra-alveolar macrophages do not (Misharin et al. 2017). Macrophages can be classified as classically activated M1 macrophages that are stimulated by interferon γ (INF-γ) and tumor necrosis factor α (TNF-α), and as alternatively activated M2 macrophages that respond to interleukins 4, 10, and 13, and TGF-β (Desai et al. 2018, Zhang et al. 2018). Simply put, proinflammatory M1 macrophages inhibit, whereas profibrotic M2 macrophages stimulate the fibroproliferation and aberrant tissue repair (Desai et al. 2018, Zhang et al. 2018). M2 macrophages have been noted to be excessively present in fibrotic lungs (Pechkovsky et al. 2010). Pulmonary administration of TNF-α to mice with bleomycin-induced pulmonary fibrosis has been noted to decrease the amount of M2 macrophages and enhance the resolution of pulmonary fibrosis (Redente et al. 2014). Depending on environmental signals, M1 macrophages can switch to M2 macrophages and vice versa (Zhou et al. 2014).

In lung biopsies, intra-alveolar macrophages are a common, non-specific finding (Rossi et al. 2017). Light brown macrophages are seen in healthy smokers' lungs, and their abundant numbers serve as the hallmark of respiratory bronchiolitis-associated ILD (RB-ILD) and desquamative interstitial pneumonia (DIP) (Ryu et al. 2005). When intra-alveolar macrophages contain coarse hemosiderin pigment, chronic hemorrhage should be suspected (Rossi et al. 2017). Macrophages filled with exogenous, sometimes birefringent particles, are seen in pneumoconiosis (Crouch and Churg et al. 1984). Foamy macrophages are a common and non-specific finding, but they have been associated with HP, OP, exogenous lipoid pneumonia, and pneumotoxic drug reaction (Rossi et al. 2017). Different types of intra-alveolar macrophages can commonly be seen in IPF samples (Katzenstein and Myers 1998). A marked accumulation of macrophages is noted from 11% to 27% of IPF biopsies (Collard et al. 2007, Rabeyrin et al. 2015). Intra-alveolar macrophages in UIP samples

seem to have a higher proliferative activity than other ILDs (El-Zammar, Rosenbaum and Katzenstein 2009).

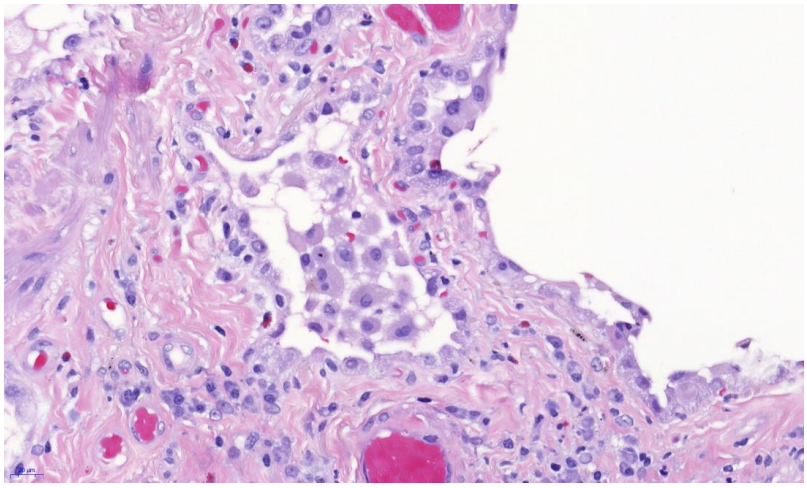


Figure 5 Intra-alveolar macrophages in idiopathic pulmonary fibrosis at 400x magnification.

According to the previous hypothesis of IPF being a result of chronic inflammation, intra-alveolar macrophages were thought to be the main culprits in “the alveolitis” driving the fibrosing process (Keogh and Crystal 1982). Even though the current understanding of the pathogenesis of IPF is that the injury of the alveolar epithelium starts the vicious circle of aberrant fibrinogenesis, the role of intra-alveolar macrophages is still elusive in IPF. As the pathogenesis of IPF initially arises from the alveolar space, intra-alveolar macrophages probably participate in the process, and altering their activity might be a potential treatment target.

The clinical trials targeted on macrophages in the IPF lungs have given some conflicting results. On the other hand, the clinical trials focusing on the suppressing M1 responses, such as by TNF-α blocker etanercept or by macrophage inflammatory response promoting chemokine C-C motif ligand 2 (CCL2) blocker carlumab, have not been able to slow the disease progression or improve survival (Raghu et al. 2008, 2015). Immunosuppressive treatment can lead to worse survival (Raghu et al. 2012). However, preliminary results have been reached on slowing the decline of lung function by using pentraxin, a blocker of monocyte differentiation into profibrotic macrophages and TGF-β production (Raghu et al. 2018c). Pirfenidone and nintedanib have anti-inflammatory properties that are poorly known; pirfenidone is known to inhibit the TGF-β pathway that is associated with M2 macrophages (Heukels et al. 2019).

2.4.4 GIANT CELLS

Giant cells are a union of histiocytes that are of part monocyte-macrophage lineage cells capable of phagocytosis (Anderson 2000). Giant cells can be divided into Langhans' giant cells (Figure 6) and foreign body giant cells (Figure 7) (Anderson 2000). Langhans' giant cells are characteristic for infectious granulomatous diseases, such as tuberculosis, and non-infectious granulomatous diseases, such as sarcoidosis (Anderson 2000). Morphologically, Langhans' giant cells consist of less than 20 nuclei that align in a circular, horseshoe-like formation within the giant cell (Anderson 2000). Foreign body giant cells consist of numerous nuclei (usually more than 20) that are diffusely located in the cell (Anderson 2000). The giant cell forms a thin layer with macrophages around the nondigestible material. Non-specific cholesterol clefts or Schaumann bodies can be observed inside giant cells.

Typically, giant cells can be seen in CHP both interstitially and in alveolar spaces (Castonguay et al. 2015). The current diagnostic criteria for HP by the ATS/ERS/JRS/ALAT state that isolated multinucleated giant cells are common in HP (Raghu et al. 2020). They should involve both peribronchiolar interstitium and peribronchiolar air spaces and can be associated with OP (Raghu et al. 2020). The evidence on giant cells in IPF samples is controversial. In thirteen SLBs of IPF patients, no giant cells were registered when compared to CHP samples (Takemura et al. 2012). Whereas in two samples out of eleven autopsy samples of IPF patients, occasional or marked giant cells were reported, with no significant difference between IPF and CHP samples (Akashi et al. 2009). In a recent study, no giant cells were observed in the samples that were confident IPF samples (n=5). Conversely the samples, whose histopathology was indefinite both for IPF and CHP, two out of eight SLBs manifested with giant cells (Wright et al. 2020). In addition, confident CHP samples had giant cells in eight out of ten samples (Wright et al. 2020). All in all, pathologists do not agree on the numbers of giant cells that are accepted in the UIP pattern.

The current guidelines exclude definite and probable UIP patterns, when giant cells are simultaneously seen with UIP features (Lynch et al. 2018, Raghu et al. 2018a), but otherwise no specification on the number of giant cells is given. A recent review focusing on the histopathological evaluation of IPF states that single multinucleated giant cells in air spaces or within fibrosis, sometimes associated with cholesterol clefts, do not exclude the definite UIP pattern (Smith et al. 2020). However, IPF is not favored by small rounded epithelioid histiocytes clustered as granulomatous inflammation or giant cells in the non-fibrotic interstitium or adjacent to the bronchioles (Smith et al. 2020). In rats, repetitive aspiration of a particulate food material or pH-neutralized gastric fluid caused a pulmonary pathology of granulomatous inflammation and multinucleated giant cells (Downing et al. 2008). In biopsies or resection samples, OP and giant cells can be observed to have a connection with aspirated organic or inorganic material (Mukhopadhyay and Katzenstein 2007). Moreover, IPF patients have signs of gastroesophageal reflux more often than

controls (Savarino et al. 2013). These findings raise the question whether giant cells in the IPF lungs are associated with clinical or subclinical aspiration in IPF.

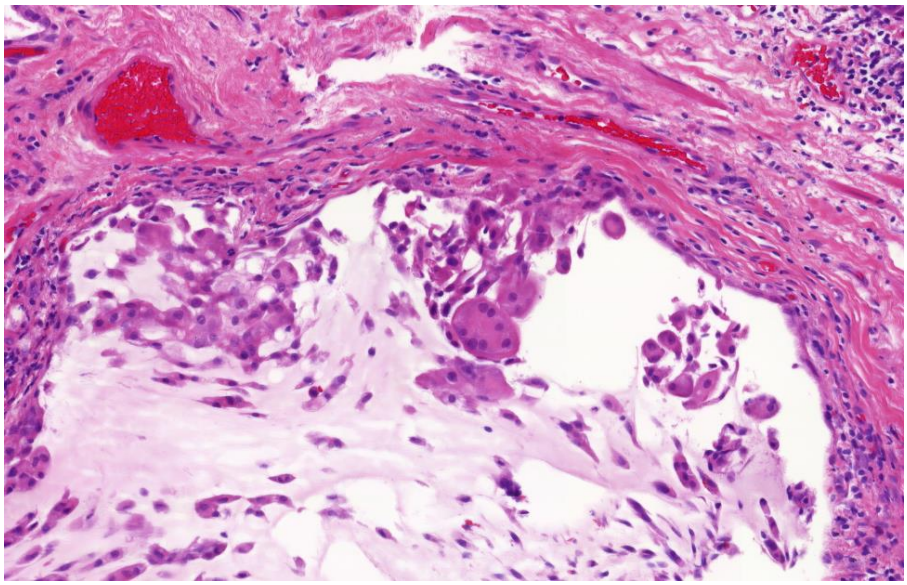


Figure 6 Alveolar Langhans' giant cell.

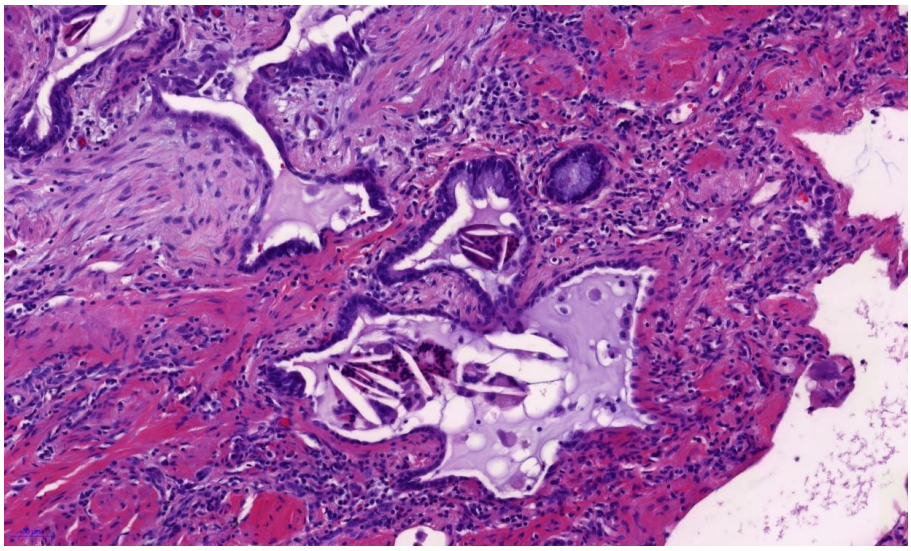


Figure 7 Alveolar foreign body giant cells. Cholesterol clefts can be seen inside giant cells.

2.4.5 DIGITAL PATHOLOGY

In conventional pathology, counting specific histopathological features is laborious and rarely necessary. For example, counting the inflammatory cells in a whole slide can be practically impossible. In the evaluation of the numbers of a histological feature, semiquantitative scoring (e.g., Study II, Table 2 and Table 3) and point-counting have been widely used. Also in these methods, both inter-and intraobserver variations exist. By point-counting, only part of the slide is analyzed, and hence, essential areas might be missed. Accurate quantitation of histological features could provide a new approach to lung pathology.

The development of digital and computational pathology offers an opportunity in the histological quantitation. Whole slide imaging (WSI) enables to analyze a scanned tissue slide in a resolution similar to a microscope (Abels et al. 2019). Machine learning is a form of artificial intelligence (AI) that enables image analysis by computer software that is taught to analyze wanted features (Abels et al. 2019). Deep learning is a type of machine learning in which the algorithm teaches itself (Abels et al. 2019). In deep learning, a network model is mathematically built with multiple connected layers, mimicking biological neural networks (Wang et al. 2019). Convolutional neural network (CNN) is a subset of deep learning that can have hundreds of convolution layers as hidden layers, between the visible input and output layers (Wang et al. 2019). CNN suits exceptionally well for image analysis for classifying and segmenting features, and it has spread widely in histological research, especially in oncology (Abels et al. 2019). CNN analyses the image by focusing on a region of interest (ROI) at a time, i.e., a field of view or a receptive field, and this window is slided along the width and height directions of the image (Wang et al. 2019). Supervised learning of CNN requires the labeling of histopathological features as annotations, which form the ground truth for the model (Wang et al. 2019). A CNN model is capable, for example, of recognizing specific histopathological features that will support the diagnostic process and even identifying novel prognostic features, when outcome data is represented to it (Abels et al. 2019).

In histopathology, pulmonary fibrosis has been analyzed with AI in murine models (Gilhodes et al. 2017, Heinemann et al. 2018, Seger et al. 2018). Automated image analysis of HRCTs has provided novel prognostic markers (Maldonado et al. 2014, Jacob et al. 2018, Robbie et al. 2019). Also, AI-based HRCT pattern recognition on ILDs has succeeded in pattern recognition in a level even comparable to radiologists (Walsh et al. 2018). To my knowledge, AI models of digital pathology have not previously been developed in IPF lung tissue.

3 HYPOTHESES AND AIMS

We hypothesized that histopathological features in IPF samples affect the survival of IPF patients. Specific hypotheses for articles were:

(I) After the 2011 diagnostic criteria, interobserver variation on the UIP pattern has decreased among pathologists. The definite UIP pattern in histopathology decreases the survival time of IPF patients in comparison with a non-definite UIP pattern. Atypical histopathological features for UIP affect the survival and the interobserver variation among pathologists.

(II) Inorganic PM in the lungs exists in IPF samples. The location of residency, exposure to environmental dust, and occupational history affect the amount and the elemental composition of PM observed in the lung tissue. The high amount of histopathological PM is associated with the disease progression of IPF.

(III) AI can be used in the histopathological analysis of IPF samples. Of specific histopathological features, FF, interstitial mononuclear inflammatory cells, and intra-alveolar macrophages have prognostic values.

We aimed to evaluate possible associations between different histopathological and clinical features in our study cohort of Finnish IPF patients. Specifically, the articles aimed at analyzing:

(I) the prevalence of the histopathological classifications using 2011 IPF criteria and the histopathological features atypical for IPF/UIP, the repeatability of the histopathological observations of the pathologists, and to compare the histopathological features to clinicoradiological information and survival of 60 IPF patients;

(II) the existence of inorganic PM in the lung tissue samples of 73 IPF samples with polarizing light microscopy, the elemental composition of inorganic PM using energy dispersive spectroscopy and field emission SEM, and comparing PM data to clinical information and survival of 73 IPF patients;

(III) FF, interstitial mononuclear inflammation, and intra-alveolar macrophages with deep learning CNN, and comparing the data to clinical information and 71 IPF patients' survival.

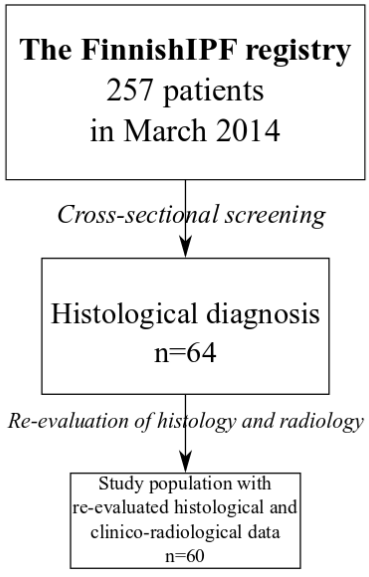
4 MATERIALS AND METHODS

4.1 STUDY POPULATION AND RE-EVALUATION OF DIAGNOSIS (I, II, III)

All of the patients in Study I, II, and III originated from the FinnishIPF registry, a nationwide prospective study that started in 2012 (Kaunisto et al. 2015). The patients came from Finnish university and central hospitals, and all of them have given written informed consent. The registry holds diagnostic and follow-up patient data collected manually from medical records, including basic information on birth, transplantation and death dates, age, gender, location of residence, smoking status and pack-years, pulmonary function test values, laboratory values, BAL fluid values, radiological data, histopathological data, and medication, among other data.

In March 2014, the registry was searched for patients with an available histopathological lung tissue sample (Figure 8). Then, the registry held 257 IPF patients. As SLBs are mainly taken in a university hospital, all available lung tissue samples were gathered from the university hospitals of Helsinki, Turku, Tampere, Kuopio, and Oulu. The search resulted in 64 samples, of which one patient had duplicate samples, and the most recent one was selected, the total was 63 cases.

Figure 8 A flowchart representing the patient selection in Study I.



In Study I, four pathologists, experienced in pulmonary pathology, investigated all of the lung tissue sample slides. The pathologists knew that the samples were from the FinnishIPF registry patients, but they were blinded to the clinicoradiological and registry data. Two pathologists analyzed all of the samples in consensus, forming observation 1 in statistical analysis. The other two pathologists analyzed samples blinded to each other's observations, forming observations 2 and 3 used in the analysis of the interobserver agreement. The histopathological classifications were re-evaluated by the 2011 criteria (Raghu et al. 2011). The disease severity was evaluated as mild, moderate, or severe in each case. Additional histopathological features, considered atypical for UIP, were also assessed; namely the presence of emphysema, respiratory bronchiolitis (RB), giant cells, inflammation, OP, DAD, and DIP-like reaction was systematically recorded. Pathologists also wrote open descriptions for other histopathological observations and possible differential diagnosis. HRCT images of 62 patients were re-evaluated by an experienced radiologist together with a radiology resident by the 2011 diagnostic criteria (Raghu et al. 2011). One of the 63 patients had HRCT images on film; thus, they were not available.

After the radiological and histopathological evaluation of 63 cases, twelve cases did not lead to the IPF diagnosis according to the diagnostic algorithm presented in the 2011 criteria (Raghu et al. 2011). All of the available data on each of these patients was assessed in a discussion between a pulmonologist and a pathologist, and so three patients were excluded from the study population. There were three reasons for exclusion: 1) "inconsistent with UIP" in HRCT, more suitable histopathology for CHP, and clinical suspicion of rheumatoid arthritis-associated ILD, 2) HRCT more suitable for NSIP and histopathological features favoring more CHP, and 3) radiology and histopathology favoring RB-ILD/DIP. The rest of the patients were considered to have IPF after MDD, leading to a study population of 60 patients in Study I. Out of lung tissue samples, 50 (83%) were SLBs, six were explant samples (10%), and four (7%) were autopsy samples. In March 2014, the SLB rate in the patient cohort of the FinnishIPF registry was approximated to be 19% (50/257).

For Study II, the registry was again screened for patients from the university hospitals of Helsinki and Oulu that would have a lung tissue sample available in January 2017. The search resulted in thirteen SLBs, and when added to the study population in Study I, the final study population consisted of 73 patients.

In Study III, the study population consisted of 71 patients, as two of the samples were not suitable for automated image analysis. One SLB had adenocarcinoma, and the other was an autopsy sample with other tissue types in the slide.

The patient characteristics of Study I, II, and III are shown in Table 8.

Table 8 Patient characteristics of Study I, II, and III. The values are expressed in mean ± standard deviation (SD) or percentages (%).

	Study I	Study II	Study III
NO of patients	60	73	71
Follow-up time (mo)	61.7±34.2	67.3 ± 40.3	72.5±42.7
End of follow-up	May 31, 2016	March 5, 2018	April 29, 2019
Age at diagnosis (y)	62.4±10.5	61.7 ± 10.4	61.5±10.4
Transplantations	13 (21.7%)	16 (21.9%)	17 (23.9%)
Age at transplantation (y)	58.7±8.71 (n=13)	56.3 ± 8.6 (n=16)	56.8±8.3 (n=17)
Deaths	23 (38.3%)	37 (50.7%)	37 (52.1%)
Age at death (y)	69.8±8.66 (n=23)	70.1 ± 8.1 (n=37)	70.5±8.2 (n=37)
Never-smokers (N/%)	23 (38.3)	27 (37.0)	26 (36.6)
Ex-smokers (N/%)	27 (45.0)	35 (47.9)	34 (47.9)
Current smokers (N/%)	10 (16.7)	11 (15.1)	11 (15.5)
Pack-year smoking (y)	23.3±13.3 (n=32)	21.8 ± 12.5 (n=41)	22.3±12.4 (n=40)
Men/women (N/%)	42/18 (70/30)	51/22 (69.9/30.1)	49/22 (69.0/31.0)
BMI (kg/m^2)	28.6±4.87 (n=55)	28.9 ± 4.9 (n=68)	29.1±4.8 (n=66)
FVC%	77.8±16.6 (n=57)	76.2 ± 16.7 (n=68)	75.6±16.7 (n=66)
DLCO%	55.6±18.5 (n=58)	56.4 ± 15.9 (n=68)	56.3±16.0 (n=67)
SLB	50 (83.3%)	63 (86.3%)	62 (87.3%)
Explant sample	6 (10.0%)	6 (8.2%)	6 (8.2%)
Autopsy sample	4 (6.7%)	4 (5.5%)	3 (4.2%)

NO, number of observations; BMI, body mass index; FVC%, forced vital capacity, % predicted; DLCO%, diffusing capacity for carbon monoxide, % predicted; SLB, surgical lung biopsy.

4.2 ANALYSIS OF PARTICULATE MATTER (II)

In Study II, we analyzed histopathological PM of HE-stained lung tissue samples of IPF patients using polarizing light microscopy and SEM. As we noted that birefringent PM co-existed with coal dust pigment in light microscopy, we also decided to analyze coal dust pigment. We developed novel scoring methods for coal dust pigment and birefringent PM using 73 IPF lung tissue samples with the most representative of UIP pathology, described in detail in Table 2 and Table 3 in Study II. First, we reviewed

each slide of lung tissue samples available for 73 IPF patients and selected the most representative slide of UIP pathology. Based on the amount of coal dust pigment and birefringent PM in the slides, we developed a novel scoring method with a polarizing light microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan). The scoring was conducted blinded to registry data and the origin of the slide. Coal dust pigment and birefringent PM were scored on a semiquantitative scale of 0 to 5 (Study II, Table 2 and 3). The score for coal dust pigment was affected by the size, amount, and distribution of accumulations, areas covered with coal dust pigment, and the sample's approximated size. With the used 100x magnification and optics, the resolution used in the coal dust pigment scoring was 1.30 µm. In addition, the score for birefringent PM was affected by the number of single particles, the amount and size of particle clusters, the distribution of particles, areas covered with particles, and the sample's approximated size affected the score. In the scoring of birefringent PM, we used a higher magnification of 200x, and the resolution used was 0.52 µm.

We analyzed PM in six IPF lung tissue samples different from those used in light microscopy. All of the samples in SEM analysis were noted to have high PM content in polarizing light microscopy. SEM analysis is described in detail in Study II. In sample preparation, titanium (Ti) plates were used, and carbon (C) plates were used for conductivity. Hence, they were excluded from further elemental analysis. We used field emission SEM (JSM-6335F, JEOL, Tokyo, Japan) for analyzing the size of particles, the distribution of different sizes, shapes, and the structures of the surface. For the analysis of the elemental composition, we used energy dispersive spectrometry. From 10 to 24 particles per sample were analyzed.

4.3 DIGITAL PATHOLOGY AND IMAGE ANALYSIS (III)

4.3.1 DIGITALIZATION OF SLIDES AND PRELIMINARY EVALUATION

As scanned whole slide images of lung tissue samples require a large amount of storing space, we scanned only one representative slide per lung tissue sample. All slides were scanned with a bright field, using Pannoramic 250 Flash II (3DHistech, Budapest, Hungary) at 400x magnification, 0.12 µm/pixel resolution, and 40X/0.95 NA objective.

In the beginning of Study III, we aimed to annotate slides manually with the image analysis software CaseViewer (3DHistech, version 2.2). The aim was annotate FF, interstitial mononuclear inflammatory cells, intra-alveolar macrophages, giant cells, cholesterol clefts, and peribronchial metaplasia (PBM). In the preliminary evaluation of the slides, we noted that quantitating interstitial mononuclear inflammation would only be possible by outlining lymphocyte infiltrations or semiquantitatively scoring the amount of interstitial inflammation. Intra-alveolar macrophages were also laborious to quantitate by manual annotation. Thus, we chose to analyze the slides with an AI-based software, Aiforia®. During the preliminary evaluation, we had noted that giant

cells and cholesterol clefts are not a common feature. Our data set did not include enough examples of them so that AI could properly recognize giant cells or cholesterol clefts. PBM was commonly seen in IPF samples. However, PBM shares similar features with bronchioles and honeycombing, which makes it challenging target for an AI model. As our primary goal in Study III was to test the use of AI in IPF lung tissue, we chose to focus on the recognition of interstitial mononuclear inflammatory cells and intra-alveolar macrophages that are easy targets for AI, and also FF that had been subjects in previous image analysis studies of IPF.

4.3.2 THE ARTIFICIAL INTELLIGENCE MODEL

A diverse set of 20 representative slides was chosen as training data to develop an AI model (Figure 9) in Aiforia® image management and analysis platform (Aiforia Technologies, Helsinki, Finland). The model was trained by supervised learning to recognize target features: lung tissue, air spaces, FF, interstitial mononuclear inflammation, and intra-alveolar macrophages (Study III, Table 2). The model included four layers that were individual neural networks, but also connected as an analysis pipeline. The first layer consisted of lung tissue. Of the lung tissue, the second layer then separated the interstitium and alveolar spaces. Finally, the third layer recognized FF and interstitial mononuclear inflammatory cells from the interstitium, whereas the fourth layer recognized intra-alveolar macrophages from the alveoli. The different layers enabled the adjustment for an optimized field of view; a large field of view was required for the first layer, so that even large bullae would be recognized as part of the tissue, and a small field of view was required for inflammatory cells (Study III, Table 2).

In supervised learning of AI, the input data (i.e., training areas) is created by the model developer. In each layer, all slides in the training data were searched for representative examples of the target features. A training area was annotated, and inside of it, the borders of a target feature were annotated (Study III, Appendix I). The model also required a lot of training for the features that did not represent the target features. For example, perivascular fibrosis shared similar features with FF; therefore, areas of perivascular fibrosis were included in the training areas but not annotated as FF. In the beginning of the AI model development, we tried to train the model to distinguish FF from OP. As OP was a rare feature in our data (Study I), we could not offer enough examples of OP to the AI model. Conflicting data between FF and OP occurred, as the model was taught similar features to represent two distinct features simultaneously. The model did neither recognize FF nor OP properly. That is why it was allowed for the AI model to recognize OP as FF, leading to better performance of the model.

Supervised training includes analyzing the output data produced by the model. When enough training areas had been conducted, the model analyzed the training slides, and the results of the analysis were then analyzed visually. After each iteration of the training, the old annotations were edited. New ones were created based on the

feedback from the results of each iteration, that is, which features the model had difficulties recognizing and which it already recognized well. More details of the development of the AI model are described in Study III.

When the model was finished, we analyzed all of the 71 samples with it. The model produced counts and surface areas of each feature, of which the percentage was derived in relation to the whole tissue area (area%) and density (Study III, Table 3).

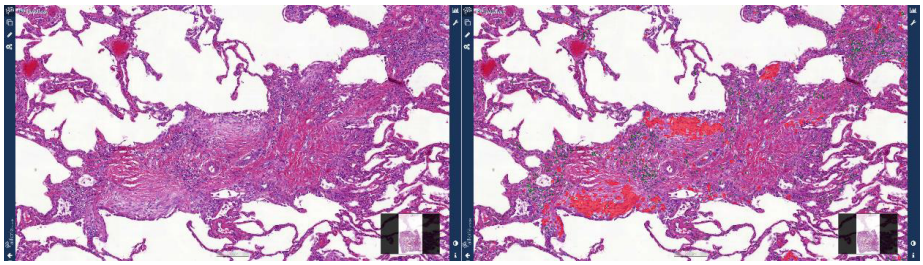


Figure 9 A view from the developmental phase of the artificial intelligence model in the Aiforia® software. The artificial intelligence model marks fibroblast foci with red and interstitial mononuclear inflammation with green.

For the validation of the AI model, we selected 30 slides that had not been included in the training of the model. Every slide was searched for a FF, and a rectangular validation area was created around it. A pathologist experienced in pulmonary pathology reviewed all the validation areas and annotated the borders of each FF. The pathologist's annotations were considered as the ground truth, and the results of the model were compared against the ground truth. In addition to visual results (Study III, Appendix 3), false positive, false negative, error, precision, sensitivity, and F1 score values were counted for each validation area. For all 30 selected validation areas, the values of true and false positive and true and false negative were counted. The statistics of the validation are described in detail in Study III. The recognition of the interstitial mononuclear inflammatory cells was evaluated only visually. Furthermore, the reproducibility of the model was tested by running the AI model three separate times in a subanalysis of five slides.

4.4 STATISTICAL ANALYSIS (I, II, III)

In all studies, statistical analyses were conducted with IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY, USA), with version 24.0 (Study I) or with version 25.0 (Study II and III). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test if the data was normally distributed. When comparing two groups of continuous measurements, the t-test or Mann-Whitney U test was used depending on the results of the normality tests. In the comparison of three or more groups, a one-way ANOVA test or the Kruskal-Wallis test were used. For categorical data, we used Fisher's exact test. Additionally, Sperman's correlation was used. In survival analysis, the survival

time was the time between the IPF diagnosis date and the death or lung transplantation dates that were considered as end-point events. The Kaplan-Meier method was used to analyze survival, and the significance of the results of the Kaplan-Meier method was evaluated with the log-rank test. In Study I, observation 1 was used in the statistical analysis of histopathological features. The agreement between observations 1, 2, and 3 was compared with Cohen's κ coefficient (Landis and Koch 1977). In Study III, the cut-point values for survival analysis were determined with the R package maxstat (Hothorn 2017) in the R software for Windows, version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria).

4.5 ETHICAL ASPECTS

The Finnish National Institute for Health and Welfare (Dnro THL/1161/5.05.01/2012, Dnro THL/1211/5.05.00/2015) approved the screening of hospital registries for patients with IPF. Valvira, National Supervisory Authority for Welfare and Health (Dnro 3317/05.01.00.06/2011), approved using the diagnostic tissue samples of the FinnishIPF registry patients for research purposes. The Ethics Committee of the Finnish University Hospital of Helsinki (HUS/2550/2017) approved the study. Also, the Ethics Committees of the Finnish University Hospitals of Turku, Tampere, Kuopio, and Oulu approved the study. All patients participating in the study provided written informed consent.

5 RESULTS

5.1 HISTOPATHOLOGICAL FEATURES OF IDIOPATHIC PULMONARY FIBROSIS (I AND III)

In the re-evaluation of IPF/UIP diagnostic categories (Raghu et al. 2011) in Study I, 63% (38/60) of the lung tissue samples of IPF patients were analyzed as “definite UIP.” Thus, a significant proportion of re-evaluated IPF patients had a non-definite UIP histology; 10% (6/60) were “probable UIP,” 20% (12/60) were “possible UIP,” and 7% (4/60) were “not UIP.” Differential diagnoses were not systematically recorded; in 12 cases, there was a differential diagnosis of CHP (n=3), NSIP (n=3), asbestosis (n=1), pneumoconiosis (n=1), and airway-centered interstitial fibrosis (n=2). Of the atypical features for UIP in Study I, abundant inflammatory cells were the most common (15/60, 25%). The distribution of inflammatory cells was mainly diffuse (n=9), but they were also noted only in lymphoid follicles and germinal centers (n=2). Giant cells were also noted to be quite a common finding (12/60, 20%), both interstitially and intra-alveolarly. OP was a rare finding (n=1). Other noted features were emphysema (n=8), RB (n=3), DAD (n=2), and DIP-like reaction (n=1). Of the atypical histological features, only giant cells were connected to UIP classifications as they existed more often in a non-definite UIP (10/22, 46% vs. 2/38, 5%, $p<0.001$). In Study III, the percentage in relation to the whole tissue area (area%) and density of FF, interstitial mononuclear inflammatory cells, and intra-alveolar macrophages were counted with the AI model (Study III, Table 3). Histograms of the area% for FF, interstitial mononuclear inflammatory cells, and intra-alveolar macrophages are expressed in Figure 10.

5.2 INTEROBSERVER AGREEMENT ON HISTOPATHOLOGICAL FEATURES (I)

In Study I, all pathologists agreed on the histopathological UIP classification by the 2011 criteria (Raghu et al. 2011) in nearly half of the cases (29/60, 48%). Out of the cases with full agreement, 28 were evaluated as “definite UIP.” The cases, whose classifications pathologists disagreed on, are shown in Table 9. Three cases were re-evaluated both as “definite UIP” and “not UIP,” and one case was re-evaluated both as “probable UIP” and “not UIP.” In these cases, one sample was noted to be of a small size, and normal parenchyma was lacking; two had giant cells, and CHP was suggested as a differential diagnosis; one was also noted to be small and have both emphysema and DIP-like reaction. There was a considerable variation in the interobserver agreement among pathologists measured with Cohen’s κ coefficient.

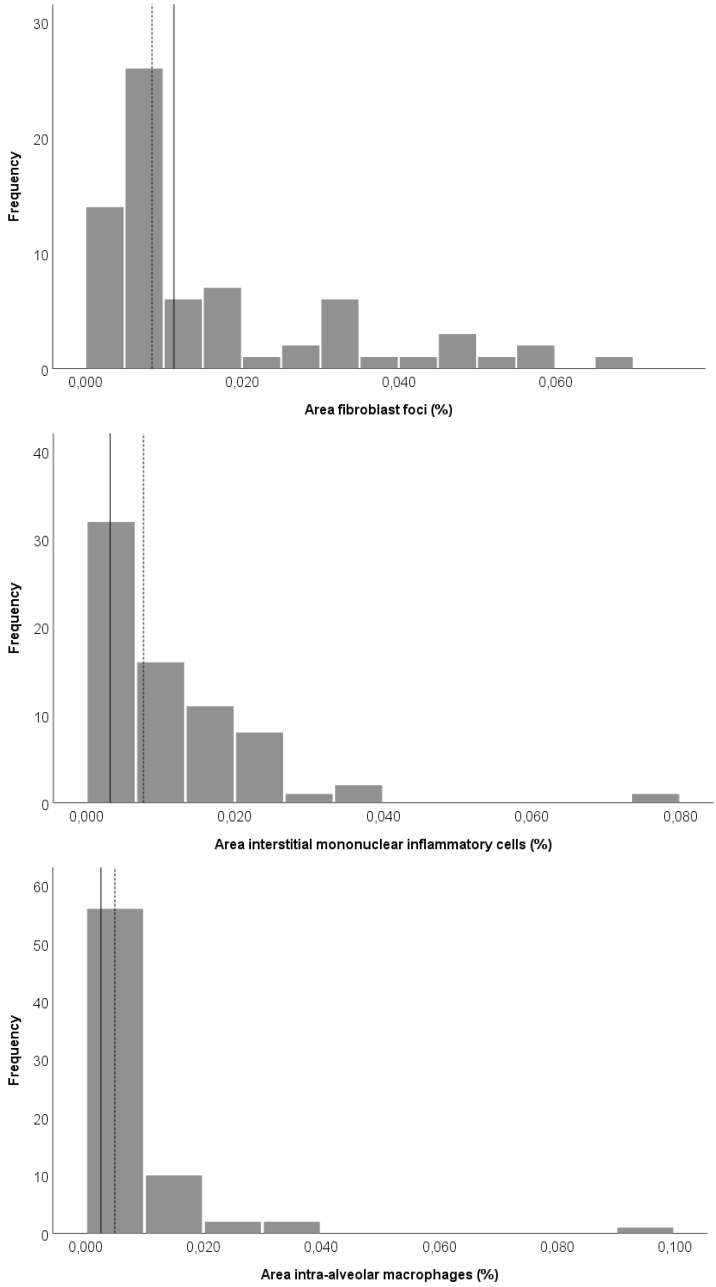


Figure 10 Histograms of the areas in relation to whole tissue of fibroblast foci, interstitial mononuclear inflammatory cells, and intra-alveolar macrophages. Cut-point values used in the survival analysis are expressed in black lines, and median values are expressed in black dotted lines.

Table 9 The histopathological classifications by the 2011 criteria of the 31 cases that pathologists disagreed in Study I.

Case	Observation 1	Observation 2	Observation 3	Sample type
1	UIP	Probable UIP	Possible UIP	Autopsy
2	UIP	UIP	Probable UIP	SLB
3	UIP	Probable UIP	UIP	SLB
4	Possible UIP	Possible UIP	Probable UIP	SLB
5	Probable UIP	UIP	UIP	SLB
6	Possible UIP	UIP	Probable UIP	SLB
7	Probable UIP	UIP	UIP	SLB
8	Not UIP	Probable UIP	Probable UIP	SLB
9	Possible UIP	UIP	UIP	Explant
10	Possible UIP	UIP	UIP	SLB
11	Possible UIP	UIP	Probable UIP	SLB
12	UIP	UIP	Probable UIP	SLB
13	Possible UIP	UIP	UIP	SLB
14	Possible UIP	UIP	UIP	SLB
15	Possible UIP	Probable UIP	Probable UIP	SLB
16	Probable UIP	UIP	UIP	SLB
17	UIP	UIP	Probable UIP	SLB
18	UIP	Probable UIP	UIP	SLB
19	Not UIP	Not UIP	Possible UIP	SLB
20	Probable UIP	UIP	UIP	SLB
21	UIP	Possible UIP	UIP	SLB
22	UIP	Probable UIP	Possible UIP	Autopsy
23	Not UIP	UIP	UIP	SLB
24	Possible UIP	UIP	UIP	SLB
25	Possible UIP	UIP	UIP	Explant
26	Possible UIP	UIP	UIP	Explant
27	Probable UIP	Possible UIP	Possible UIP	SLB
28	Not UIP	UIP	UIP	SLB
29	UIP	Not UIP	Possible UIP	SLB
30	Probable UIP	Probable UIP	UIP	Autopsy
31	UIP	Probable UIP	Possible UIP	Autopsy

UIP, usual interstitial pneumonia; SLB, surgical lung biopsy.

The crosstabulation between observations 1 and 2 are shown in Table 5 in Study I. The κ coefficient between observations 1 and 2 was slight (κ1=0.14), between observations 1 and 3 slight (κ2=0.04), and between observations 2 and 3 fair (κ3=0.37). The interobserver agreement was similar or slightly increased when only the agreement on SLBs (n=50) was measured (κ1=0.13, κ2=0.18, and κ3=0.41). A substantial interobserver agreement (κ=0.78) was reached for SLBs (n=50) when classifications were dichotomized into “definite and probable UIP” and “possible and not UIP.”

Of the additional histopathological features, interobserver variation was the greatest in the presence of giant cells (κ1=0.06, κ2=0.69, and κ3=0.20). Even though the κ2 value was only slight for histopathological categorization, the κ2 value for giant cells was substantial. Giant cells were detected with a different sensitivity; observations 1 and 3 recorded giant cells in 12 samples, whereas observation 2 recorded giant cells only in three samples. In samples categorized as “definite UIP,” giant cells existed in

two out of 12 samples by observation 1, two out of three samples by observation 2, and nine out of twelve samples by observation 3. The presence of giant cells was associated with “non-definite UIP” in observation 1 ($p<0.001$), but the association in observations 2 and 3 was statistically insignificant.

5.3 OBSERVATIONS ON THE DEVELOPMENT OF THE ARTIFICIAL INTELLIGENCE MODEL (III)

One of the biggest challenges for the CNN-based AI model was the recognizing lung tissue and air spaces, which are very simple tasks for the human eye. Alveolar space is similar to the blank background of the slide. Hence, the recognition of air spaces required a lot of training to avoid conflicting data problems. Furthermore, big bullae required a large enough field of view, whereas narrow alveolar septae required a smaller field of view. Finding an optimal field of view for tissue recognition took more time than expected. Surprisingly, detecting intra-alveolar infiltrates, or air spaces of compressed alveoli seemed not to be a major issue for the model.

During the development of the model, false-positive FF were a major issue. Especially perivascular fibrosis, bronchiolar epithelium, and honeycombing areas, if the recognition of an alveolar space had failed, were misinterpreted as FF. As discussed in section 4.3.2, teaching the difference between OP and FF was not possible due to the relatively small amount of OP lesions. Therefore, it was accepted to recognize OP as FF. The further development of alveolar space recognition solved the problem with honeycombing areas. The recognition of FF was also improved by introducing abundant examples of fibrotic lesions lacking the subepithelial location characteristic for FF, such as perivascular fibrosis and bronchiolar epithelium. The observation alleviated the importance of introducing CNN representative examples of features that were falsely interpreted as the target feature.

Detecting inflammatory cells both in the interstitium and in alveolar spaces were simple tasks for the AI model. However, it turned out to be more difficult to detect single interstitial mononuclear inflammatory cells than to detect lymphocytes in aggregates. The most significant source of error in recognition of intra-alveolar macrophages was the misinterpretation of alveolar space as the interstitium. An additional issue was narrow alveolar septae that sometimes resembled intra-alveolar macrophages. Hence, further developing the correct recognition of alveolar spaces was crucial for detecting intra-alveolar macrophages.

Consistency was one of the most vital elements of the training. Thus, only one person was responsible for annotating training areas. If conflicting data seemed to occur in borderline features, that is, if it was also difficult for a human observer to label the feature correctly, we did not train the model this feature, but let the model decide how to interpret the lesion. Even though we tried to teach the different intensities of HE

staining and artifacts as diversely as possible to the model, the model's performance was visually best in slides that resembled training slides. In addition, the quality of the sample seemed to affect the performance of the model.

5.4 VALIDATION OF THE ARTIFICIAL INTELLIGENCE MODEL (III)

For all 30 different validation areas, false positive, false negative, error, precision, sensitivity, and F1 score values are shown in Table 10. The visual results of all 30 validation areas are shown in Study III, Appendix 3, and visual matrices of each validation area are shown in Study III, Appendix 2. By a visual evaluation, most of the FF annotated by the pathologist were recognized by the model. According to the statistical confusion matrix of all 30 validation areas, the sensitivity of the model was 56.4% (the area of FF recognized both by the AI model and the pathologist, 0.480mm^2/the area of FF recognized by the pathologist, 0.851mm^2), and the specificity was 95.0% (the non-FF area recognized both by the AI model and the pathologist, 9.709 mm^2/the non-FF area recognized by the pathologist, 10.225 mm^2). In the AI model's repeated analysis for a subset of five slides, the results were similar between three runs of the AI model. The measurements are shown in Study III, Appendix 4.

Table 10 The validation of the recognition of fibroblast foci of the artificial intelligence model against the pathologist in 30 selected areas. Areas of fibroblast foci analyzed by the artificial intelligence model were compared against pathologist's annotations of fibroblast foci.

Value	Minimum	Maximum	Median
Validation area (mm^2)	0.002	0.1	0.03
False positive (%)	0	6.7	1.4
False negative (%)	0.1	5.2	1.0
Error (%)	0.6	9.9	2.9
Precision (%)	7.3	98.2	54.5
Sensitivity (%)	7.0	87.3	65.2
F1 score (%)	7.4	85.5	55.7

5.5 PROGNOSTIC MARKERS (I, II, III)

In Study I, II, and III, the mean follow-up time, i.e., the time between diagnosis date and lung transplantation, death or the end of follow-up date, lasted 5.1 years, 5.6 years, and 6.0 years, respectively. During the follow-up, 23 out of 60 (38%) patients, 37 out of 73 (51%) patients, and 37 out of 71 (52%) patients had succumbed, respectively. In each study, 13 (22%), 16 (22%), and 17 (24%) lung transplantations had occurred. In Study I, one patient, and in Study II and III, two patients were deceased after lung transplantation. The survival rates, namely the percentage of patients alive without transplantation at the end of follow-up, in Study I, II, and III were 42%, 30%, and 27%, respectively.

Of all the histopathological features studied in this thesis, the areas of FF, interstitial mononuclear inflammatory cells, and intra-alveolar macrophages had the strongest association with survival (p=0.01, p=0.01, and p=0.01, Study III). High amounts of FF marked a poor survival, whereas high numbers of inflammatory cells were associated with a prolonged survival (Figure 2, Study III). In Study I, however, we did not find abundant inflammation to be associated with survival. For evaluating the connection between FF and interstitial mononuclear inflammation in each sample, we divided the area of FF by the area of interstitial mononuclear inflammatory cells to create an FF/interstitial mononuclear inflammatory cell index value, which also was associated with survival (p=0.001, Study III). In addition to a poor survival, a high density of FF was associated with a low DLCO% at the time of diagnosis (p=0.03, Study III). A high area of intra-alveolar macrophages was associated with high FVC% (p=0.03, Study III).

The survival estimate of IPF patients with a histopathological pattern of “definite UIP” was not different from patients with non-definite UIP (p=0.8, Figure 1B, Study I). FVC% at the time of diagnosis was lower in the patients with a definite UIP pattern than those with a non-definite UIP pattern (mean 75.7%±13.5% vs. 81.4±20.7%). However, the difference was statistically insignificant (p=0.2, Study I). Otherwise, the UIP pattern in histopathology was not connected to lung function (Study I). None of the additionally analyzed histopathological features were associated with survival (Study I). Neither was the histopathological PM score associated with survival of IPF patients (p=0.08, Study II). When comparing survival between IPF patients that had either occupational history without known exposures (n=41), occupational history with known exposure to inorganic dust, chemicals or diesel (n=16), or occupational history with known exposure to organic dust (n=10), patients of the latter group had the shortest survival time (p<0.001). In radiology, “definite UIP” was associated with poor survival (p=0.03, Figure 1A, Study I). Moreover, patients with a histopathological pattern of “definite or probable UIP” and definite UIP on HRCT had a trend towards poor survival in comparison with patients having “inconsistent with UIP” on HRCT (p=0.008, Study I, Figure 1C).

5.6 PARTICULATE MATTER IN THE LUNGS OF IDIOPATHIC PULMONARY FIBROSIS PATIENTS (II)

In all of the samples, we observed both coal dust pigment and birefringent PM (Study II, Supplementary data, Table A.1). Coal dust pigment and inorganic PM scores correlated with each other (Spearman correlation, R=0.75, p<0.01), and we saw birefringent PM often within coal dust pigment (Figure 11). We observed both PM considered silicon dioxide (SiO_2) because of its weak birefringence, triangular form and small size, and PM considered silicates because of its strong birefringence, needle- or platy-shaped form. PM could be detected either widely distributed as single

particles or focally in high numbers as clusters, both among the fibrosis and adjacent to the bronchovascular bundles.

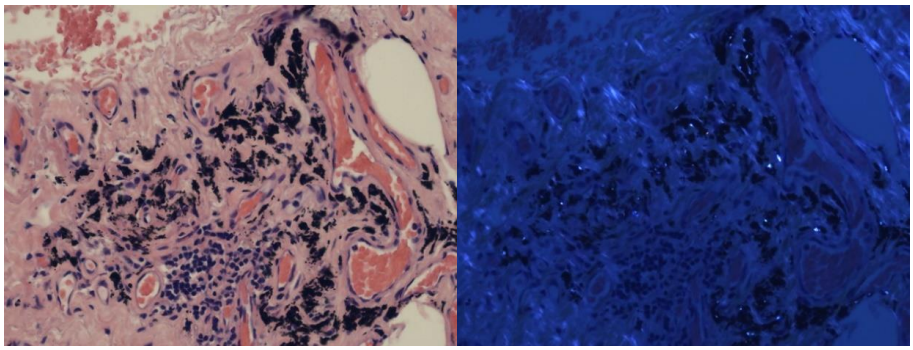


Figure 11 Birefringent inorganic particulate matter in polarizing light microscopy at 200x magnification.

Table 11 shows the population densities and mean PM2.5 and PM10 levels by university hospital districts and the whole Finland. In 2016, the mean $PM_{2.5}$ levels were higher in Helsinki, Turku, and Tampere compared to Kuopio and Oulu (6.0 ± 1.2 µg/m3 vs. 4.4 ± 1.7 µg/m^3, p=0.002), while the difference between the mean PM_{10} levels was insignificant (The Finnish Meteorological Institute). In 2018, the population density was 46 people/km^2 in southern university hospital districts, whereas in the northern districts, it was 7 people/km^2 (Statistics Finland). Inorganic particulate matter scores by five university hospital districts are shown in Table A.2 in the Supplementary Data of Study II. Lung tissue samples from Helsinki, Turku, and Tampere that have southern geographical location, had more often PM scores from 3 to 5 when compared to university hospital districts of Kuopio and Oulu (31/50, 62.0%, vs. 7/23, 30.4%, Fisher's exact test, p=0.02).

Table 11 Population densities, mean levels of particulate matter less than 10 µm in diameter (PM_{10}), and mean levels of particulate matter less than 2.5 µm in diameter ($PM_{2.5}$) in each Finnish university hospital district and in the whole Finland. The minimum and maximum particulate matter values are in brackets.

University hospital district	Population density *(people/km²)*[a]	Mean PM_{10} level *(µg/m³)*[b]	PM_{10} measuring sites *(n)*[b]	Mean $PM_{2.5}$ level *(µg/m³)*[b]	$PM_{2.5}$ measuring sites *(n)*[b]
Helsinki	88	13.1 (6.2-21.4)	23	6.4 (4.5-8.3)	19
Turku	33	11.2 (6.8-14.8)	12	4.9 (3.9-6.7)	3
Tampere	27	11.1 (10.3-12.0)	5	5.1 (4.6-5.3)	3
Kuopio	13	12.7 (2.7-25.3)	13	3.9 (1.9-5.3)	5
Oulu	5	10.0 (2.8-17.1)	9	4.8 (2.6-7.1)	5
Finland	18	12.0 (2.7-25.3)	62	5.6 (1.9-8.3)	35

[a] Statistics Finland [Internet]. Population density by area 1.1.2018 [cited 2018 Nov 5]. Available from: http://pxnet2.stat.fi/PXWeb/pxweb/en/StatFin/StatFin__vrm__vaerak/?rxid=ba16c87d-002c-4d08-96e5-de65713bc945.

[b] The Finnish Meteorological Institute [Internet]. Ilmanlaadun seurannan vuositilastot 2016 lähtien [cited 2019 Feb 14]. Available from: https://ilmatieteenlaitos.fi/ilmansaasteet.

Most of the patients had occupational data available (67/73, 92%, Study II, Table 1). Forty-one patients had a work history with no known exposures, sixteen had occupations related to inorganic dust exposure, and ten had occupations related to organic dust exposure. Seventeen patients had reported a history of known exposure to inorganic dust, including asbestos (n=6), metal dust (n=3), stone dust (n=3), glass dust (n=2), cement dust (n=1), diesel exhaust fumes (n=1), and sand dust (n=1). Eleven patients had a known exposure to organic dust: farming (n=7), cotton dust (n=2), flour (n=1), and wood dust (n=1). The highest PM scores of 4 and 5 in the samples were associated with the patients' known history of exposure to inorganic dust (p=0.004) and male gender (p=0.03). The PM score was correlated inversely with pack-years of smoking (R=-0.401, p=0.01, n=41); the patients with a sample of the highest PM score of 4 and 5 were either ex- (11/15) or never-smokers (4/15, p=0.048).

As an additional analysis, we tested if the PM score had any connection to the histological parameters analyzed in Study III, that is, the areas in relation to the whole tissue area of alveolar spaces, FF, interstitial mononuclear inflammation, and intra-alveolar macrophages. The area of alveolar spaces in relation to whole tissue was lower in samples with the highest PM score of 4 and 5 compared to samples with lower scores (mean of 25.1%±8.8%, n=14 vs. 35.0%±12.4%, n=57, t-test, p=0.007). The other parameters had no significant associations with the PM score. However, the area of interstitial mononuclear inflammatory cells tended to be higher in the samples that had the highest PM score of 4 and 5 in comparison with samples with lower PM scores (median of 0.015%, range of 0.026%, n=14 vs. 0.007%, 0.074%, n=57, Mann-Whitney U test, p=0.07).

6 DISCUSSION

6.1 HISTOPATHOLOGICAL FEATURES

As expected, we found the majority of the lung tissue samples of IPF patients to represent the definite UIP pattern. Studies reporting the prevalence of the histopathological classifications according to the 2011 guidelines (Raghu et al. 2011) have been limited. In a cohort of 241 biopsied IPF patients, Yagihashi et al. (2016) reported the prevalence of the histopathological "definite UIP," "probable UIP," "possible UIP," and "not UIP" pattern to be 78.0%, 17.4%, 3.7%, and 0.8%, respectively. The level of confidence for the UIP pattern was a little higher than in our smaller patient cohort of 60 patients. In the light of the results in Study I and the study by Yagihashi et al. (2016), it appears that from 20% to 30% of IPF lung tissue samples manifest with a non-definite UIP pattern. If the current criteria (Raghu et al. 2018a) had been used in Study I, the prevalence of non-definite UIP patterns would probably have been higher. As "possible UIP" required the absence of UIP features (Raghu et al. 2011), but "indeterminate for UIP" includes a UIP pattern with overlapping features suggesting an alternative diagnosis (Raghu et al. 2018a), probably some samples considered as a definite or probable UIP pattern would have had "indeterminate for UIP". In a recent study, the prevalence of the histological UIP pattern by the 2018 guidelines (Raghu et al. 2018a) was evaluated in two four-year periods before and after 2011 (Eldersveld et al. 2020). From two institutions, patients biopsied due to other causes than a tumor or other localized processes were searched. SLBs were evaluated by two pathologists. The number of biopsies had fallen by 51%. The number of biopsies that represented either a "definite UIP" or "probable UIP" pattern decreased from 34.5% to 16.7%. The result is not surprising, as the current guidelines (Raghu et al. 2018a) offer an option for definite IPF diagnosis without histopathological confirmation for IPF suspected patients with the probable UIP pattern on HRCT. Hence, it is likely that also in study population of IPF patients, the prevalence of non-definite UIP pattern in SLBs has increased. The finding highlights the challenges in diagnosis for ILD patients undergoing SLB. Therefore, the role of MDD as the gold standard in IPF diagnosis is justified, even though interobserver variation between MDD groups has been reported (Walsh et al. 2016b).

Even though we had a well-defined cohort of IPF patients, the histopathological features varied among samples. Abundant inflammation was seen in 25% of the samples (Study I). Our observation is, however, consistent with other studies (Takemura et al. 2012, Rabeyrin et al. 2015, Yagihashi et al. 2016). In a recent study, the percentage of IPF patients that had a tissue sample manifesting with histological autoimmune features (plasma cell infiltration, lymphoid aggregates, and germinal

centers) was 35% (Kim et al. 2020). When compared to the UIP-like pattern in CHP, the prevalence of interstitial inflammation or lymphoid follicles in IPF lung tissue is lower (Takemura et al. 2012). In a small recent study comparing IPF and CHP, minimal to mild interstitial inflammation was found in all samples and the number of lymphoid aggregates did not associate with CHP diagnosis (Wright et al. 2020). According to the current histopathological criteria of IPF, areas of interstitial inflammation, not adjacent to fibrosis, lead to the pattern of indeterminate for UIP or alternative diagnosis (Raghu et al. 2018a). The current criteria do neither make a clear statement on the amount of the interstitial inflammation or lymphoid follicles that are allowed in IPF/UIP nor for other single histological features. In Study III, we showed that quantitating FF and inflammatory cells in IPF samples is possible. However, giving solid values or proportions for a single histological feature is challenging or even impossible, as the biopsy needs to be assessed compehensively and in relation to other histological features. Since patients with suspected IPF are biopsied less than before, the amount of atypical histological features for UIP will increase. The guidelines should be updated on the atypical features, and more detailed instructions how these features affect the histopathological diagnosis of IPF are warranted.

6.2 INTEROBSERVER VARIATION BETWEEN PATHOLOGISTS

Despite using the well-defined diagnostic criteria for IPF (Raghu et al. 2011) in a cohort of patients of which IPF diagnosis had been re-evaluated, there was a marked interobserver variation among pathologists in Study I. In the few studies using the current diagnostic criteria (Raghu et al. 2018a, Troy et al. 2020, Nemoto et al. 2021), the interobserver agreement on SLBs has been moderate, which is higher than in most of the studies using the 2011 criteria (Raghu et al. 2011). Thus, interobserver variation would probably have decreased in Study I if the current criteria (Raghu et al. 2018a) had been used. The most critical situation in interobserver variation is when one pathologist evaluates a sample as “definite UIP,” whereas another pathologist would interpret it as “not UIP.” Three cases in our cohort of 60 samples (5%) represented this situation. In the study of Hashisako et al. (2016), in which 20 fibrotic IIP lung tissue samples were re-evaluated by 11 pathologists, over half of the cases had been assessed both as “definite UIP” and “not UIP” by different pathologists. In studies of the interobserver agreement on UIP features, the study setting is usually blinded, like in our study as well, which is not the case when evaluating the samples in real life. It would have been interesting to analyze the interobserver agreement after exposing the pathologists to clinical and radiological data, and also to each other’s observations.

The use of κ coefficient is one explanatory factor for generally low interobserver agreement in our study. Most cases, where the pathologists were in disagreement, were classified either “definite UIP,” “probable UIP,” and “possible UIP.” After MDD,

all of these classifications could lead to IPF diagnosis, whereas “not UIP” in histopathology is considered to exclude IPF diagnosis (Raghu et al. 2011). The unweighted κ coefficient does not take into account the weight of the differences between classifications. However, using the weighted κ coefficient is statistically more complicated and is often not comparable to other studies. The moderate κ values (≥0.40) are considered acceptable for a diagnostic test (McHugh 2012).

The maximum interobserver agreement seemed to be reached when the classifications as “definite and probable UIP” and “possible and not UIP” were dichotomized, similarly to the study of Hashisako et al. (2016). In the COLDICE study (Troy et al. 2020) in which the results of SLBs and TBLCs taken simultaneously from the same patients were compared, the interobserver agreement between pathologists was calculated both using the 2018 diagnostic guidelines for UIP (Raghu et al. 2018a) and the individual intepretation of the ILD pattern. By individual interpretation, 60% of SLBs were defined as “UIP-IPF,” whereas by the 2018 guidelines (Raghu et al. 2018a) only 33% of SLBs were categorized as “UIP” or “probable UIP” (Troy et al. 2020). Perhaps a dichotomized approach to the UIP patterns simulates the best way for pathologists to interpret samples in routine pathology, which is why it seems to produce the best interobserver agreement. It is unknown why the level of confidence decreases when the four categories of the 2018 IPF guidelines are used (Raghu et al. 2018a) in comparison with the traditional histopathological approach. It is a phenomenon that should be further investigated. In the next update of the diagnostic criteria, combining the current diagnostic categories of “UIP” and “probable UIP” as one and “inderminate for UIP” and “alternative diagnosis” as an another might be an option to consider.

In Study I, pathologists mainly agreed on the definite UIP pattern, and atypical histological features seemed to cause the most significant interobserver variation. Atypical features in IPF seem to be a source of discrepancy also in other studies (Yagihashi et al. 2016, Jo et al. 2019). Especially the interobserver agreement on giant cells varied. There was particular variation between the sensitivity to detect them and the effect of their presence on the level of confidence of the UIP pattern. To my knowledge, this is a novel finding and reflects the diagnostic challenge in differentiating IPF from CHP. None of the modern diagnostic criteria of IPF (Raghu et al. 2011, Lynch et al. 2018, Raghu et al. 2018a) make a detailed statement on the relationship of giant cells to IPF. In the current guidelines, the presence of giant cells suggests patterns of “indeterminate for UIP” or “alternative diagnosis” (Raghu et al. 2018a). The presence of giant cells strongly favors the diagnosis of CHP. However, approximately one-third of both IPF and CHP patients do not reach a high confidence on diagnosis in MDD even after SLB (Takemura et al. 2012, Wright et al. 2020). Out of 31 samples from 15 patients clinically suspected CHP, five samples expressed the UIP pattern (Trahan et al. 2008). In the study of Morell et al. (2013), nearly half of IPF patients were re-diagnosed as CHP after thorough re-evaluation. The recent diagnostic criteria for HP by the ATS/ERS/JRS/ALAT do not give a description of the quantity of giant cells needed for the diagnosis of fibrotic HP/CHP either (Raghu et

al. 2020). In non-fibrotic HP, giant cells or loose clusters of epithelioid cells are required for the definite cellular HP pattern and they should be located both in peribronchiolar intestitium and peribronchiolar air spaces and possibly be associated with OP (Raghu et al. 2020). For fibrotic HP, giant cells are not required but if present, they support the finding of fibrotic HP (Raghu et al. 2020). Focusing on the differentiation of IPF and CHP in the next update of the diagnostic criteria of IPF might be useful in increasing interobserver agreement. The current guidelines (Raghu et al. 2018a) do not mention the interobserver variation neither among pathologists nor radiologists or clinicians. The issue should be addressed in the next update of the criteria, perhaps as a recommendation for a getting a second opinion in biopsies that do not manifest with the definite UIP pattern.

In addition to not giving specific details on quantities of specific histological features, the current diagnostic guidelines do not provide exact instructions on what the significance of a certain feature is in relation to other histological features. By the current guidelines (Raghu et al. 2018a), all of the “features favoring either a pattern other than UIP or features favoring UIP secondary to another cause” exclude “UIP” or “probable UIP.” This statement neither takes into account how many of these features can exist in “indeterminate for UIP,” nor when “alternative diagnosis” would be more suitable. The guidelines by the Fleischner Society state more strongly that “a UIP pattern with ancillary features strongly suggesting an alternative diagnosis” leads to “features most consistent with an alternative diagnosis” (Lynch et al. 2018). If the diagnostic guidelines are strictly followed, “alternative diagnosis” in histopathology exludes the diagnosis of IPF in MDD. Atypical histopathological features, such as granulomas, OP, and inflammatory cells, have been associated with the diagnosis of CTD instead of IPF (Moua et al. 2014). In a recent study, non-IPF UIP biopsies were seen to have more extensive lymphocytic infiltration, non-caseating granulomas, airway centered inflammation, small airway disease, and acute lung injury (Lee et al. 2021). However, these features are also seen in the lung tissue of patients with a re-evaluated diagnosis of IPF, and more detailed instructions how to differentiate IPF from non-IPF UIP histopathologically are required. Recently, a combination score of plasma cell infiltrations, lymphoid aggregates, and germinal centres in biopsies of IPF patients predicted a more favorable disease course than with IPF patients lacking them (Kim et al. 2020). This alleviates the importance of combinations of different histopathological features, instead of analyzing single features. The next guidelines could be improved by giving a detailed analysis of each histopathological feature that can be seen in biopsies of patients with IPF and by explaining what kind of combinations of different features would favor an alternative diagnosis.

6.3 ARTIFICIAL INTELLIGENCE IN HISTOPATHOLOGY

In Study III, the AI model was capable of learning to identify FF and inflammatory cells, even in a small training set of 20 samples. To my knowledge, the histopathology

of IPF has not been analyzed previously with AI. The help of AI seemed to be especially useful when analyzing inflammatory cells. Manually, the absolute numbers of inflammatory cells would be practically impossible to count. They are structurally more simple objects than FF and easy to learn for AI. In addition, AI could identify the interstitium and alveolar spaces and quantitate inflammatory cells from these spaces without immunohistochemistry. Since our finding of high inflammatory cells being associated with prolonged survival of patients with IPF was a novel one, it indicates that the association could not have been found without the help of AI. Recently, the density of FF was noted to vary between UIP-IPF and non-IPF UIP (Lee et al. 2021), which supports our observation of the quantitation of histological features being useful (Lee et al. 2021). Hence, AI provides new approaches to the histopathological analysis of prognostic biomarkers of IPF.

In the validation of FF in the 30 selected areas, our AI model reached only a sensitivity of 56.4%, which indicates just a moderate performance of the model. When inspecting the model's visual results, however, the recognition of FF seemed to be at an acceptable level for most FF. In the validation of the model, we used pathologists' annotations as the ground truth that the AI model's performance was compared against. In AI studies, pathologist's evaluation is often used as the reference standard. The phenomenon of "gold standard paradox" affects the validation of algorithms for histopathological analysis (Aeffner et al. 2017). Manual pathology scoring is more qualitative or semiquantitative instead of truly quantitative as AI can be. Even the most experienced pathologists are prone to multiple unintentional biases and to both intraobserver and interobserver variation (Aeffner et al. 2017). Moreover, AI might even find endpoints that are beyond human visual perception. For example, an AI model analyzed the surrounding stroma of breast ductal carcinoma in situ lesions and found novel morphological markers that could predict the grade of the tumor (Dong et al. 2014). In the future, validation of the AI models might be more often compared with other AI models instead of a pathologist. Therefore, the visual evaluation of the AI model's results is crucial instead of only relying on the model's performance statistics.

The moderate sensitivity of our AI model has also other explanations. Firstly, our training set was small, although increasing the sample size does not always improve the deep learning model (Holland et al. 2020). However, the intensity and quality of HE staining varies between slides and pathology laboratories, which alleviates the need to teach all kinds of HE staining intensities and artifacts in slides. In addition, a small training set might have caused data overfitting. This means that the AI model does not function optimally for slides that it has not previously encountered but works well for training slides. Data overfitting is a problem that CNNs often encounter, and one solution to manage the issue is to increase the training set. A larger training set could also allow recognition of other rarer histological features, as more examples of the feature could be provided. Secondly, FF and interstitial mononuclear inflammatory cells were included in the same layer of the model. The recognition of FF would have benefitted from a larger field of view, since we had to keep a small field of view for

inflammatory cell detection. The small field of view probably increased the counting of FF-like spots towards FF. Overall, the tissue inspection is different between AI and the human eye; a human observer is capable of changing the field of view from large to small, whereas our model was restricted by the field of view it was given. Thirdly, FF is quite a complex structure, and its morphology varies a lot between samples, mainly depending on the intensity of HE staining. Thus, giving more examples of different FF in multiple slides would probably improve the model's sensitivity.

To my knowledge, Study III is the first to represent the use of AI in the histopathological analysis of IPF lungs. To develop an AI model suitable for clinical use would require much more additional work. In the field of AI, many issues need to be solved before implementing AI to routine pathology. There are still many technical obstacles. For example, each slide's file size is giga-pixels, so the storing of multiple slides requires a lot of storage space, and the analysis of a slide requires a lot of computing power. Currently, whole slide imaging (WSI) with CNN can even take hours, which is not the case for routine analysis of a slide by a pathologist. Naturally, WSI of CNN and a pathologist are not fully comparable, as the tasks vary; for example, the absolute quantitation of inflammatory cells would be practically impossible for a human observer. Furthermore, the inspection of AI is limited to its field of view. AI inspects the whole slide "through a small window," whereas a pathologist can acquire much information by the "first look" at a low magnification. The ability to mimic the pathologist's way of examination, that is, first inspecting the slide with low magnification and then selecting regions of interest for the use of high magnification, could speed up WSI of AI. As we observed during the AI model development, what can be easy for the human eye, such as the recognition of lung tissue and alveolar spaces, might be difficult for AI. To conclude, a CNN-based AI model trained with supervised learning is as good as its training is. The AI model's capacity is limited to data that it has been taught. Due to the limitations related to the use of AI, it seems very unlikely that AI models could overrule pathologists soon. Combining AI's ability to produce accurate, reproducible, and quantitative data with the pathologist's skills could provide synergy in the histopathological evaluation, especially in IPF or other rare ILDs as the patients are biopsied more rarely than in the past.

6.4 HISTOPATHOLOGICAL PROGNOSTIC MARKERS

Histopathological UIP itself is a useful marker of poor prognosis when compared to other histopathological ILD patterns (Bjoraker et al. 1998, Nicholson et al. 2000). In Study I, the level of confidence on the UIP pattern by the 2011 diagnostic criteria (Raghu et al. 2011) did not impact on the survival of IPF patients, similarly to the study of Hashisako et al. (2016). In Study I, however, patients with "definite and probable UIP" in histopathology combined with "definite UIP" in radiology seemed to lead to a more reduced survival than the patients with "definite and probable UIP" in histopathology and HRCT of "inconsistent with UIP." The results of previous studies

concerning the histopathology-radiology concordance have been controversial (Flaherty et al. 2003c, Sumikawa et al. 2008, 2014, Yagihashi et al. 2016). Altogether, the level of confidence on the radiological UIP pattern seems to be a better marker of survival than the level of confidence on the histopathological UIP pattern (Sumikawa et al. 2014, Romei et al. 2015, Salisbury et al. 2017).

In Study III, we could confirm the previous finding of FF associating with a dismal survival (King et al. 2001, Nicholson et al. 2002, Enomoto et al. 2006, Tiitto et al. 2006, Lee et al. 2011, Harada et al. 2013). FF probably represent aberrant wound healing essential to IPF pathogenesis, as FF have been noted to associate with minute lesions of alveolar damage (Emura et al. 2015). The exact role of FF in IPF pathogenesis is not fully known, and besides, many studies have not been able to confirm the association between FF and poor prognosis for IPF patients (Flaherty et al. 2003a, Collard et al. 2007, Hanak et al. 2008, Triantafillidou et al. 2011). Recently, FF were shown to be linked with AEs, in addition to mortality (Kishaba et al. 2020), which supports the theory of FF participating in the pathogenesis of IPF. To my knowledge, there are no results on FF being associated with prolonged survival. Patient and sample selection probably explain some of the controversial results. As the diagnostic criteria of IPF have been evolving only since the 2000s, it is possible that other non-IPF ILD patients were included in the earlier studies. For example, in some studies (King et al. 2001, Collard et al. 2007) the enrollment of IPF patients had begun in 1984 prior to the release of international diagnostic criteria of IPF. Also in the earlier studies, the definition of FF was not as specific as it is today. King et al. (2001) scored “interstitial young connective tissue” that can be considered as FF, and also “intra-alveolar granulation tissue” that can be considered as intraluminal fibrosis or OP. In the survival analysis of Flaherty et al. (2003a), only when nine CTD-UIP patients were included in the group of 99 IPF patients, FF were associated with poor survival. In the study of Collard et al. (2007), the study material was a subset of SLBs in the study of King et al. (2001); however, only the earlier study of King et al. (2001) reported the association between FF and a poor prognosis. Hanak et al. (2008) selected only clinically stable IPF patients, that is, no AEs or death within one month after SLB. The method was justified by the unreliability of the evaluation of FF during AE. Moreover, biopsies with extensive OP or DAD overlying UIP were excluded, and intraluminal FF were not counted towards the FF score (Hanak et al. 2008). The number of biopsies is also often small, which is why the lack of statistical power might make it impossible to show the association with survival. The IPF patient cohort in Study III (N=71) is relatively large compared to other studies on FF and IPF survival.

Different methodologies explain some of the variation in the study results of FF and the survival. Semiquantitative scoring, which is always subjective and prone to intra- and interobserver variation, has been primarily used in older studies (King et al. 2001, Nicholson et al. 2002, Flaherty et al. 2003a). However, Nicholson et al. (2002) showed a strong positive correlation between counting the FF and a semiquantitative score. Nicholson et al. (2002) used a semiquantitative Brompton score of 0-6, whereas Flaherty et al. (2003a) used a semiquantitative Michigan score of 0-3. Enomoto et al.

(2006) showed that even though quantitative FF scores correlated with semiquantitative scoring methods, quantitative scores within semiquantitative scores varied. In a study population of 114 biopsied patients with IPF, a high FF score was shown to be associated with increased mortality in a univariate Cox analysis, but not in a multivariate Cox analysis (Kim et al. 2020). Besides, the difference in FF score between alive and deceased patients with IPF was statistically insignificant, which alleviates the statistical method's effect (Kim et al. 2020). When quantitating FF, some studies have analyzed only the areas that have been most prominently affected by UIP (Nicholson et al. 2002, Lee et al. 2011). In some studies, honeycombing areas have been excluded from the analysis (Nicholson et al. 2002, Enomoto et al. 2006).

The novel finding of Study I was the prognostic impact of inflammatory cells in IPF lung tissue. Both increased interstitial mononuclear inflammatory cells and intra-alveolar macrophages were associated with prolonged survival, and intra-alveolar macrophages were also associated with high FVC% (Study III). The finding was not explained by increasing FF numbers replacing tissue affected by inflammation, as FF and interstitial mononuclear inflammatory cells positively correlated with each other. Besides, high FF/interstitial mononuclear inflammatory cell index values were connected with a poor survival. Most of the previous evidence favors interstitial lymphocytes to be markers of a poor survival and accelerated disease progression (Nicholson et al. 2002, Daniil et al. 2005, Parra et al. 2007, Todd et al. 2013, Balestro et al. 2016). However, the only evidence for a link between increased histological interstitial mononuclear inflammation and poor survival in IPF has been from a study of 20 patients before the 2011 guidelines (Parra et al. 2007, Raghu et al. 2011). In larger studies, no such association has been demonstrated (Nicholson et al. 2002, Collard et al. 2007). In BAL fluid, decreased numbers of lymphocytes have been shown to be associated with dismal survival for IPF patients (Ryu et al. 2007, Song et al. 2011) and ILD patients with AE (Salonen et al. 2020a). Furthermore, anti-inflammatory therapies in IPF have mainly been proven either inefficient or even harmful (Raghu et al. 2008, 2015, Raghu et al. 2012, Parker et al. 2018, Raghu et al. 2018b).

Recently, IPF patients with histological autoimmune features, defined as plasma cell infiltration, lymphoid aggregates, and germinal centers, were proven to have a better prognosis than IPF patients lacking these features (Kim et al. 2020). Concordant to our novel finding, a low germinal center score was associated with increased mortality in a multivariate Cox analysis (Kim et al. 2020). In addition, patients that had succumbed had a lower score for lymphoid aggregates ($p=0.03$), plasma cell infiltration ($p=0.11$), and total inflammation ($p=0.07$) (Kim et al. 2020). Evidence supporting these findings also exists in older studies. Lymphocytic inflammation outside fibrotic areas has been associated with improvement in FVC% at six months (Collard et al. 2007). In a study by Selman et al. (2007), the numbers of interstitial inflammatory cells in SLBs did not differ among rapid and slow progressors. In areas of loose fibrosis adjacent to the alveolar epithelium, compared to areas of dense fibrosis, increased interstitial lymphocyte numbers have been noted (Nuovo et al.

2012). The genes associated with lymphocyte migration are upregulated in the preserved alveolar epithelium, and on the other hand, downregulated in the dense fibrosis (Jonigk et al. 2019). High numbers of mononuclear interstitial inflammatory cells are seen in the lung tissue of IPF patients in areas away from dense, end-stage fibrosis. It might indicate a compensatory mechanism against the fibrosing process. Yet, the role of interstitial inflammation as the promoter of pulmonary fibrosis cannot be excluded. Interstitial mononuclear inflammatory cells can have either profibrotic properties, such as Th2 cells, or antifibrotic properties, such as Th1 cells, or both like Tregs. Investigating the subtypes of lymphocytes in the IPF lung tissue would probably provide more information. In all, interstitial mononuclear inflammation seems not to be a significant contributor in the end-stage pulmonary fibrosis, whereas it is seen in the preceding phases of pulmonary fibrosis.

The prognostic impact of intra-alveolar macrophages in IPF lung tissue has not been widely studied. In two studies preceding the international consensus criteria of IPF, “desquamation”/alveolar space cellularity or intra-alveolar accumulation of pigmented macrophages was not associated with survival (King et al. 2001, Collard et al. 2007). A semiquantitative score reflecting the numbers of intra-alveolar macrophages did not associate with survival of IPF patients (Kim et al. 2020). Antifibrotic treatment does not seem to affect the amounts of intra-alveolar macrophages (Zhang et al. 2019b). A very low positive correlation has been noted between intra-alveolar macrophages and change in FVC% at six months follow-up (Nicholson et al. 2002). In a small study of twelve IPF patients, macrophages both in the interstitium and in alveolar spaces correlated inversely with FVC% and FEV1% (Daniil et al. 2005). High circulating monocyte counts lead to poor survival for IPF patients (Scott et al. 2019). Intra-alveolar macrophages in IPF lungs that are deficient in transferrin receptor (CD71-) have been associated with worse survival (Allden et al. 2019). However, in BAL fluids of ILD patients with AE, a decrease in the numbers of macrophages has been recently noted (Salonen et al. 2020a). The controversial findings might suggest that different types of macrophages, from different origins, could have a different impact on the IPF lung tissue. Further investigations on the role of inflammation in IPF lung tissue conducted with the current well-defined diagnostic criteria are needed.

6.5 INORGANIC PARTICULATE MATTER

We did not confirm our hypothesis of the histological inorganic PM being a marker of a poor survival (Study II). In an additional analysis, the highest PM scores in lung tissue samples were associated with a low amount of alveolar spaces. A trend towards high numbers of interstitial mononuclear inflammatory cells was noted. It is indeed likely that PM and interstitial mononuclear inflammation can be more easily spotted from more fibrotic tissue. If the high amount of birefringent PM is seen in more fibrotic lungs, does causality exist, and which comes first? PM is also known to promote inflammation, and the association suggests that histologically observed PM is not a bystander but has direct effects on the lungs. To my knowledge, Study II was

the first to report the amounts of PM seen in polarizing light microscopy in a study population of IPF patients sized like ours. A key finding was that inorganic PM scores were higher in samples from patients from the southern Finnish university hospital districts (Helsinki, Turku, and Tampere) compared to samples from the northern districts (Kuopio and Oulu). The histological PM could be a sign of chronic exposure to air pollution, which is known to increase the incidence of IPF (Conti et al. 2018). The finding is also in line with the previous studies that have reported an association between the location of residence and increased IPF mortality (Johnston et al. 1990, Hubbard et al. 2000, Harris, Cullinan and McDonald 2001) and also increased fibrotic lesions (Souza et al. 1998, Brauer et al. 2001, Churg et al. 2003). In a recent study in the Catalan region of Spain, the most significant air pollutant sources, PM levels, and patients with IPF were mapped, and the areas with high PM levels had a higher prevalence of IPF in comparison with the areas of low PM levels (Shull et al. 2021). In global comparison, the PM levels in Finland are low (World Health Organisation 2018). In southern Finland, the accepted daily $PM_{2.5}$ concentrations are episodically exceeded due to long-range transport fine particles (Niemi et al. 2009). The finding suggests that a threshold level of exposure to air pollution exists when PM starts cumulating in IPF lungs.

The highest inorganic PM scores were associated with a history of inorganic dust exposure, male gender, and ex-smoker status (Study II). However, no current smokers had the highest inorganic PM scores, and PM score correlated inversely with pack-years (Study II). Tobacco smoke seems not to be the primary source of histopathological PM in IPF. It also raises the question of whether occupational and environmental exposure history is taken into account to the required extent when diagnosing IPF. The relevance of an exposure history when suspecting IPF is also hard to estimate (Trethewey and Walters 2018). Elderly patients might not recall past exposures, and besides, the results of case-control studies might also be affected by a recall bias (Trethewey and Walters 2018). Our registry data was gathered from medical records, and its systematicness on the assessment of exposure history cannot be guaranteed. The lack of systematicness when gathering occupational or environmental exposure history is an issue, and future investigations with a detailed exposure history are required. The guidelines for diagnosing IPF could also give more detailed instructions on the evaluation of exposure history when suspecting IPF.

6.6 STRENGTHS AND LIMITATIONS

The FinnishIPF registry, from which our study population was gathered, is a strength for this study. The diagnoses of IPF have been re-evaluated for every patient, which is essential when studying IPF (Kaunisto et al. 2015). Concerning the approximated prevalence of IPF in Finland, the participation rate of IPF patients in Finland is high. Patients originated from all of the five university hospitals. However, the most fragile patients do not usually participate in studies that require informed consent as the FinnishIPF registry does. Hence, our study population mainly represents patients with

a stable, slowly progressing disease. In Finland, the survival of patients is easy to follow via The Population Information System. Still, patient registries also have limitations. The gathering and maintenance of the data in a registry is laborious and prone to human error. The study population in the registries is heterogeneous compared to clinical trials. This is also their strength as they reflect real-life patient populations better (Culver et al. 2019). Especially when evaluating disease course and prognosis, long-term follow-up provided by the registry is beneficial (Culver et al. 2019).

Our study cohort of IPF patients with lung tissue samples was relatively big, when it is taken into account that IPF is a rare disease and and only a minority of patients undergo SLB. In Study I, the SLB rate was 19%, which is acceptable when considering that approximately one-third of IPF suspected patients would need a histopathological confirmation for the diagnosis (Kaarteenaho 2013). It creates a selection bias if only patients with lung tissue samples are investigated. The most fragile patients do not undergo SLB. IPF patients undergoing SLB represent patients that have an atypical clinicoradiological manifestation of the disease. This probably explains some of the high variation of histopathological features and low interobserver agreement. However, we had a significant amount of patients with definite UIP on HRCT (25/60, 25%) and also explant and autopsy samples that might represent typical IPF better. In addition, biopsies represent only a fraction of the lungs. This limits the interpretation of the results in Study II and III, in which only one representative sample was analyzed. However, we aimed at selecting the most representative slide for UIP pathology.

One of the greatest limitations is the lack of control groups. As fibrotic lungs are rarely biopsied, that is practically never done to completely healthy lungs. Possible sources for control lung tissue could have been: 1) lung tissue from autopsies from patients that have no known respiratory disease, but their poor quality is an issue, 2) bullous emphysema samples that are also limited in numbers, 3) cancer samples, 4) explant samples from other than IPF patients, which also forms only a small group of patients, and 5) SLBs of other ILD patients, also the small number of samples being a limiting factor. In particular, the PM amount in samples from non-IPF patients would have been of interest in Study II. Furthermore, the association between the location of residence and PM amount in the lung tissue would have been interesting to analyze in greater detail.

The lack of validation is a significant limitation in Study II. Neither was the AI model in Study III validated in an external validation set that would be required for the development of a generalizable AI model. In Study III, the detection of inflammatory cells was based only on morphology in HE staining. For example, intra-alveolar macrophages and desquamated alveolar epithelial cells cannot be indubitably differentiated from each other without immunohistochemistry. Moreover, the methods in Study II and III were only developed in lung tissue samples of patients with IPF. The results are, therefore, merely suggestive, and no firm conclusions can be drawn.

However, in Study II and III, our aim was not to create clinical applications but to introduce novel methods. As a result of the novel methods in Study II and III, we were able to produce new, hypothesis-generating information on IPF lung tissue.

6.7 IMPLICATIONS AND FUTURE PERSPECTIVES

Histopathological features in the lung tissue of IPF patients can affect the survival. The strongest markers of survival are FF, which is supported by our results and previous evidence. Even though it is the most studied prognostic marker, the evidence for the association with a poor prognosis is not explicit. In addition, inflammatory cells also seem to be markers of survival in IPF despite the fact that inflammation is currently considered as an epiphenomenon in the pathogenesis of IPF. Contradicting the previous evidence, we found elevated numbers of interstitial mononuclear inflammatory cells and intra-alveolar macrophages to be associated with prolonged survival. The finding was probably enabled by our novel AI-based method, and it also encourages developing further AI models for the analysis of IPF patients' lung tissue. The quantitative power of AI can provide new approaches to the disease whose etiopathogenesis is still obscure.

The role of histopathology in the diagnosis of IPF has decreased during the past two decades, and it is likely that the trend will continue. However, a need for lung tissue might increase the number of biopsies if they can be used as a source of molecular markers for different ILDs (Pankratz et al. 2017, Raghu et al. 2019, Kheir et al. 2020). Recent evidence suggests that patients with other types of progressive pulmonary fibrosis also benefit from antifibrotic medication (Distler et al. 2019, Flaherty et al. 2019, Maher et al. 2020, Wells et al. 2020). In progressive fibrosing ILDs other than IPF, antifibrotic medication is currently an option after first-line therapy (Wijsenbeek and Cottin 2020). The findings do not yet eliminate the requirement of a histopathologically confirmed diagnosis, which was stated in a recent position paper (George et al. 2020). Patients with systemic sclerosis-associated ILD, CHP, autoimmune ILD, idiopathic NSIP, and unclassifiable progressive pulmonary fibrosis, that formed the study population in the trials, had undergone prior, ineffective treatments and manifested a progressive disease (Distler et al. 2019, Flaherty et al. 2019, Maher et al. 2020, Wells et al. 2020). Therefore, the study populations did not represent the situation of the first manifestation of the disease. At the first manifestation of a radiological probable UIP pattern, 34% of the patients have other IIP than IPF (Fukihara et al. 2020) and might benefit from immunosuppressive treatment. The exact timing of the antifibrotic treatment is not fully known but starting the antifibrotic treatment in a mild disease severity is likely be more helpful than later in the disease course. Hence, early definite diagnosis still remains important. In the end-stage pulmonary fibrosis phase, a diagnosis of "chronic progressive pulmonary fibrosis" might be enough. In a small recent study, IPF patients that had histopathologically confirmed diagnoses had prolonged survival (Pannu et al. 2019). Although the finding should be interpreted with caution, the prognostic aspects of

histopathological confirmation should be taken into account in the next update of IPF guidelines.

TBLC is a very promising, less invasive type of histopathological confirmation in comparison with SLB. TBLC seems to be a safer procedure, at least when it comes to mortality, and the diagnostic yield is acceptable (Romagnoli et al. 2019, Troy et al. 2020, Zaizen et al. 2019). Its sensitivity on the UIP pattern seems reasonable, but the differential diagnosis, especially between IPF and CHP, can be difficult when there is less tissue in TBLC samples (Troy et al. 2020). The data on the safety of TBLC has been mainly conducted on study populations that have been evaluated to be eligible also for SLB. Thus, we do not know if TBLC would be a safer procedure also for more fragile IPF suspected patients. In future studies, if the mortality associated to TBLC is low, and no alarming evidence appears on the safety profile of TBLC, SLB might only be performed for patients whose previous TBLC was non-diagnostic. Further research on patients undergoing both TBLC and SLB is necessary, although exposing study patients for risks of both TBLC and SLB is challenging.

In the histopathology of IPF, interobserver variation still exists, even though the diagnostic criteria have been specified during the last decade. Different interpretations of features that are considered atypical for IPF, such as giant cells, might explain the finding. The UIP pattern associated with atypical histological features is categorized as “indeterminate for UIP” (Raghu et al. 2018a). In the situation of having both radiological and histopathological “indeterminate for UIP,” the guidelines offer no help in differentiating IPF from non-IPF, as shown in Table 3. Here, the meaning of MDD is emphasized the most. Instead of distinct diagnostic assessment pathways for clinicians, radiologists, and pathologists, the guidelines could offer statements on how clinicoradiological information affects the histopathological analysis (Smith et al. 2020). In the next update of the histopathological criteria of IPF, having a focus on specifying the relationship of atypical histopathological features to IPF would decrease the interobserver variation. Nevertheless, MDD in the diagnosis of IPF is essential.

In the future, investigating the histopathological features in non-fibrotic parenchyma might help to understand IPF pathogenesis and perhaps in developing the histopathological IPF criteria. Macroscopically normal IPF lung tissue has been seen to represent areas of OP and NSIP (Todd et al. 2016). In the non-fibrotic areas of IPF lung tissue, minute lesions of alveolar damage can be observed (Emura et al. 2015). The gene expression differs between the fibrotic and non-fibrotic areas in UIP lung tissue (Jonigk et al. 2019). The numbers of macrophages and lymphocytes are increased in normal-appearing IPF lung tissue in comparison with non-fibrotic control lung tissue (Nuovo et al. 2012). The analysis of the normal-appearing lung tissue might help in the differential diagnosis of IPF (Jonigk et al. 2019). In addition, incidental interstitial lung abnormalities in patients without clinical pulmonary fibrosis are acknowledged as predictors for mortality (Hatabu et al. 2020). Further investigations of the progression of abnormalities into clinical pulmonary fibrosis could

help in the understanding the pathogenesis of IPF. AI might also help with these features that may be overlooked in a clinical setting.

Inorganic dust in the lung tissue of IPF patients was not a marker of survival in Study II. Its high amounts were associated with living in an area of high PM and population density, even in Finland that is a country of low air pollution levels, and also with an exposure history, despite the idiopathic nature of the disease. It is possible that inorganic dust contributes to or modulates the pathogenesis of IPF, and more information on this potential relationship is required. Our finding also emphasizes the meaning of documenting detailed occupational and exposure history from IPF suspected patients.

Like the past and outdated concepts of the etiology and pathogenesis of IPF have shown, it is likely that the understanding of IPF as disease changes in years to come. With the current methods used, we might be unable to identify the cause behind the disease. As the murine models of IPF represent pulmonary fibrosis of a known cause, the analysis of IPF lung tissue might provide essential insight into pathobiology and targets for treating the fatal disease.

7 SUMMARY AND CONCLUSIONS

1. Most of the lung tissue samples in a well-defined cohort of IPF patients represent a definite UIP pattern. Still, a significant proportion of the samples manifest with a non-definite UIP pattern. Interobserver agreement among pathologists varies on the UIP pattern. Marked inflammation and giant cells are a relatively common finding in IPF lung tissue, even though they are considered atypical for IPF. A different sensitivity to detecting and interpreting histopathological features, especially giant cells, partly explains the high interobserver variation among pathologists. The histopathological UIP pattern or additional histopathological features were not associated with survival, whereas the radiologically definite UIP pattern was strongly associated with a dismal survival. The findings alleviate the importance of MDD in the diagnosis of IPF.

2. Using polarizing light microscopy and SEM, coal dust pigment and inorganic PM were found in varying amounts, and the elemental composition of PM in the IPF lung tissue was analyzed. The difference in PM amounts was partly explained by the air pollution levels and population density. High amounts of PM was connected with known exposure to inorganic dust. The histological PM did not associate with the survival of IPF patients. There might be a threshold limit of exposure to air pollution that leads to the accumulation of inorganic PM in the fibrotic lungs.

3. With an AI-based method, we confirmed FF being markers of a poor survival in IPF. We found a novel association between high numbers of inflammatory cells and prolonged survival. AI could measure the interstitial and alveolar inflammatory cells separately, which is a benefit compared to manual methods. AI can help in the quantitation of histopathological features in IPF lung tissue samples. Evaluating the role of inflammation in IPF lung tissue requires further research.

In all, histopathology still has a role in the diagnosis of IPF despite the development of HRCT. However, IPF suspected patients should be biopsied only electively and before the end-stage phase of the disease to avoid the mortality associated with the biopsy procedure. A biopsy does not eliminate the need for MDD, nevertheless. The clinical relevance of independent histopathological features in the lung tissue of patients with IPF is still obscure. Defining the diagnosis of IPF suspected patients has an impact on the treatment strategy. Studying the histopathology of IPF, possibly with the help of AI, gives information on the prognosis, and might help in search for the etiology and the cure of the disease.

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