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Clinical characteristic, psychometric properties, novel diagnostic methods and outcomes in interstitial lung diseases.

(Klinische Charakteristik, psychosoziale Merkmale, neue Diagnostikmethoden sowie Überlebensanalysen bei interstitiellen Lungenerkrankungen)

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1. Introduction

1.1. Interstitial Lung Diseases. Idiopathic Pulmonary Fibrosis

Interstitial lung diseases (ILD, also diffus parenchymal lung diseases, DPLD) comprise over 200 heterogeneous entities with lung fibrosis as a common trait [1]. The group is very diverse regarding etiology, therapy, and outcomes. ILD may occur due to a known cause such as drugs, connective tissue disease (CTD), hypersensitivity to inhaled organic antigens (hypersensitivity pneumonitis, HP), or sarcoidosis, whilst others, the idiopathic interstitial pneumonias (IIPs), have no identifiable origin [2]. Of them, idiopathic pulmonary fibrosis (IPF) is associated with most burden and poor prognosis [3–5]. Globally, the incidence of ILD and especially of IPF is rising, which is also associated with an economic healthcare burden [6].

The overwhelming fibrotic processes and the distortion of the lung´s ultrastructure result in a progressive loss of pulmonary compliance and a decline in gas exchange properties [7]. Thus, the natural history of progressive ILD is characterized by a decline in lung function, worsening of symptoms and health-related quality of life, and early mortality, especially in familial forms [5, 8]. Greater impairment in forced vital capacity (FVC) or diffusion capacity of the lungs for carbon monoxide (DLco), and a greater extent of fibrotic changes on a highly resoluted computed tomography scan (HRCT), are known predictors of mortality in ILD patients [9].

Although significant progress in the understanding of the pathogenesis of ILDs has been made, the natural course, progression factors, biomarkers, and the response to the treatment of an individual patient can still not be reliably predicted [4, 10, 11].

Many of the ILD entities are characterized by a progressive- fibrosing phenotype, with a worsening of respiratory symptoms, faster decline of lung function, limited response to antifibrotic or immunomodulatory therapies, decreased health –related quality of life (HRQoL) and, as a consequence, early death [12]. In some cases (e.g. IPF), the patient’s survival is still limited despite the introduction of novel antifibrotic therapies [3, 13].

1.1.1. Epidemiology and genetics

The most progressive fibrosing ILD type, Idiopathic pulmonary fibrosis (IPF) is a chronic and usually fatal type of ILD that typically affects elder patients beyond 60 years of age [14]. The prevalence of the disease has been estimated at 2–29 per 100000 persons and its incidence is about 3-9 per 100 000 persons per year [15]. The natural course of IPF ranges from slow progression with long periods of stable disease to a rapid progressive fibrosis, with successive lung function impairment and death within first 2 years upon diagnosis; the average survival from the time of diagnosis is estimated as three to five years [16].

The incidence of IPF is increasing worldwide [17].The most important known prognostic determinants for mortality in IPF are decline in lung function, acute exacerbations as well as pulmonary hypertension [18]. Nadrous et al found that a younger age at onset of the disease isn't associated with a better prognosis, observing no clinical, radiological, and histopathological differences to the patients over 50 years of age [19]. It is also obvious that men are more likely to be affected by IPF than women [13]. Additionaly, the vast majority of IPF patients (apart from familial cases) are current or previous smokers [20].

In IPF, myofibroblasts progressively accumulate in the lung interstitium in so-called "fibroblast foci", which leads to an increased deposition of collagen and other extracellular matrix in the lung interstitium. To date, some signaling pathways have been linked to persistent fibroblast activation in IPF; strong evidence suggests that TGF-β-1 plays a key role in these processes [21]. It is also known, that tyrosine kinase-induced intracellular signaling cascades stimulate fibroblast proliferation, in part via vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), connective tissue growth factor (CTGF) as well as platelet- derived growth factor (PDGF) [22].

In IPF development, the persistent epithelial cell damage of the lung plays the key role in genetically susceptible individuals [23]. The publications of study group of Guenther et al showed that the type II alveolar epithelial cells (AEC II), which synthesize, secrete and recycle all components of pulmonary surfactant, are of outmost importance in the IPF pathogenesis [24]. An impaired function of surfactant protein could be determined as an additional pathogenetic factor, leading to an alveolar collapse [25]. Surfactant proteins A (SP-A) and D (SP-D) of alveolar epithelial cells type II, could potentially be a biomarkers of mortality prediction, exacerbations and progression of IPF [23].

According to a currently favored concept, IPF is a “two-hit” disease, comprising a genetic predisposition affecting epithelial homeostasis, and an environmental stressor, including exposure to known noxious dusts and fumes (e.g. asbestos, silica, outdoor pollution, and cigarette smoke) [26, 27]. The strongest epidemiological evidence for a genetic predisposition is the observation of familial clustering, which has been reported in monozygotic twins raised apart, in consecutive generations of families, and in family members separated at an early age [28]. As opposed to sporadic IPF (s-IPF), familial form of IPF (f-IPF) is defined by the presence of at least two cases of IPF in the same family [29]. In families of IPF patients, 2% to 20% of subjects reported to have a first degree relative with ILD [29, 30]. Despite the clear proof of monogenetic transmission in the pathogenesis of f-IPF’s, the penetrance appears to vary substantially, resulting in a clinically apparent disease in some cases within four months after birth, whereas others members of the same family were over 60 years of age at first diagnosis [31, 32].

In fact, any IIP of known or unknown cases can occur in a familial setting and be related to genetic cause [33]. Around 80% of all patients with familial idiopathic interstitial pneumonia (f-IIP) receive the diagnosis IPF, about 10% of f-IIP patients might have the diagnosis of NSIP and another 10% of IIP patients stay as unclassifiable IIP or diagnoses split between remaining IIP entities [34–36]. The reported degree of relation for a diagnosis of f-IPF ranges from 3rd degree, to 2nd degree and to a required first degree relationship [35, 37]. Other authors describe the degree of relationship with "from the same primary family" or "from the same biological family" [38–40]. The relative frequency of f-IPF varies between 0.5% to 9.5% of all IPF patients [3, 33, 41]. An f-IIP appears to be present in 10% to 19.5% of all IIP cases [42, 43].

Many studies have been looking for genetic associations in IPF; during the last years, mutations in components of the shelterin or the telomerase complex as well as in the surfactant proteins A and C have been identified to cause IPF [44, 45]. About a quarter of familial IIP cases can be explained in some studies by specific gene mutations [46]. The telomere length seems to play an important role in IPF development in general [47, 48]. Telomere shortening was found to be below the 10th percentile in 15% of all f-IIP patients and in 10% of s-IIP patients [49]. As a result, cells may be prone to cellular senescence, which in return may provoke fibrogenesis [50]. The presence of short telomere length is associated with poor prognosis in IPF patients [28].
The protein portion of telomerase is encoded by the TERT gene (TERT = telomerase reverse transcriptase), mutations of TERT are the most frequently identified in f-IPF and

are present in up to 15% of cases [29, 37, 44]. Other mutations responsible for monogenetic transmission of IPF affect the coding genes for the surfactant proteins C and A2, which result in expression of misfolded proteins that accumulate in the endoplasmic reticulum (ER) or the lysosomal compartment of alveolar type II cells, thereby causing ER stress and apoptosis [36, 51].

Last but not least, the influence of comorbidities (e.g. pulmonary hypertension, gastroesophageal reflux, chronic obstructive pulmonary disease, lung cancer, pulmonary embolism, diabetes, as well as psychosocial impairments) has to be taken into account, negatively influencing the course of the disease [5, 52–55]. Of them, pulmonary hypertension is especially associated with increased mortality in IPF [56, 57].

1.1.2. Diagnostics in ILD (IPF)

The diagnosis of ILD involves the teamwork of multiple specialists, together with the ability to interpret and to implement complex clinical data patterns, as well as to integrate uncertain or conflicting information. The interpretation of the history and physical examination of the patient to develop a clinical context, is an essential first step to the correct diagnosis, followed by recognition of the HRCT pattern and, if necessary, histological results seen on lung biopsy samples [58].

Safe diagnosis of ILD's can be notoriously difficult, since the number of differential diagnoses is usually large and the clinical and radiographic (HRCT) phenotype patterns can be incomplete or overlapping [4, 58]. For this reason invasive procedures such as cryobiopsy during bronchoscopy or open lung biopsy are regularly needed, and are crucial for setting the diagnosis, but are also prone to serious side effects including bleeding, pneumothorax, triggering of exacerbation and death [59]. Prior to IPF diagnosis, alternative causes of fibrosing ILD, broadly grouped into systemic and exposure-related disorders, need to be excluded [16].

Differential diagnosis of ILD can be challenging, and requires detailed consideration of clinical, radiological and histopathological features by a multidisciplinary team (MDT) [60]. Hence, there is a great need for novel and specific, non-invasive diagnostic methods. Reliable, sensitive, and objective diagnostic and prognostic biomarkers could allow distinction between different forms of ILD and assessment of the risk of

deterioration [11]. Up to now, however, there is no such biomarker being in clinical routine use (except HRCT) to support such diagnostic process.

In up to 20% of ILD patients, a specific diagnosis cannot be defined, even after thorough MDT discussion. In such situation the disease should be labeled as an “unclassifiable ILD” (uILD) [61]. An ILD might be labelled unclassifiable for various reasons. As suggested by Hyldgaard et al., in most cases, the inability to certainly classify ILD is the result or combination of the following aspects: 1) a biopsy was not performed (and clinical and radiological data were insufficient for accurate diagnosis); 2) histopathological results were inconsistent or overlapping; 3) discrepancy between clinical, radiological and pathological findings; and 4) significant overlap between clinical and/or radiological features [61]. Common histological patterns of uILD are often overlapping, including e.g. usual interstitial pneumonia (UIP) with bronchiolo-centric fibrosis, NSIP with organizing pneumonia, UIP with non-specific interstitial pneumonia (NSIP)-like changes and UIP with diffuse alveolar damage [62, 63]. Further studied factors for diagnostic assessment are a fibrotic score and the percentage of fibroblastic foci [64].

Traditionally, physiological measurements are used for the evaluation of disease severity and these are characterized by a restrictive phenotype (reduced forced vital capacity (FVC) and vital capacity (VC)), where declines of lung volumes are linked to disease progression [9]. Typically, also the diffusing capacity (DLco) is reduced and a decline of DLco may frequently precede decreases of lung volumes [65]. Furthermore, the gender-age-physiology (GAP) staging system is an easy to-use predictor of the progression course in ILD [66]. The GAP stage, which is based on the Fine-Gray competing risk models by Ley et al uses gender, age, and two pulmonary function results (percent predicted FVC and percent predicted DLco) [67]. Advanced GAP stage has been significantly associated with aging, reduced lung function, low PaO2, and higher mortality rate [66, 68].

The use of HRCT has been suggested for the further identification of progressive disease, providing a direct morphological measure for disease extent [4, 69]. The actual ATS/ERS/JRS/ALAT guideline has clearly outlined the use of HRCT as an essential component for the identification of a pattern of usual interstitial pneumonia (UIP) as the morphological correlate of progressive ILD [13].
On HRCT, UIP is characterized by honeycombing, which is crucial for definite diagnosis, reticular opacities and traction bronchiectasis as well as absence of other patterns (e.g. consolidation) suggestive of other diseases. The preferred distribution is typically basal

and peripheral, though often patchy [69, 70]. The term “ground glass attenuation” denotes the presence of a diffuse homogeneous increase in lung density. In other conditions, ground glass attenuation is almost uniformly associated with the presence of inflammatory cells in the alveolar or interstitial space (i.e. alveolitis). In IPF, it is thought that ground glass attenuation is predominantly associated with patchy fibrotic thickening of alveolar septa and less related to alveolar inflammation. This is proven by existence of traction bronchiectasis in the respective region [71].

Biomarker research has been undertaken using peripheral blood, bronchoalveolar lavage fluid (BALF), and exhaled breath condensate (EBC) [72]. Numerous molecules involved in alveolar epithelial cell injury, proliferation and matrix remodeling as well as immune regulation have been proposed as potential biomarkers [11]. As example, in previous studies in IPF, epithelial proteins such as KL-6, Surfactant protein (SP)-A, SP-D and other factors, e.g. indicating oxidative stress (e.g. serum hydroperoxide), have been suggested as biomarkers able to discriminate between stable and progressive disease (e.g. CCL18 in serum) or to indicate response to therapy (e.g. Toll interacting protein) [73–76]. Likewise, tissue samples based biomarkers, such as the number of fibroblast foci, the level of Ki-67(a marker of tissue proliferation), and caspase-3 (a marker of tissue apoptosis) were falso ound to have prognostic implications [77].

However, there is currently no established biomarker in routine use for diagnosis and assessment of prognosis in ILD, and this holds particularly true for non-invasive markers. With regard to non-invasive markers, one pathway of interest for non-invasive detection is the free radical nitric oxide (NO), which is regulated by three isoforms of nitric oxide synthase (NOS), neuronal NOS, inducible NOS and endothelial NOS (eNOS). Several reports showed that NO plays an important role in the development of pulmonary fibrosis [78, 79]. NO is an important physiological and pathophysiological messenger that belongs to the reactive nitrogen species and acts in the pulmonary system as vaso- and bronchodilatory neurotransmitter, also showing some inflammatory properties and being a marker of oxidative stress.

The fractionated exhaled nitric oxide (FeNO) can be detected with commercially available portable devices. FeNO has been shown to be useful for the diagnosis of eosinophilic airway inflammation especially in asthma, and for determining steroid responsiveness in chronic respiratory inflammation, with a focus on avoiding unnecessary steroid therapies [80–82]. With regard to the role of exhalative biomarkers in ILD, some investigations have already been carried out. For example, increased FeNO

values had been previously demonstrated in HP and IPF patients. Other volatile substances of potential interest are prostaglandin E2 (PGE2) and 8-isoprostane. PGE2 is a known antifibrotic mediator that plays an important role in pathogenesis of wound resolution and also been shown to be an important negative regulator of fibroblast activation and collagen expression released by an intact alveolar epithelium [83, 84].

Inhalation of PGE2 in combination with selected siRNA(s) was recently proposed in some experimental studies for the treatment of IPF [85]. 8-isoprostane also acts a marker for oxidative stress, in which case the substance is produced by peroxidation of the arachidonic acid. This biomarker shows biological activity as a pulmonary and renal vasoconstrictor. It has also been shown to contract human smooth muscle cells from the lungs in vitro and to increase platelet activity [86, 87]. Some studies have been done on 8-isoprostane in ILD patients, but its role as a marker of oxidative stress remains unknown [88].

Further of interest are electronic noses (eNoses) , which are artificial sensor systems, usually consisting of a range of sensors for various chemicals of interest; being able to detect patterns of volatile organic compounds (VOC) in exhaled breath and then use pre-established algorithms for classification of the ‘breath print’ for comparison with previously recorded samples [89]. The concept of the eNose is that metabolic and biochemical processes occurring in different diseases give rise to specific patterns of endogenous VOC, resulting in a “volatolome” or a VOC signature which could be evaluated by eNose’s chemical sensors, and serve as possible markers of some inflammatory, microbial, oxidative and neoplastic conditions [11, 90]. Applications of the eNose technology has already been implemented in the food and beverage industry, in monitoring air quality, as well as in the detection of explosive and chemical agents [91].

Lung cancer (LG) is not only a relevant ILD comorbidity, but also one of the most common causes of cancer-related death worldwirde. CT-based LC screening programs have proven to be effective in reducing cancer-specific mortality, but with high number of false positive results. Therefore, LC diagnostic would profit from a reliable non-invasive and radiatio-free test; as such, the eNose technology could represent a valuable and complementary addition [89].

1.1.3. Treatment options in ILD (IPF).

The therapy of ILD is challenging because of the heterogenitiy and despite continuous development of therapeutic approaches; still, it has not yet been possible to establish a definite curative treatment option [92, 93]. In general, the treament regimes vary, depending on underlying cause. For example, ILD due to HP would be treated with antigen removal and immunosuppressants; cryptogenic organizing pneumonia (COP) can be effectively treated with steroids [94, 95]. The progressing fibrosing interstitial manifestations of rheumatic diseases, e.g. systemic scleroderma (SSc-ILD) or rheumatoid arthritis can be slowed down by causal treatment of underlying disease, e.g. with help of anti-TNF-a-blockers, mycophenolate mofetil (MMF), rituximab, as well as with other immunmodulators and immunsuppressants, being subject to recent clinical trials [96, 97].

Changes in the knowledge on etiology and pathogenesis of IPF, technical improvements in radiological diagnostics, as well as new treatment modalities resulted in a modification of the existing ATS/ERS/JRS/ALAT diagnostic guidelines in 2011 as well as in 2018 [13, 98]. Based on the findings of new therapeutic studies, a new ATS/ERS/JRS/ALAT Clinical Practice Guideline for treatment of IPF was revised in 2015 [99]. Here, a triple therapy with prednisolone, azathioprine, and acetylcysteine was not classified as being suitable for IPF; furthermore, the patients also should not receive any anticoagulation with warfarin or imatinib as causal therapy option due to low to moderate confidence in effect [99].

Although it is still not possible to cure IPF, it is now promising to be able to slow down the progression of the disease with use of novel antifibrotics: pirfenidone (Esbriet®) and nintedanib (Ofev®) [100–102]. The survival expectancy under antifibrotics may increase by 2.47 years and more as compared to best supportive care [103].

Pirfenidone is an oral antifibrotic drug with pleiotropic effects, which has been shown to regulate important profibrotic and proinflammatory cytokine cascades by inhibiting fibroblast proliferation, collagen biosynthesis, and production of TNF-α and TGF-ß [93, 104, 105]. In both, CAPACITY and ASCEND studies pirfenidone slowed the FVC decline and improved survival in IPF patients [106–108]. Nintedanib (previously known as molecule BIBF 1120) is an intracellular inhibitor of several tyrosine kinases that targets multiple profibrotic kinase-mediated signaling cascades, including vascular endothelial growth factor, fibroblast growth factor, and PDGF receptors [109, 110].

The last remaining treatment option is the lung transplantation. The International Society for Heart and Lung Transplantation recommended in recent guidelines that suitable recipients have a DLco >39%, FVC decline ≥10% within last six months, reduction of oxygen saturation (SaO2) below 88% in the 6-minute walk test (6MWD), as well as a honeycomb pattern in the HRCT [111–113]. The relative contraindication is a patient age over 65 years; the 5-year survival is estimated at about 50-56% [114, 115].

As the natural course of IPF is quite heterogeneous, and as the response to the novel anti-fibrotic drugs has been reported to show great variability, it is essential to identify reliable predictive factors indicating the risk of deterioration and the response to medical treatment, as well as side effects in a broad non-selected patient cohort. In addition, data collected in the frame of the numerous controlled clinical trials undertaken so far in IPF, here especially in the placebo arms, have provided important insights into the clinical course of IPF. It should, however, be kept in mind that patients recruited into these studies represent a rigorously selected population and do therefore not necessarily reflect the characteristics of IPF subjects seen in clinical routine.

2. Aims of this research

The aims of this research were to provide clues for a better understanding of clinical characteristics and natural course of ILD patients (using baseline and follow up data from eurIPFreg as data source), evaluating dependence of ILDs on environmental factors, comorbidities, progression factors, as well as response to therapy.

Also we intended to profoundly evaluate phenotypical differences and outcomes of familial and unclassifiable ILD cases. Further, we addressed psychometric characteristics in IPF using the Short Form-36 Health Status Questionnaire (SF-36), providing detailed information about psychosocial impairments and HRQoL in ILD patients.

Moreover, we analysed to usefullness of novel diagnostic methods in ILD, such as diagnostic and prognostic role of diverse exhalative breath biomarkers together with the analysis of ability of electronic nose technology to recognize ILD and LC.

3. Results

In the study "The European IPF registry (eurIPFreg): baseline characteristics and survival of patients with idiopathic pulmonary fibrosis" we assessed baseline data of 525 IPF subjects (attachment 1) [3]. The European IPF Registry (eurIPFreg) and the European IPF Biobank (eurIPFbank) were set up in the frame of the European IPF Network, a research consortium funded by the European Commission under the FP7 program from 2008 until 2011 (see www.pulmonary-fibrosis.net) in order to better explore the pathogenesis and natural course of ILD, especially of IPF, and also in order to facilitate translational research in biomaterials from IPF subjects.

The eurIPFreg is an Internet-based, multicenter registry and is now core element of the project "Registries and Biobanks in Pneumology" of the TransMIT, the Intellectual Property Agency of the Justus-Liebig-University Giessen, with Prof. Andreas Günther as the main Coordinator [3]. In the eurIPFreg, a broad range of clinical data and biomaterials from European IPF patients is collected in a longitudinal fashion [1, 116]. The objectives of the registry are to provide clues for a better understanding of IPF phenotype sub-clusters, triggering factors and aggravating conditions, regional and environmental characteristics, and of disease behavior and management [116].

The patients were eligible for the enrolment if they were at least 18 years old, had IPF (prevalent or incident cases) or other ILDs (as comparator group) as diagnosed by the expert site, and had provided written informed consent prior to the inclusion. In order to facilitate research, inclusion of other lung diseases such as LC, chronic obstructive pulmonary disease (COPD) as well as healthy subjects or family members as additional comparator groups was made possible in the frame of an amendment in March 2013.

On a local level, each patient's IPF diagnosis was evaluated in a MDT discussion including at least chest physicians, pathologists and radiologists on the basis of the respective ATS/ERS/JRS/ALAT guidelines [13, 98]. The registry had no explicit exclusion criteria, thereby reducing selection bias. The clinical data acquisition took place primarily via patient and physician baseline questionnaires, comprising 1700 parameters, which could be retrieved upon logging in to the website www.pulmonary-fibrosis.net at the time of enrolment (baseline) and in intervals 3 to 12 month thereafter (as dictated by clinical routine) [3]. The patient questionnaire included patient's demographics, a detailed medical history making use of the WHO classification, complaints as well as report of co-morbidities. Alongside with this questionnaire, each patient also received HRQoL- questionnaires, among them the EQ-5D, the SF-36 and

the Mahler Index questionnaire [5, 117, 118]. These documents were available in different languages and were printed out on e-paper format and with an individual pseudonym on each page, allowing scanning and automated computer entry upon manual fill-out of the form.

The physician questionnaire contained data of physical examination and laboratory tests, pulmonary function, radiology, echocardiography, results of 6 MWD as well as other information concerning relevant patient's diagnosis and therapy; the form had to be filled out online in English language. Import of historical medical data was also possible. Medication was assessed making use of the official WHO list of drugs 2018 from WHO Collaborating Centre for Drug Statistics and Methodology, allowing categorial (group-wise) analysis of co-medication (www.whocc.no/atc_ddd_index).

Next to the baseline data, follow-up data were obtained in a similar way, making use again of patient and physician questionnaires. These questionnaires consisted of a smaller number of items of the baseline questionnaires, but also included additional aspects relevant for the further course of the disease such as information on intermittent respiratory infections, working status, transplantation or any changes in the medication. In case a patient was deceased, the site investigator was asked to document this in the registry, including the underlying reasons of death (if known). In addition to the data as provided by the questionnaires, HRCT and any other digital formatted images could be uploaded; during this procedure, the patient's personal data were replaced by the pseudonym.

Biological materials such as blood, bronchoalveolar lavage fluid (BALF) and tissue samples as well as exhaled breath condensates together with eNose profiles were centrally recorded and managed through generation of patient-, time-, and specimen-specific Lab IDs and they were stored both, locally as well as in the centralized eurIPFbank located in Giessen.

After entry into the registry, each case was checked by a documentation officer for data quality and for internal plausibility of medical data and the diagnosis of IPF (e.g. hints for collagen/vascular diseases or HP in patient questionnaires). Also, in 2015 and 2016, all previous IPF cases diagnosed as having IPF according to the previous guideline from 2000, were evaluated if they would also fulfil the guideline criteria from 2011. No central reading of HRCT or histology samples was performed.

Quality of data was improved by introduction of internal plausibility checks, in which different items were put into a logical context, causing the generation of queries in case inconsistent entries were noted (e.g. if physician´s and patient´s report were not consistent with regard to signs of underlying collagen/vascular disease). These queries were addressed to the respective site investigator, asking for clarification of the issue. In addition, site investigators were asked to conduct on-site data verification.

In the period between 2009 and 2019, the following sites recruited patients: Universities of Giessen and Marburg Lung Center, Germany and the nearby Agaplesion Lung Clinic Waldhof-Elgershausen; Competence Center for Rare Pulmonary Diseases of Hopital Bichat in Paris, France; Interstitial Lung Disease Unit of Royal Brompton Hospital in London, United Kingdom; Reference Center for Rare Pulmonary Diseases, Centre Hospitalier Universitaire Dijon-Bourgogne, France; Dept. of Clinical and Molecular Biomedicine of Università degli Studi di Catania, Italy; Vienna University Hospital, Austria; Hospital Clinico San Carlos in Madrid, Spain; Department of Pulmonology Semmelweis University in Budapest, Hungary; Thomayer Hospital in Prague, Czech Republic; Ospedale San Gerardo in Monza, Italy; Università degli Studi di Napoli Federico II, Italy, and Ruhrlandklinik, University Hospital of Essen, Germany.

The software solution underlying the registry (secuTrial®) was developed by the German Parkinson Network and is certified by the Food and Drug Administration (FDA) as well as the European Medicines Agency (EMEA). The patients' data were transferred via a secure internet-based data collection form and all data entries were based on the unique patient's encrypted ID number ("pseudonym"). The data protection concept was confirmed by local and national networks such as the TMF (Technology, Methods, and Infrastructure for Networked Medical Research e.V.) and official authorities (e.g. Hessian Data Protection Officer, Protocol Nr. 412101 from 25.08.2008).

Both, eurIPFreg and eurIPFbank have also been reviewed and received positive votes from institutional review boards in Germany (e.g. Ethics Committee of Justus-Liebig-University of Giessen; 111/08), France, Italy, Austria, Spain, Czech Republic, Hungary and the UK. The research was conducted strictly according to the principles of the Declaration of Helsinki. The eurIPFreg and eurIPFbank are listed in ClinicalTrials.gov (NCT02951416).

Between November 2009 and October 2016, a total of 1086 ILD patients were enrolled into the eurIPFreg. The distribution of ILD is shown in Figure 1: next to the 525 patients

with IPF, there were 561 patients with other ILD, who had a mean age of 65.2 ± 12.9 years, of them being 63.7% male. The most common clinical symptoms in these 561 non-IPF ILD cases were insidiously increasing dyspnea (85.9 %), dry cough (50.4%) as well as fatigue (70.6%). A smoking history was reported by 64.7% of participants and 6.3% continued to smoke at the time of the enrolment. Despite profound evaluation, 158 patients (14% of all recruited patients) were found to be unclassifiable after MDT discussion.

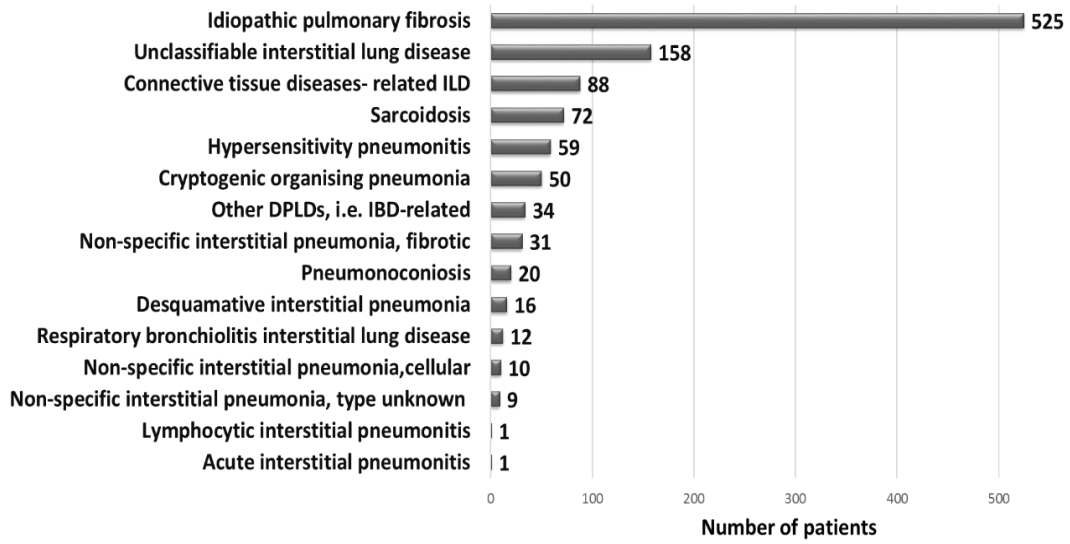

Figure 1. Distribution and diversity of ILD diagnoses in the eurIPFreg cohort. Data are presented as patients' numbers per diagnosis. IBD inflammatory bowel diseases; DPLD diffuse parenchymal lung diseases.

With regard to the 525 IPF patients included in the eurIPFreg, the baseline characteristics are outlined in Table 1. Of all IPF patients, 18.64 % had a familial history of IPF or other ILD (Grades A-C).

Table 1. Clinical baseline characteristics of the IPF cohort

Demographic parameters	Value
Ethnical origin (% of the whole IPF cohort)	
Caucasian	67.6
African	3.8
Indian	1.3
Polynesian	1.0
Missing data (Patients did not provide the ethnic origin)	26
Male (%)	73.7
BMI	
mean value + SD (kg-m²)	27.2 ± 4.6
Familial IPF (% of the whole IPF cohort)	
Grade A - Direct relative suffers or died at IPF / NSIP	9.5
Grade B - Direct relative suffers or died from IIP	4.4

Grade C - Direct relative suffers / died from non-classified lung disease	4.8
Age at diagnosis mean value ± SD (years)	 65.2 ± 11.6
Age at enrollment into the registry mean value ± SD (years)	 68.1 ±11.1
Time between onset of symptoms and inclusion into eurIPFreg median; q1-q3 (months) mean value ± SD (months)	 36.5; 19.2 - 70.3 59 ± 3.85
Time between onset of symptoms and diagnosis median; q1-q3 (months) mean value ± SD (months)	 6; 1 - 25.5 21.8 ± 3.49
Smokers/ Ex-Smokers/ Never-Smokers (%)	4.0/ 65.4/ 30.6

Abbreviations: BMI - body mass index, SD - standard deviation

The most common clinical symptoms reported by the patients are shown in Figure 2 and included insidious dyspnea, dry cough and fatigue. The mean time between self-reported onset of symptoms and IPF diagnosis was 21.8 months. The most common findings during the physical examination were crackles (95.5%), finger clubbing (30.8%), as well as pretibial oedema (9.1%).

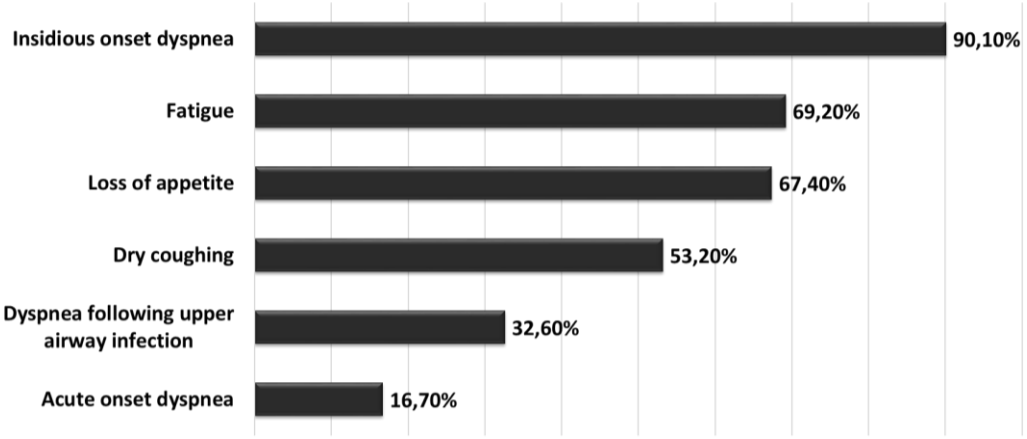

Figure 2. Distribution of self-reported symptoms of IPF patients. Data are presented as percentage of all patients with reported symptoms.

To classify dyspnea in a patient questionnaire, we graded its severity and impairment of physical activity in analogy to well-known NYHA classification [20]. Our cohort showed following distribution of severity of dyspnea in the IPF patients upon enrolment in the registry: Grade I-12.6%, Grade II-45%, Grade III-33.6%, and Grade IV-8.8%.

Diagnoses of IPF were made based on the respective ATS/ERS/JPS/ALAT guidelines 2000 and 2011 [6, 7, 16]. In our study cohort, 151 patients were diagnosed according to the ATS/ERS Consensus Statement/2000, and 351 patients were diagnosed using

criteria of ATS/ERS/JPS/ALAT guidelines released in 2011. Based on a retrospective review, the IPF patients diagnosed according to the guidelines 2000 also fulfilled the 2011 criteria.

Two different scales were used for the grading of HRCT. Prior to the release of the 2011 guidelines, the scale used in the eurIPFreg consisted of four grades and all the 151 patients diagnosed according to the 2000 guidelines were, independent of the existence of an open lung biopsy, classified into highly probable UIP (>90% probability; 72.6% of patients), somewhat probable (75-90% probability; 20.5% of all patients), weakly probable (50-75% probability; 4.8%) and not probable UIP pattern (<50% probability; 2.1% of all patients).

After the publication of the 2011 guidelines, the new HRCT classification (definite UIP pattern, possible UIP pattern and pattern inconsistent with UIP) was applied [58]. In this regard, a definite UIP pattern was encountered in 63.7% of all IPF cases, a possible UIP pattern in 27.7%. In 8.6% of the cases an “inconsistent with UIP” pattern was found. In this cohort covering the time span between 2003-2014, 79% of the patients received lung biopsy incl. VATS; 3.5% did show features of UIP in coincidentally larger transbronchial biopsies, for which reason VATS was not recommended anymore. In all these cases, histological findings were consistent with a pathologic UIP pattern. In 17.5% of patients a surgical procedure was not recommended because of the general condition of the patients, but longitudinal follow up and discussion in MDT rounds was very suggestive of UIP/IPF, for which reason this diagnosis was the preferred differential diagnosis.

Data regarding bronchoscopy were available for 455 IPF patients (86.7%). According to these, bronchoscopy was performed as part of the diagnostic procedure in 309 out of these 455 patients (67.9%) and biopsies (incl. cryobiopsies) were taken in 128 cases, corresponding to 24% of all IPF patients. BALF was performed in 263 cases (85.1%). The BAL differential revealed elevated neutrophil ($14 \pm 15.7\%$) and eosinophil ($5.4 \pm 8.5\%$) counts in face of normal lymphocyte ($9.8 \pm 10.7\%$) and reduced macrophage counts ($71.2 \pm 20.1\%$ of all cells). 20-30% of all patients diagnosed as having IPF in 2010 or 2011 underwent open lung surgery or video-assisted thoracoscopy (VATS), but these numbers went down in following years, possibly due to the increasing use of cryobiopsy, as shown in Figure 3.

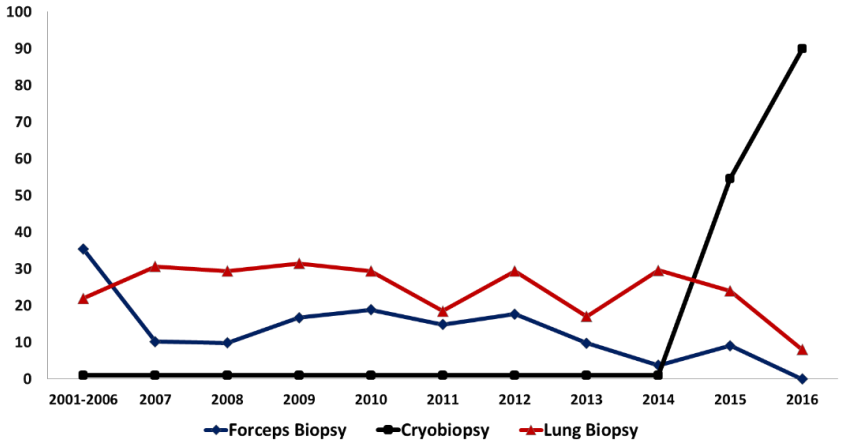

Figure 3. Change in biopsy procedures in IPF over time. Data are given as percentage of the respective bioptic procedure of all IPF subjects with first diagnosis in the respective year.

When evaluating the whole ILD cohort for the entire observation period (1086 patients from 2009 until 2016), we found that a pattern “inconsistent with UIP” was documented in 177 cases. Next to VATS, cryobiopsy was undertaken in 19 (10.7%) of these cases and forwarded criteria of an UIP pattern (n=12; 10 of which revealed fibroblast foci, spatial and temporal heterogeneity and absence of another pattern, hence only missing information as to the reference to the pleural surface), NSIP (n=5), HP (n=1), and uILD (n=1). In addition, while we did not observe any exacerbation with a temporal association to the conductance of cryobiopsy, but we have seen two exacerbations following VATS.

Baseline lung function data of IPF patients are displayed in Table 2. The mean FVC at the time of inclusion in the eurIPFreg was 2.39 ± 0.87 liters, corresponding to $68.4\% \pm 22.6\%$ of predicted value. 135 patients of the entire IPF cohort received long term oxygen treatment (LTOT), with a median flow of 2 l/min (range of 1-10l/min). Of these 135 patients, only 18 received flow rates above 4l/min. Six patients received LTOT with flow rates 8-10l/min. Fifteen patients received non-invasive ventilation (BiPAP), with a mean EPAP of 7.5 ± 1.78 cmH20 and a mean IPAP of 17.6 ± 2.7 cmH20. A six-minute walk test was performed and was available at baseline for 420 patients (80%). The mean distance walked was 388 ± 122 m.

Table 2: Results of lung function and gas exchange data in the IPF cohort.

Parameters	Mean value ± SD
VC (% predicted)	69.1 ± 21.5
FVC (% predicted)	68.4 ± 22.6

FEV 1 (% predicted)	74.1 ± 31.7
FEV 1 % FVC (% predicted)	110.2 ± 4.6
RV (% predicted)	74.2 ± 42.0
TLC (% predicted)	70.0 ± 38.4
DLco (% predicted)	42.1 ± 17.8
pO2 (mm Hg) at rest	60.2 ± 20.1
pCO2 (mm Hg) at rest	37.7 ± 10.6

Abbreviations: FEV1 - Forced expiratory volume; RV - Residual volume, TLC - Total lung capacity, VC - Vital capacity, FVC - Forced vital capacity, DLco - diffusing capacity of the lung for carbon monoxide, pO2 - partial pressure of oxygen, pCO2 - partial pressure of carbon dioxide.

Results of baseline echocardiography were available for 362 patients. An enlargement of the right heart was reported in 20.4% of the cases; an enlargement of the left ventricle or the left atrium was encountered in 12.9% of the patients. Signs of pulmonary hypertension were found in 16.8% of the patients, with systolic pulmonary arterial pressure (sPAP) values exceeding 50 mmHg (64 ± 18.9 mmHg; mean ± SD). Tricuspid annular plane systolic excursion (TAPSE) of less than 1.5cm was found in 14 patients (3.8%). The n-pro brain natriuretic peptide (nBNP)-value was assessed in 273 patients; the mean n-pro BNP was 301.24 pg/ml (range <1-6716 pg/ml). 41 IPF patients (15%) showed an n-pro-BNP value exceeding 150pg/ml.

Co-morbidities were common in patients with IPF and were assessed via both, physician- and patient questionnaires. Because co-morbidities are often exclusion criteria for clinical trials, this real-world data represents a broad IPF population without selection bias. As shown in Figure 4, the most common co-morbidity in our cohort was arterial hypertension, followed by gastro-oesophageal reflux.

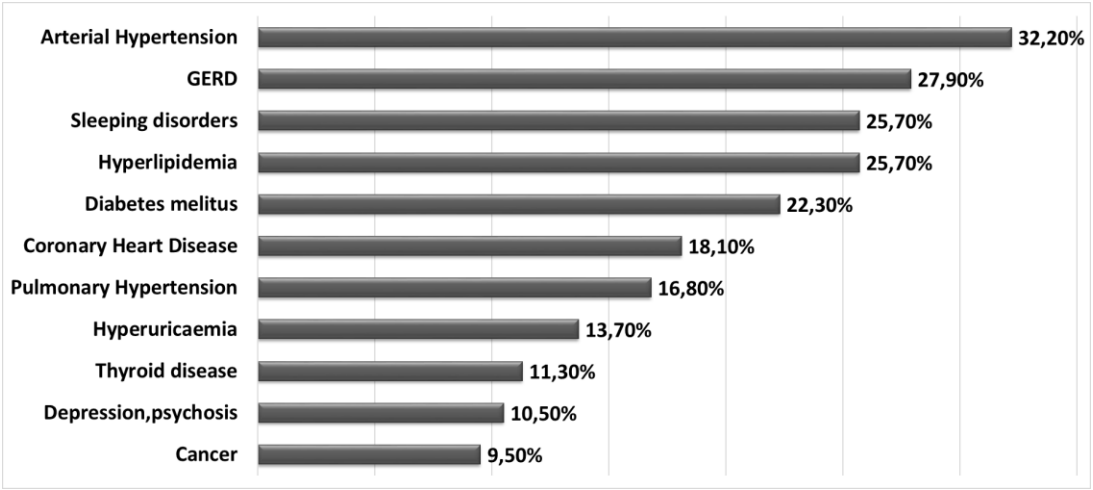

Figure 4. Spectrum of co-morbidities in the IPF cohort. Data are given as percentage of all patients. Multiple co-morbidities could be reported.

During the entire enrollment time, various therapeutic regimes were used throughout Europe. Prior to the commercial release of anti-fibrotic drugs pirfenidone and nintedanib, a significant number of the patients were treated with diverse immunosuppressants and/or N-acetylcysteine.

In our study, the most used IPF medications were classified into following groups: antifibrotics (pirfenidone, nintedanib), N- acetylcysteine, prednisolone, azathioprine, as well as mycophenolic acid. Figure 5 displays the use of these therapeutic regimes in all participating European centers over time and in percentage of all treated patients, showing a quantitative replacement of any other therapy by the two antifibrotic drugs.

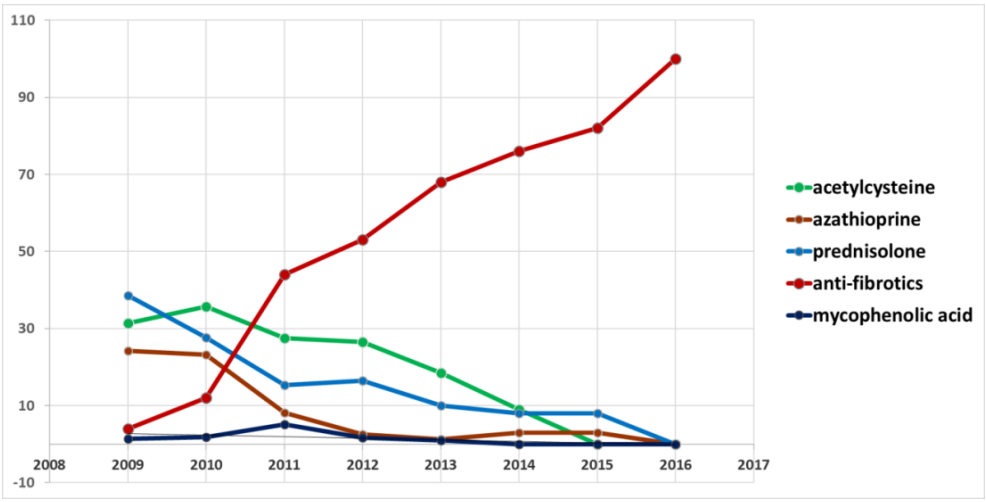

Figure 5. Change in IPF treatment over time. The graph shows various therapeutic regimes (acetylcysteine, azathioprine, prednisolone, mycophenolic acid and anti-fibrotics) in percentage of all treated patients.

Of the 525 IPF patients included in the registry from November 2009 until baseline cut-off in October 2016, definite outcome data (date of death or last known visit) were available for 210 cases. Of those, eight patients underwent lung transplantation (corresponding to 3.9%) and 78 patients (corresponding to 38%) had died until data cut-off in 2016. These 78 patients were enrolled in the registry at a mean age of 63.5 years, with a range of 42-88 years. The mean age of these subjects at the time of death was 71 years (range 44.5-90 years). The most common reasons for death were bronchopulmonary infections, i.e. pneumonia, leading to sepsis and multiorgan failure, followed by right heart failure due to progressive pulmonary hypertension.

When assessing survival via Kaplan-Meier analysis in correlation to the date of first IPF diagnosis, our results indicate that the median survival on antifibrotics was 123.1 months (censored cases inclusive, range 84-162 months), as compared to a median survival of 68.3 months in patients treated with any other medication (censored cases inclusive, range 54-83 months).

Figure 6 shows Kaplan-Meier analysis displaying improved survival in patients on anti-fibrotic medication vs. those receiving prednisolone or other treatment (p<0.001).

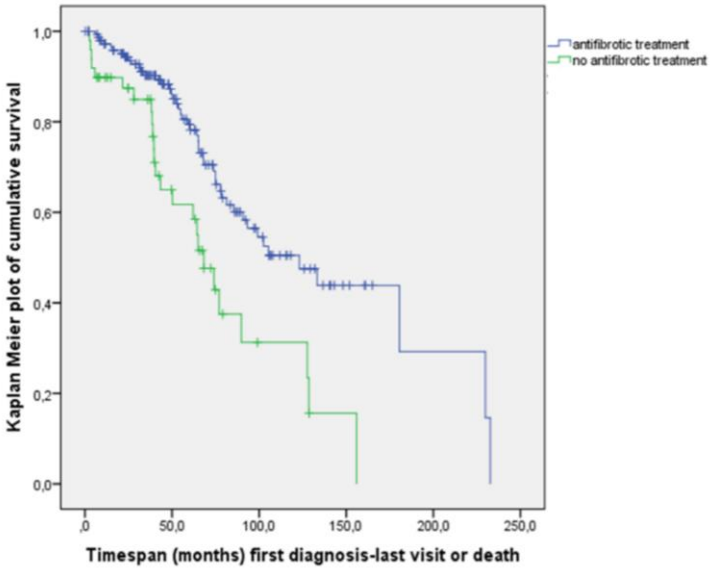

Figure 6. Overall survival of IPF patients upon first diagnosis depending on treatment. Given are Kaplan-Meier curves for cumulative survival, based on definite outcome data (survival status definitely known as per end of 2016), as well as on last visit data. A statistically significant difference in survival was encountered between patients receiving anti-fibrotic treatment and those not receiving antifibrotics (p<0.001). Within the group of patients receiving antifibrotic treatment, 83% of patients received pirfenidone and 17% received nintedanib.

In the study "Clinical characteristics of patients with familial idiopathic pulmonary fibrosis (f-IPF)" (attachment 2), we aimed to analyze relative frequency of f-IPF in our patients' cohort in relation to the sporadic form, as well as to profoundly characterize clinical differences between familial IPF (f-IPF) and sporadic (s-IPF) [8]. We also studied occurrence of the anticipation phenomenon (increasing severity of illness and / or earlier manifestation of the disease in younger generations), as well as the types of inheritance by analysing pedigree charts of our f-IPF cohort.

The phenotyping data from IIP patients and their relatives were collected via the eurIPFreg to evaluate the differences between f-IIP and s-IIP groups (with a focus on

IPF as a subgroup). All patients were recruited at our UGMLC sites in Giessen and Greifenstein. The purpose was to identify all patients with familial background. Initially, we employed wide range criteria of f-IPF (e.g. relatives who presumably died of some kind of parenchymal lung disease). After narrowing down the search to occurrence of IIP in at least one first grade relative, 28 index patients were finally identified, prospectively interviewed and examined. Their family members were phenotyped, alongside with an establishment of pedigree charts. The following exclusion criteria were applied: patients under 18 years of age, missing informed consent, death of the patient, other lung diseases apart from IIP, adoption of the patient, as well as evidence level C of f-IPF.

Within the 28 IPF families, overall 79 patients with f-IPF were identified. In the same observation period, 286 f-IIP and s-IIP patients were recruited into the eurIPFreg at our UGMLC sites, corresponding to a percentage of familial cases of 9.8% in regard to the whole IPF cohort. All 28 index patients were diagnosed accordingly ATS/ERS/JRS/ALAT Guidelines 2011 [98]. The definition and evidence level of f-IIP have been established in accordance to the recent publications and guidelines [13, 98, 99, 119, 120].

• Grade A - First line relative lives with or died from IPF / NSIP

• Grade B - First line relative lives with or died from DPLD

• Grade C - First line relative died because of a lung disease not further specified

The 28 index patients were prospectively interviewed, examined, and analyzed using the patient and physician questionnaires of the eurIPFreg, including establishment of pedigree charts. The patient questionnaire included patient's demographics, a detailed medical history and complaints. The physician questionnaire contained data of physical examination and laboratory tests, pulmonary function, HRCT, echocardiography, 6 MWD, co-morbidities as well as other information concerning relevant patient's diagnosis and therapy [3]. In addition, blood samples were obtained and archived in eurIPFbank.

Adult relatives of the f-IPF index patients were also recruited into the eurIPFreg, and were invited to our ILD outpatient clinic in order to undergo examination as part of this study. On site, a detailed medical history, a physical examination, a blood collection, a whole body plethysmography, a DLco test and a blood gas analysis were performed. In total, 52 healthy relatives were included, 26 of whom have carried out all the above-

mentioned examinations. One of the 52 family members was diagnosed with an early form of IPF and was re-classified as an affected relative.

The pedigree charts were developed in collaboration with affected families during the clinical presentation of the patient either in our outpatient clinic or via telephone interview. All family members from all known generations were taken into account. The following data were collected by each individual subject: family relationship, date of birth, date or at least age at death, cause of death, complaints and all known illnesses. In particular, we were looking for the presence of lung diseases. Individuals with known or suspected IIP were a subject to a more extensive survey: the explorative diagnostics with evaluation of the entity and stage of pulmonary fibrosis, age at first manifestation and diagnosis, smoking status, exposure to occupational and environmental risks. In 24 ILD patients, included in the pedigree charts, only medical history data could be collected. The pedigree charts were generated with the program GenoPro, version 2.5.4.1.

In total, 314 IIP patients (both, familial and sporadic forms) were recruited at our two UGMLC sites. The 28 f-IPF index patients represented 8.92% of all IIP cases. The confidence levels of IPF diagnosis were defined according to the ATS/ERS/JRS/ALAT Guideline 2011 [98]. When relating the diagnosis of all familial cases to the sporadic comparator group, percentage values from 1.4% (u-IIP) up to 23% (IPF) were encountered. Of the 28 identified index patients, all had the diagnosis IPF; together with the 51 affected family members (then total of 79 patients), these patients were diagnosed of having IPF (56%), NSIP - 3%, unclassifiable IIP (u-IIP, 1%) and other ILD (40%). The both groups showed no difference in demographics (61 vs. 79% males), smoking history, and exposure to any environmental triggers known to cause lung fibrosis.

All IPF index patients underwent HRCT for diagnosis prior to this study as a part of diagnostic routine. In all cases, the radiological findings were consistent with an UIP, of them definite UIP pattern was present in 66.7%. In total, 14 index patients additionally underwent a lung biopsy for histopathological diagnosis; a VATS was performed in 11 patients; in all of the 14 cases UIP pattern was seen. Twenty-one index patients received a bronchoscopy with a BALF; alveolar macrophages were present in 74.8 $\pm$ 37.47%, neutrophilic granulocytes in 10 $\pm$ 31.81%, eosinophilic granulocytes in 6.54 $\pm$ 0.71% and lymphocytes in 8.2 $\pm$ 4.95% of the cases (mean values $\pm$ SD). In 25 cases autoantibodies (among others anti-nuclear antibodies - ANA, extractable nuclear antibodies - ENA, rheumatoid factor – RF) were tested and not found to be abnormal; in general, prior to IPF diagnosis, connective tissue diseases-associated ILD were excluded.

Of the 147 sporadic IPF patients in 97% of the cases lung function tests were performed, 98% underwent HRCT, 76% performed 6 MWD, and 33% of the diagnoses were additionally confirmed by histology. Bronchoscopy was performed in 73% of the patients, echocardiography in 63% of patients. In comparison of f-IPF and s-IPF groups, both groups showed no difference in demographics (61 vs. 79% males) and smoking history. The f-IPF group differed by an earlier age at the onset of the disease (55.4 vs. 63.2 years; $p<0.001$) and broader age range (36-72 years). With an average of 67.0 years, f-IPF index patients died earlier than sporadic cases (71.8 years), although the result was not statistically significant ($p=0.059$). The results are shown in Table 3.

The leading onset symptom of f-IPF index patients was dyspnea (79%), similar to that in the s-IPF group. Interestingly, f-IPF patients reported more frequently on persistent, dry cough without expectoration, as compared to s-IPF group (58 vs. 11%, respectively; $p<0.001$). During physical examination, "velcro-like" crackles were found in 96 % of f-IPF patients, nail widening in 74 % and finger clubbing in 13 % of all cases. Long-term oxygen treatment (LTOT) was applied to 4.76% of the f-IPF patients with mean flow of 2.5l, as compared to 22.13% of the s-IPF group (mean flow 3.01l/min.).

On average, f-IPF index patients showed a milder extent of functional impairment at the time point of diagnosis vs. the s-IPF group (VC $p=0.027$, FVC $p=0.011$, RV/TLC $p=0.026$, DLco $p=0.006$, pO2 $p=0.015$). The most common co-morbidity in the f-IPF group was pulmonary hypertension (26%); obstructive sleep apnea was seen in 7% of the cases, and pulmonary embolism in 7% of the patients. The percentage of patients ultimately treated with anti-fibrotics was similar in both groups ($p=0.208$). Lung transplantation was performed in 15% of f-IPF patients, as compared to 5% in s-IPF patients ($p=0.094$). The average age of death in f-IPF group was 67 years, as compared to 71.8 years in s-IPF group ($p=0.059$).

Table 3. Characteristics of f-IPF and s-IPF patients.

Patients characteristics	f-IPF index patients (n=28) (mean value ± SD)	s- IPF (n=147) (mean value ± SD)	p-value
Male (%)	61	79	0.068
Height (cm)	170 ± 8	173 ± 7	0.058
Weight (kg)	80 ± 14	85 ± 16	0.314
BMI (kg/m²)	28 ± 5	28 ± 6	0.630

Current tobacco consumption (%)	0	2	1.000
Former tobacco consumption (%)	64	73	0.514
Pack years (n)	14 ± 18	19 ± 20	0.341
Age at onset of symptoms (years)	55.4 ± 10	63.2 ± 11	0.001
Age at first diagnosis (years)	58.3 ± 10	65.1 ± 10	0.001
Age at death (years)	67.0 ± 8	71.8 ± 8	0.059
Death due to IIP (%)	90	95	1.000

Abbreviations: IIP - idiopathic interstitial pneumonia, IPF - idiopathic pulmonary fibrosis, SD - standard deviation, BMI - body mass index, pack years (number of packs per day x smoker years), occupational exposure - occupational contact with potentially lung-damaging substances

The f-IPF group displayed diverse inheritance patterns, mainly autosomal-dominant with variable penetrance. In the f-IPF, the younger generations revealed a tendency for earlier manifestation of IPF vs. the older generation, thus displaying a phenomenon of anticipation (58 vs. 66 years, p=0.013). Comparing FVC decline between f-IPF and s-IPF patients, FVC levels measured after therapy start with antifibrotics or after lung transplantation were excluded. These criteria were met by 21 index f-IPF patients and 54 s-IPF patients. The last recorded FVC value was the last measurement before lost to follow-up, death of the patient, therapy start on antifibrotics or lung transplantation. Patients with f-IPF (n=21) showed an average, relative FVC decline of 0.028% per day (95% CI: 0.018-0.039%). This corresponded to an average, relative decline of 4.94% (95% CI: 3.155 - 6.71%) in 6 months, or 9.88% per year. The mean percentage starting point of the FVC of f-IPF was 73.63% predicted (95% CI: 65.76 - 82.47%).

The s-IPF group (n=54) lost 0.014% (95% CI: 0.009-0.019%) of the FVC on average per day (relative decline). Corresponding to this, the average decline in 6 months was 2.48% (95% CI: 1.568-3.382%), or 4.96% per year. The mean value of the starting point of the FVC in s-IPF group was 62% predicted. However, possibly due to the broad scattering of data, the difference in FVC decline between the groups did not reach statistical significance (p=0.12). In a logarithmic analysis of FVC decline, the starting value in the f-IPF group was 4.30 (95% CI: 4.19-4.41). In contrast, the logarithmic starting point of FVC for sporadic IPF was 4.18 (95% CI: 4.11% - 4.25). The interpolated slope of the

FVC for f-IPF was -0.00028 (95% CI: -0.00018 – -0.00039), and for s-IPF group was -0.00014 (95%-CI: -0.00009 – -0.00019). The data are shown in Figure 7.

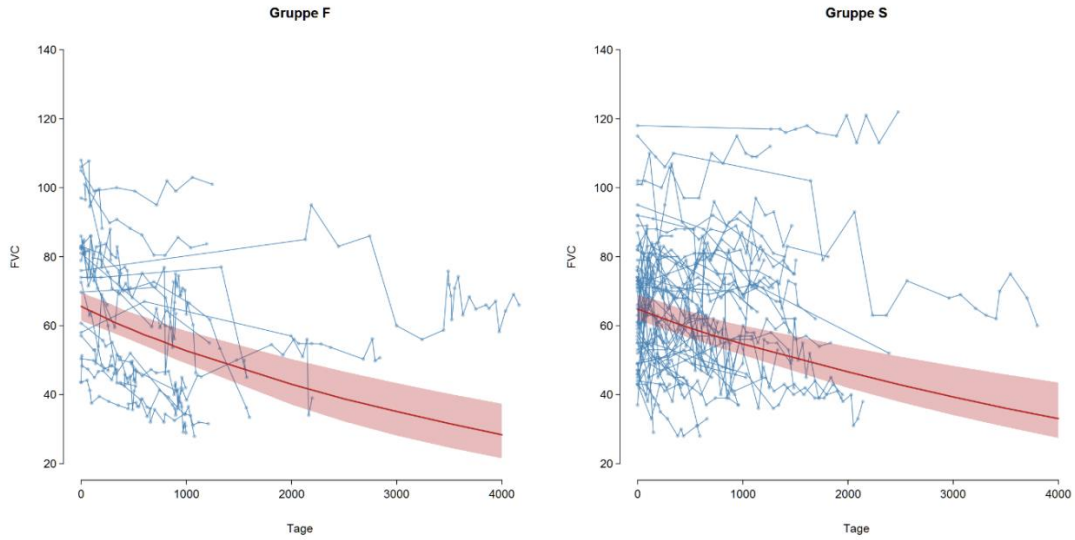

Figure 7. Decline in forced vital capacity (FVC) over time in both groups. Abbreviations: Patients with f-IPF, n=21; s-IPF patients, n=54. Day 0 corresponds to the first lung function test and approximately to the day of the first diagnosis. Each line represents the FVC course of a one patient; the points on the lines mark the individual values of FVC.

Evaluating the occurrence of malignancies and disease progression in f-IPF group, we could find that lung cancer was diagnosed in nine families, in one of the families in two members. Incidence of multiple malignancies of different organs was found in two families. In six families, patients were rather young when receiving first diagnosis of lung cancer (32, 38, 39, 48, 50 and 50 years, respectively).

In one family, it was noticed that all four affected IIP patients died within a very short time after diagnosis due to disease progression. Furthermore, it was observed in five pedigree charts that the younger generation became ill over a decade earlier than the generation of the parents (phenomenon of anticipation). The average age of death in f-IPF group was 67 years vs. 71.8 years in s-IPF group (p=0.059). The f-IIP group displayed diverse inheritance patterns, mostly autosomal-dominant with variable penetrance. In the f-IPF, the younger generations showed a tendency for earlier manifestation of IPF vs. the older generation (58 vs. 66 years, p=0.013). The 28 f-IPF index patients presented an earlier onset and more aggressive natural course of the disease.

The inheritance pattern of f-IPF has not yet been sufficiently recognized; in general, an autosomal-dominant pathway with reduced penetrance is suspected, although,

autosomal recessive inheritance could not be ruled out as well [33, 34, 37].The disease seems to affect consecutive generations at a younger age [8]. Analyzing the pedigree diagrams of f-IPF patients, we detected altogether 79 cases of ILD including the 28 index patients, as well as 51 further patients with ILD (44 IPF patients, two NSIP, one unclassifiable IIP, and 32 other ILD). In fourteen families, two members were affected; another seven families had three affected members per family, six families had four ILD cases, and one family had six relatives with ILD. Pedigree charts could be completed for 25 of the 28 f-IPF patients.

In four pedigree charts, a father to son inheritance was observed. In two pedigree charts, the disease appeared to originate from both parental sites. In contrast, in 16 families (ten times inheritance mother to child and six times inheritance father to child), the disease only seemed to originate from one parent. Of these, in one family, children of the same mother (but different father) were affected. Only one generation was affected in seven families (three times exclusively brothers, four times mixed genders).
In the following, three pedigree charts are presented as example. The index patient is marked with an arrow. All members suffering from ILD are marked yellow with a blue border. Patients suffering from another lung disease are marked with a blue border. Family members without ILD are marked with a magenta border. The relationships in pedigree charts always refer to the index patient.

Pedigree chart one.

This family tree comprised four generations with 13 individuals. The grade of evidence of f-IPF was level A, because the index patient (III: 3) had at least one immediate relative diagnosed with IPF or NSIP. In this family, the brother (III: 5) fulfilled this condition.

The disease seemed to have its origin on the maternal side. In the mother (II: 3) no diagnosed pulmonary fibrosis could be detected. However, shortly before her death she began suffering from progressive dyspnea and cyanosis without history of nicotine consumption. The aunt of the index patient suffered from pulmonary fibrosis. In the third generation, all three members were affected by an IPF. Inheritance pattern here seems to be autosomal dominant, since all children were affected, despite the fact that they have different biological fathers. The data are shown in Figure 8.

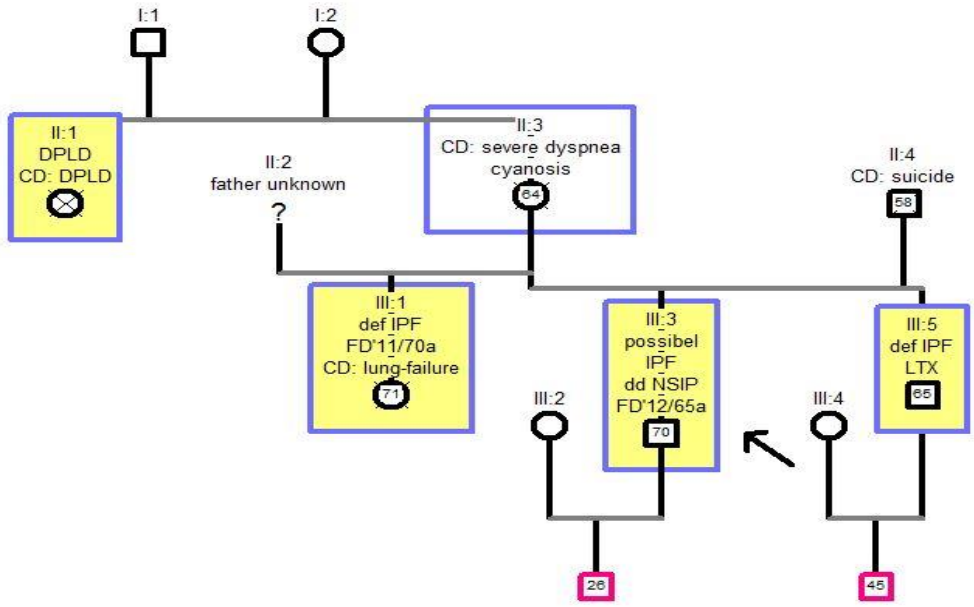

Figure 8. Pedigree chart one. Generations (I-IV) with 13 members, index patient III: 3

Abbreviations: DPLD - diffuse parenchymal lung disease, IPF - idiopathic pulmonary fibrosis, def. - definite, NSIP - nonspecific interstitial pneumonia, CD - cause of death, FD - first diagnosis year / age at first diagnosis in years; LTX - lung transplantation

Pedigree chart two.

This family included six generations with 39 members. The index patient is in position III: 4. There was an evidence level A (index patient III: 4 and sister III: 5). One cousin was also affected by diffuse parenchymal lung disease (III: 7). He was accidentally diagnosed with lung fibrosis because of auscultation of velcro-crackles in the routine checkup. His daughter (IV: 2) also did not have any complaints at the time of first diagnosis of pulmonary fibrosis. In total, ten other blood relatives of the index patient suffered from some form of lung disease. In particular, childhood pneumonia was very common (IV: 8, V: 3, V: 4).

Since only one generation was affected with IPF, inheritance mode is challenging to interpret in this example. The results are shown in Figure 9.

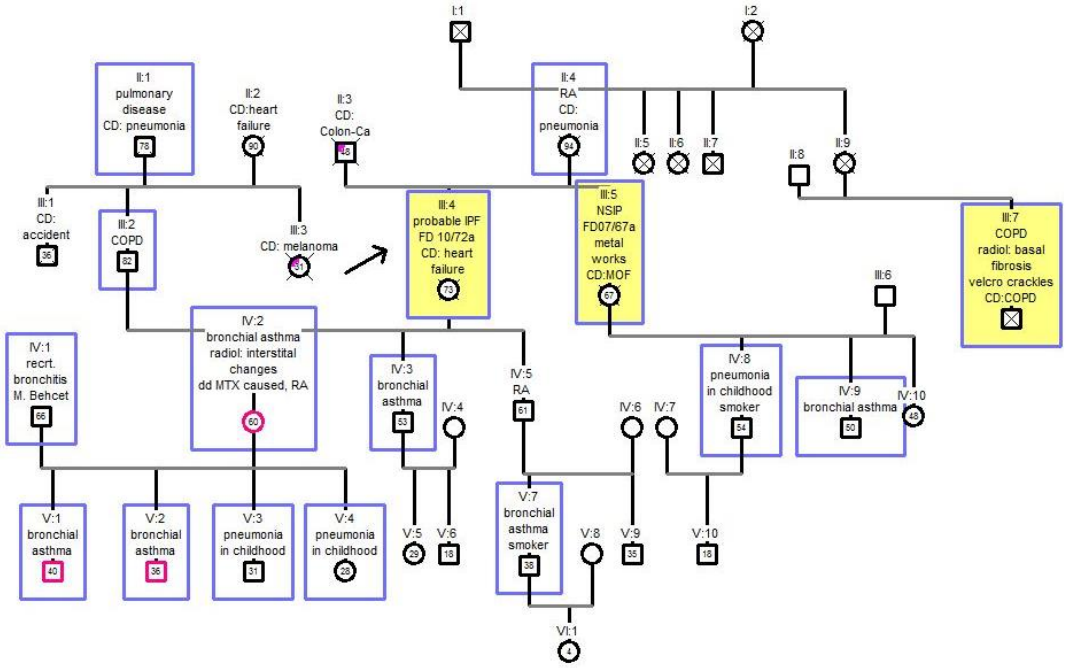

Figure 9. Pedigree chart two. Generations (I-VI) with 39 members, index patient III: 4;

Abbreviations: IPF = idiopathic pulmonary fibrosis, NSIP = non-specific interstitial pneumonia, CD = cause of death, colon Ca = colon carcinoma, RA= rheumatoid Arthritis, COPD = chronic obstructive pulmonary disease, FD = initial diagnosis year / age at first diagnosis in years, MOF = multi organ failure, radiol. = radiological, recrt = recurrent, DD = differential diagnosis, MTX = methotrexate

Pedigree chart three.

The pedigree chart three evaluated five generations, consisting of 53 individuals. Evidence level B was met by the index patient (III: 5) and her father (II: 6). Three generations were affected by pulmonary fibrosis (I, II and III). The sister (III: 4), the daughter (IV: 9) and two nieces (IV 14 and 15) of the index patient had shown no evidence of diffuse parenchymal lung disease at the time of the study.

In the oldest generation (I), the grandmother (I: 4) died at the age of 86. In the following generation (II), the father and the uncle died earlier than the grandmother. The father was diagnosed with IPF at the age of 79 years and so the uncle (II: 7) was diagnosed with IPF with 67 years. At age 53, the index patient (III: 5) was diagnosed much earlier than relatives in the previous generations. Due to the rapid progression of the disease, the index patient received a lung transplant at age 64. Up to now, no cases of pulmonary

fibrosis have been observed in generations IV and V. This family tree demonstrates the phenomenon of anticipation. The younger the generation, the sooner the disease was diagnosed, but also the sooner the patients died or become lung transplant.

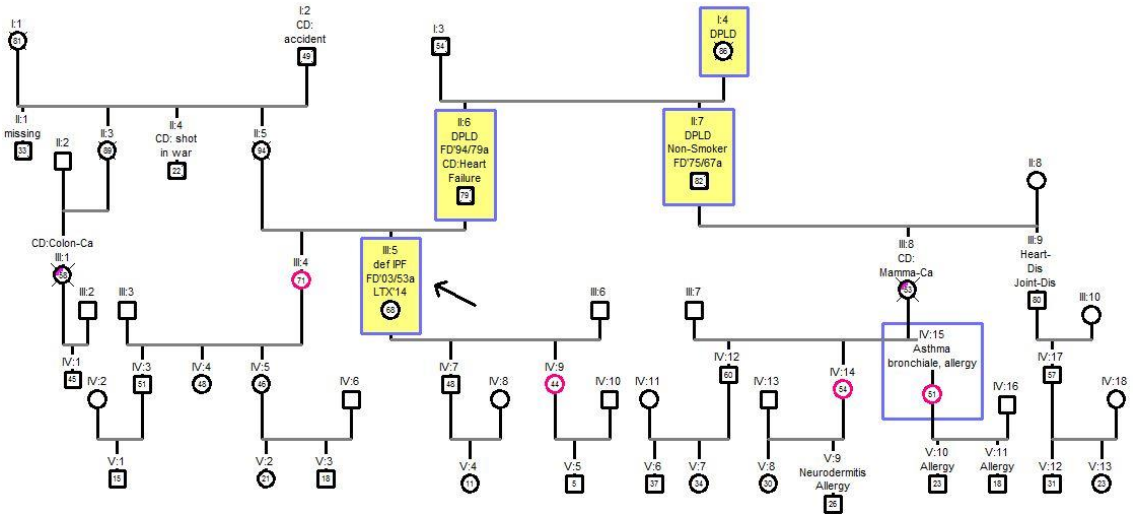

Figure 10 Pedigree chart three. Generations I-V with 53 members, index patient III: 5.

Abbreviations: def IPF = definitive idiopathic pulmonary fibrosis; DPLD = diffuse parenchymal lung disease; CD = cause of death; FD = initial diagnosis year / age at first diagnosis in years; colon Ca = colon carcinoma; LTX = lung transplantation; Mamma Ca = breast cancer; Heart Dis = heart disease; Joint Dis = joint disease

In the generation analysis, the youngest generation was defined as the youngest family member affected by ILD. Upstream of this generation was the parents' generation, the grandparents' generation and so on. The youngest generation in our cohort affected 49 members (40%). In this generation, the age at first diagnosis was 58 $\pm$ 11 years (mean $\pm$ SD values), which was significantly lower as compared to the year of first diagnosis in the parents' generation (66 $\pm$ 13 years, p=0.013). The affected family members of youngest generation died on average at the age of 66 years. In the parental generation, 25 persons were diagnosed with ILD (38%), and they died on average at the age of 71 years. The grandparent generation was first diagnosed at the age of 72 and died on average at age 79 (see Table 5).

In summary of our results, patients in f-IPF group showed an earlier age at the onset of the disease, reported more frequently on persistent, dry cough without expectoration, and presented with a milder extent of functional impairment at the time point of inclusion, but showed a significantly faster decline of lung function and gas exchange thereafter.

Our work was continued with the analysis of psychosocial impairments in ILD. In the study "Psychometric properties and minimal important differences of SF-36 in Idiopathic Pulmonary Fibrosis" (attachment 3), HRQoL was measured at the baseline visit using the Short Form Health Survey (SF-36) [5]. The SF-36 is a validated instrument for assessing HRQL and has been applied to a variety of chronic medical conditions, including IPF with the aim to characterize the relationship between selected baseline clinical covariates, the physical component score (PCS) and mental component score (MCS) as well as to provide disease specific minimally important differences (MID)[5].

The psychometric properties of the SF-36 were evaluated based on objective clinical measures as well as subjective perception of the patients. We analyzed acceptance, feasibility, discrimination ability, construct and criterion validity, responsiveness and test-retest-reliability. MIDs were estimated via distribution and anchor-based approaches. The SF-36 contains 36 items categorised into 8 dimensions (vitality (VITAL), physical functioning (PFI), physical pain (PAIN), general health perceptions (GHP), physical role functioning (ROLPH), emotional role functioning (ROLEM), social role functioning (SOCIAL), mental health (MHI)) and a physical as well as a mental component scores (PCS and MCS), which can be calculated for individuals providing all dimensions. The dimensions range from zero to 100; higher values imply higher functional health and well-being. The PCS and MCS are adjusted to normal distribution (mean equal 50, standard deviation (SD) equal 10) with higher values for better functional health and well-being.

For purposes of examining the validity of the SF-36 in IPF, we used the following anchors at baseline and follow-up: 6MWD, FVC % pred., DLco % pred., and also modified NYHA grade, evaluated by the physician (Grades I-IV). Baseline Dyspnea Index (BDI) (scale 0-12; the lower the more impaired, baseline only) and Transitional Dyspnea Index (TDI; scale -9 to 9, the lower the more impaired; follow-up only), long-term oxygen therapy (LTOT; baseline only), Modified Medical Research Council (mMRC), Dyspnea Scale (1-5, the higher the more impaired; baseline only), and an item of the SF-36 which indicates perceived change in health during the previous year. Considering the flexible intervals between the visits, the period between baseline and follow-up could not be defined a priori. As the SF-36 evaluates the HRQoL of the last four weeks, the interval between baseline and follow up needed to be of at least 28 days.

The study population included 258 individuals (73.3% male) with a mean age of 67.3 years (SD 10.7) and on average 2.6 years since first diagnosis (SD 2.8). In spite of a tolerance time frame of plus/minus 45 days between SF-36 and anchor, it was not

possible to provide all anchors for each patient. HRQoL presented in MCS and PCS was considerably reduced compared with norm values (mean 45.3, SD 11.8 and mean 34.6, SD 10.5 versus mean 50.0, SD 10.0). Except for ROLEM and ROLPH all HRQoL measures were normally distributed based on visual validation. Gender and severity of disease were of no significant influence. Almost half of the study population rejected (answer: 'definitely false') that their 'health is excellent' (45.8%, item five of GHP, possible answers: definitely true; mostly true; don't know; mostly false; definitely false) (appendix 2).

The tests showed significant lower HRQoL in individuals with LTOT except for MCS, MHI, and PAIN (Table 4). Cronbach's alpha ranged from 0.85 (SOCIAL) to 0.87 (ROLEM). In longitudinal analysis, SF-36 follow-up data were available of 161 individuals with the mean time between baseline and all considered follow-ups of 1.3 years (SD 0.88, range 0.1-5.0 years). Analyses for test-retest-reliability did not show significant differences of HRQoL except for SOCIAL and the anchor FVC % pred. Individuals with relevant changes of the health status based on the anchors had significant changes in all SF-36 dimensions and summary scales except for PAIN (responsiveness). Physical component score (PCS) correlated significantly and moderately with several anchors, whereas the correlations of mental component score (MCS) and anchors were only small. The tests showed mainly significant lower HRQoL in individuals with long-term oxygen therapy. Analyses in stable individuals did not show significant changes of HRQoL except for one dimension and anchor. Mean MIDs of the dimensions ranged from seven to 21. SF-36 appeared to be a valid instrument to measure HRQoL in IPF and can therefore be used in RCTs or individual monitoring of disease.

Table 4. Validity criteria analysed via correlation variables.

	n[c]	PCS	MCS	PFI	ROLEM	ROLPH	GHP	MHI	VITAL	SOCIAL	PAIN
VC % pred[a]	232-257	0.35**	-0.01	0.35**	0.11	0.35**	0.26**	<0.01	0.20*	0.11	0.07
DLco % pred[a]	208-227	0.36**	0.05	0.39**	0.14*	0.37**	0.22*	0.04	0.20*	0.23*	0.02
6MWD[a]	167-180	0.44**	0.17*	0.53**	0.18*	0.37**	0.29*	0.22*	0.32**	0.31**	0.17*
mMRC[b]	180-196	-0.48**	-0.11	-0.61**	-0.14	-0.3**	-0.32**	-0.13	-0.37**	-0.31**	-0.16*
BDI[b]	131-140	0.25*	-0.05	0.38**	-0.02	0.11	0.09	0.01	0.22*	0.14	0.14

NYHA[b]	206-228	-0.33**	-0.09	-0.41**	-0.17*	-0.37**	-0.22*	-0.13*	-0.3**	-0.18*	-0.09

a Pearson correlation
b Spearman correlation
c sample size varying depending on temporal relation of anchors and filled in SF-36, number of missing items within SF-36 and the possibility to calculate dimensions and summary scores
* $p<0.05$
** $p<0.0001$

Abbreviations: FVC pred forced vital capacity percent predicted, DLCO pred diffusing capacity of the lungs for carbon monoxide percent predicted, 6MWD 6 minute walking distance, mMRC Modified Medical Research Council Dyspnea Scale, BDI Baseline Dyspnoea Index, NYHA New York Heart Association, GHP general health perceptions, PFI physical functioning, ROLPH physical role functioning, ROLEM emotional role functioning, SOCIAL social role functioning, MHI mental health, PAIN bodily pain, VITAL vitality.

The SF-36 seems to provide adequate psychometric properties to assess HRQoL in an IPF cohort. Our analysis demonstrated an increased number of missing items in older patients. It is well known, that in an older population the number of missing items is higher. Especially items containing the wording 'work or other regular daily activity' (dimensions ROLEM and ROLPH) led to a higher number of missing values in our study as well as in the studies of Hayes et al. and Mallinson [121, 122]. As 75.2% of participants completed the questionnaire without any missing values in our study, we assumed that the higher age of most of the patients suffering IPF is not necessarily a limiting factor.

As we expected in IPF subjects, there was a floor effect of the items regarding limitations in 'vigorous activities' and 'climbing several flights of stairs' (dimension PFI), as well as the statement 'my health is excellent' (dimension GHP). As the dimension PFI contains ten items and considers different levels of activities, the floor effect of two items may be acceptable. Surprisingly, 4.4% and 7.9% of our study population declared to have no limitations at all in these two physical activity categories and 1.6% rated their health as excellent. Furthermore, the influence of dyspnea and physical activity measured via mMRC, BDI, NYHA, and 6MWD on HRQoL was higher than the influence of clinical parameters as vital and diffusion capacity. Other studies also showed similar results with varying interpretation of the relevance of the correlation between pulmonary function and HRQoL.

The strength of this study is in the international multicentre population of the IPF individuals of all ages and disease stages without strict inclusion and exclusion criteria,

which provides a ‘real life’ setting and transferable results. We investigated a potential influence of the study sites and countries on HRQoL. After adjusting for age, gender, DLco, FVC and 6MWD there was no correlation with HRQoL. In general, SF-36 appears to be a valid instrument to measure HRQoL in IPF and can therefore be used in RCTs or individual monitoring of this disease. Our findings have a potential impact on the evaluation of IPF patients in clinical trials as well as individual disease monitoring.

As in up to 20% of ILD patients, a specific diagnosis cannot be defined (unclassifiable ILD (uILD)), the aims of our next study “Clinical and functional characteristics of patients with unclassifiable interstitial lung diseases: Long term follow-up data from European IPF Registry (eurIPFreg)” (attachment 4) were to describe the detailed clinical characteristic of the Giessen uILD cohort included in the eurIPFreg, as well as to define the prognostic factors of pulmonary function decline, impact on HRQoL and survival analysis. The disease progression was defined as change in annual rate of FVC decline of ≥10% predicted value [123].

At baseline, uILD patients showed a mean age of 68.25 years. 81% were male, 50.7% were previous or current smokers; mean FVC was 67.8% ± 22.1% pred., DLco was 43.8% ± 26.3% pred. In 46.4%, the uILD diagnosis was due to conflicting clinical, radiological and pathological data. A lung biopsy was performed in 75% of the patients (of which 71.4% transbronchial forceps biopsy, 19.4% VATS, 7.1% cryobiopsy, and 2% open lung biopsy). A pulmonary hypertension was present in 19.3%, arterial hypertension in 54.3%, coronary heart diseases 23.8% of the patients. Of all patients, 14% received pirfenidone, 16.4% azathioprine, 32.9% prednisolone.

By applying the diagnostic criteria of UIP based on CT patterns (Fleischner Society), 22.2% of the patients showed a typical UIP pattern, 11.1% a probable UIP pattern, 12.8% an indeterminate UIP pattern and 53.8% CT features most consistent with a non-IPF diagnosis [58]. In a Cox regression analysis for survival we could show that FVC% pred. at baseline ($p=0.008$, Figure 11), FVC decline ≥10% p.a. ($p<0.0001$, Figure 12), smoking ($p=0.033$), and a DLco ≤55% pred. at baseline ($p<0.0001$) were significantly associated with progressive disease and fatal outcome.

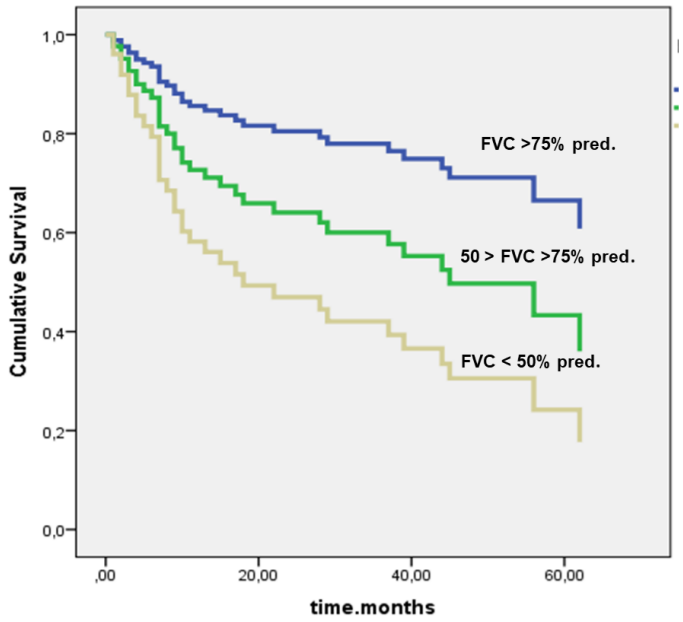

Figure 11. Cumulative survival in dependency of FVC at baseline (p=0.008). Given are Kaplan-Meier curves for cumulative survival, based on definite outcome data. Abbreviations: FVC- forced vital capacity, % pred.- percentage of predicted value.

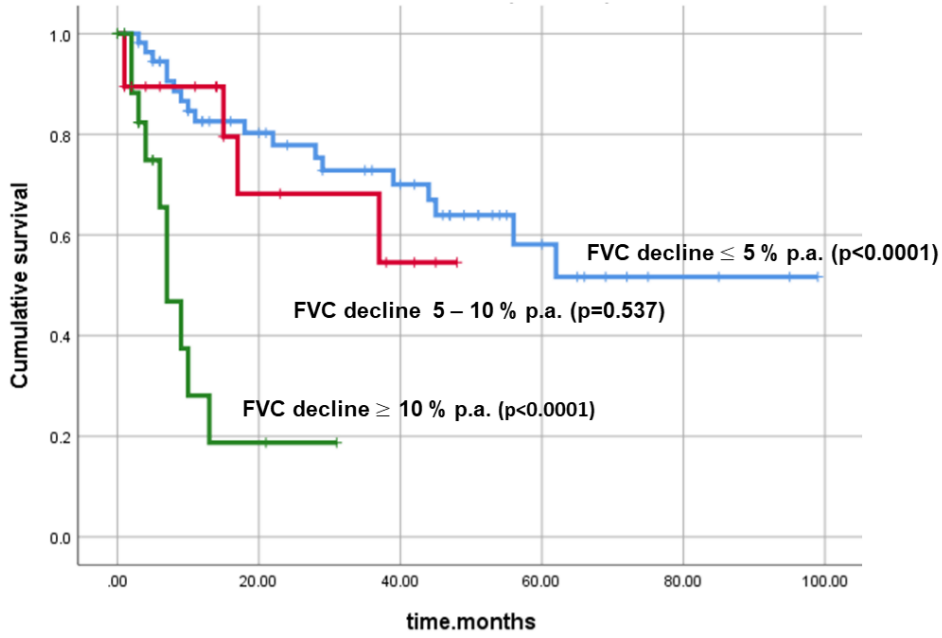

Figure 12. Cumulative survival with regard to FVC decline p.a. (p<0.0001). Abbreviations: FVC- forced vital capacity, % pred.- percentage of predicted value.

We conclude that the most important known prognostic factors in uILD remain the decline in lung function and smoking. The use of Fleischner diagnostic criteria allows further

differentiation and accurate diagnosis together with new therapeutic options for patients, who currently have no established approach to therapy.

New biomarkers are urgently needed to facilitate diagnosis in ILD, thus reducing the need for invasive procedures, and to enable tailoring and monitoring of medical treatment. In the study "Exhalative Breath Markers Do Not Offer for Diagnosis of Interstitial Lung Diseases: Data from the European IPF Registry (eurIPFreg) and Biobank" (attachment 5), a total of 120 patients from the Giessen site of the eurIPFreg have been analyzed on the basis of clinical data as well as obtained biomaterials [10]. We investigated if patients with IPF (n=21), non-IPF ILDs (n=57) and other lung diseases (24 COPD and 16 LC patients) as well as 20 healthy subjects show relevant differences in exhaled NO (FeNO; Niox MINO), or in eicosanoid (PGE2, 8-isoprostane; enzyme-linked immunosorbent assay (ELISA)) levels as measured in exhaled breath condensates (EBC) and BALF.

Biological materials such as blood, BALF, tissue samples as well as EBC were centrally managed through generation of patient-, time-, and specimen-specific Lab IDs; they were stored in our eurIPFbank. Patients were asked to withdraw from food and nicotine intake a few hours prior to the measurements, as well as to rinse the mouth before analyses. A nose clip was consistently used.

The FeNO measurements were carried out with the device NIOX Mino (Aerocrine, Bad Homburg, Germany). EBCs were collected according to the recommendations of the ATS/ERS Working Group with the turbo-DECCS device (ItalChill, Parma, Italy) [124]. The turbo-DECCS device has a thermoelectric module that works through the Peltier effect. The exhaled breath of the patient is passed through disposable polyethylene tubes into the collection tube of the device and is cooled there. The DECCS Polyethylene tube system (ItalChill, Parma, Italy) has been changed for each patient. After the collection, the EBC was quickly filled into cryocups and frozen at -80 °C.

The quantitative detection of PGE2 and 8-Isoprostan in biomaterials was performed using a commercially available immunosorbent assay (ELISA, Cayman Chemical Company, Michigan, USA). It is based on an enzymatic immune assay (EIA) principle. In case of BALF, samples were directly transferred to the wells of the plate. In case of EBCs, we analyzed EBC fluid directly, as well as after concentrating its content. For this purpose, 600 µl of each sample were lyophilized in the vacuum centrifuge and dissolved thereafter in distilled water at a volume of 120 µl.

In total, 94 FeNO measurements were made in the different groups of patients. All groups showed a similar range of FeNO values (Figure 13). In order to assess the extent to which FeNO values relate with the clinical progression of pulmonary fibrosis, FeNO values for IPF and other ILDs were correlated with PFT values, blood gas analysis, 6MWD and spiroergometry in a linear regression analysis. In IPF, FeNO values were not significantly correlated to PFT values, with the exception between KCO and FeNO of uncertain relevance.

Likewise, the total ILD group including IPF showed no significant correlation of lung function values and FeNO as well. However, an inverse, significant correlation between FeNO and ITGV (r=0.44, p=0.03) as well as an inverse, highly significant correlation between FeNO and RV / TLC (r=0.69, p=0.0003) were observed.

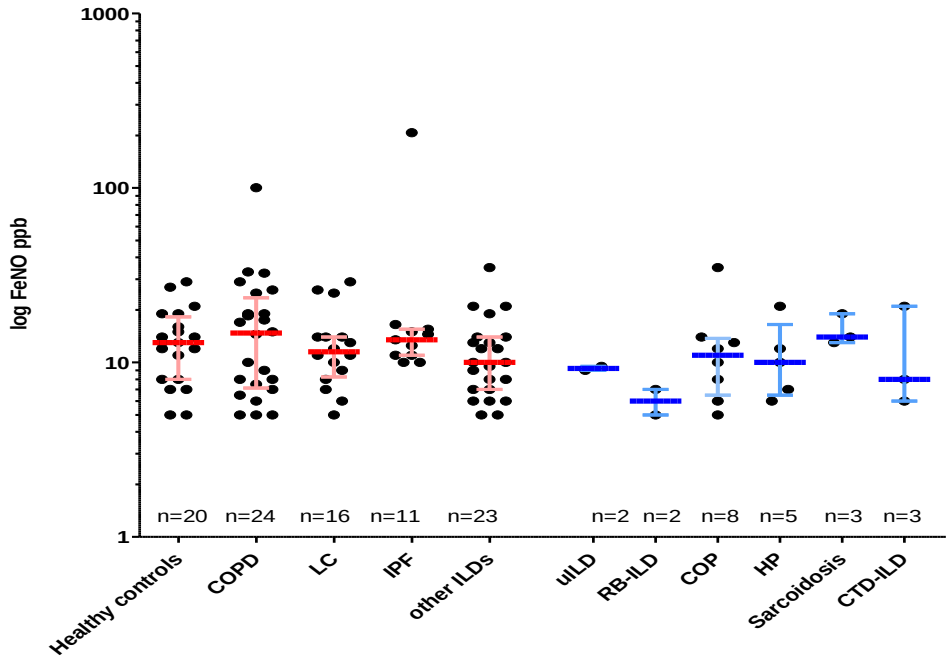

Figure 13. FeNO values in the different lung disease cohorts vs. healthy controls. Given are the median (horizontal bar) with interquartile range as well as single data (dots). uILD, RB-ILD, COP, HP, sarcoidosis and CTD-ILD have been summarized as “ILD” and are shown separately in blue on the right margin. Abbreviations: IPF - idiopathic pulmonary fibrosis, ILD - interstitial lung diseases, HP - hypersensitivity pneumonitis, COPD - chronic obstructive pulmonary disease, COP - cryptogenic organizing pneumonia, CTD-ILD – connective tissue disease- associated ILD, LC - lung cancer, NSIP - non specific interstitial pneumonia, RB-ILD – respiratory bronchiolitis ILD, uILD - unclassifiable ILD. If data was not available from all patients, the number (s) was specified.

To further analyze the relationship between exercise capacity and FeNO in IPF, the values were correlated with the walking distance of the 6MWD and the maximum oxygen uptake (VO2max) values of the spiroergometry. There was no relevant correlation of the FeNO values with the 6MWD, either when restricted to IPF patients alone or when analyzed for all ILD patients including IPF. The VO2max in spiroergometry showed a positive, although insignificant correlation to the FeNO values for the IPF (r=0.6) as well as for the total ILD cohort (r=0.02), possibly related to one outlier. To clarify the extent to which smoking behavior and drug intake influence the FeNO values, the FeNO values for all patients were analyzed in dependency of the following subgroups: smoker status (never, active, former), as well as intake of medication (NSAID, steroids and proton pump inhibitors (PPI)). To this end, actively smoking patients showed significantly lower FeNO levels than former smokers and never smoked patients. The median FeNO values observed in patients with intake of NSAID, steroids or PPI were not significantly different than in those without the above mentioned medication.

The intraindividual variability of FeNO was determined by repeated analyses (from 1 to 11 days between the measurements) in the IPF and COPD cohorts. Overall, a good reproducibility of the FeNO measurements was shown. To evaluate the impact of exacerbation on FeNO, we analyzed FeNo values of the whole cohort as well as of COPD patients with regard to the following exacerbation criteria: formal diagnosis of exacerbation in the patient file, colored sputum, intake of antibiotics, and proof of causative pathogen in sputum or bronchial suction samples. However, none of these criteria was associated with a meaningful difference in measured FeNO values.

The free 8-isoprostane in the EBC was measured with a commercial ELISA (Cayman). In general, when analyzing EBC directly, the values were in the lower range of the standard curve and in a number of cases even below that. We therefore analysed EBC after concentrating its content. In Figure 14 (right panel) it is shown that for those samples which were measurable after concentration, no significant difference was visible between IPF, other ILDs and healthy controls. In the EBC, PGE2 was difficult to detect and each single patient sample yielded values below the smallest standard. Samples were therefore concentrated by lyophilization and again analyzed for PGE2 determination, all of which were measurable for PGE2 (Figure 12, left panel). Healthy controls showed the highest PGE2 median value (9.9 pg / ml), while the IPF (7.03 pg/ml) and the ILD (6.61 pg/ml) appeared to have slightly lower values. There was no significant difference between the groups.

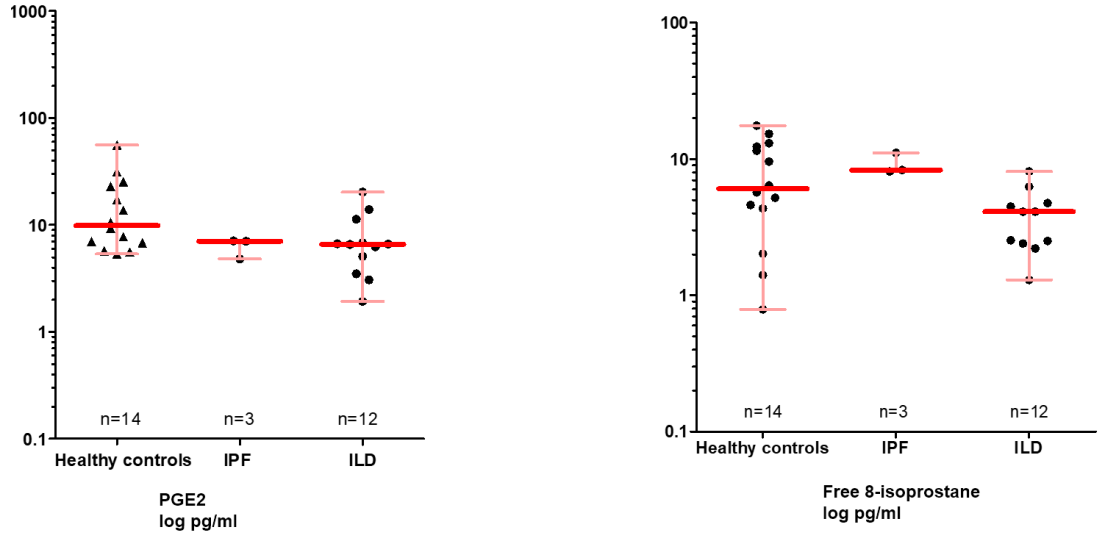

Figure 14. Free 8-isoprostane and PGE 2 in the EBC. Given is median with interquartile range. HP, uILD, DIP, COP, sarcoidosis as well as CTD-ILD have been summarized as ILDs. Abbreviations: IPF- idiopathic pulmonary fibrosis, ILD- interstitial lung diseases. EBC was analysed after concentrating its content. If data was not available from all patients, the number (s) was specified.

In a complementary approach, we analyzed PGE2 in BALF, which was found to be detectable in all samples. Overall, the medians for healthy subjects (22.08 pg/ml), IPF patients (23.34 pg/ml) and ILD patients (24.51 pg/ml) were quite similar, so there was no significant difference between the groups. Looking more closely at the ILD subgroups, NISP patients (27.33 pg/ml) showed the highest PGE2 values, followed by HP (24.51 pg/ml) and sarcoidosis (22.18 pg/ml) (Figure 15). Again, there was no significant difference here either.

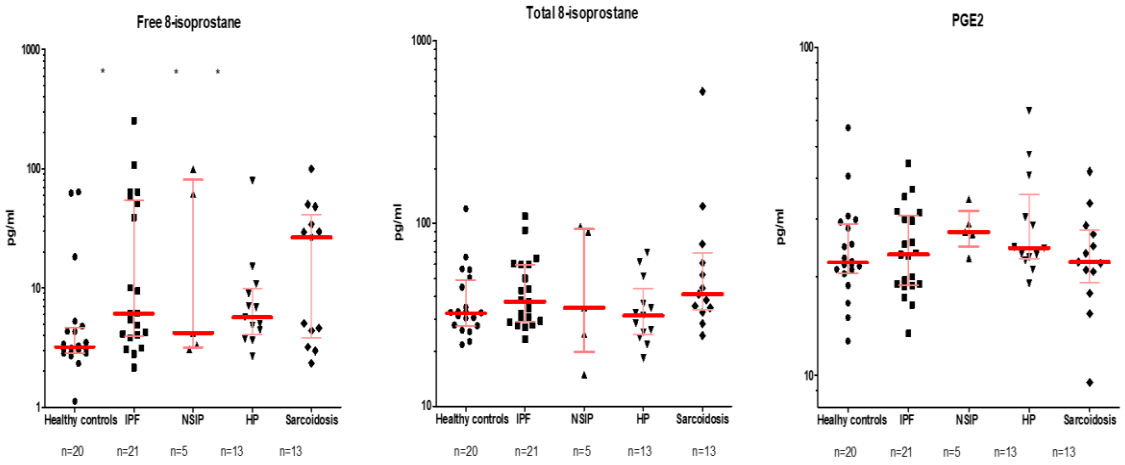

Figure 15. Distribution of PGE2 and 8-isoprostane in BALF. Data are given as median with interquartile range. Abbreviations: IPF- idiopathic pulmonary fibrosis, ILD- interstitial lung diseases, NSIP - non specific idiopathic pneumonia, HP- hypersensitivity pneumonitis. If data was not available from all patients, the number (s) was specified.

In order to assess the extent to which 8-isoprostane and PGE2 levels from BALF samples reflect the disease progression, the annual change in FVC (Figure 16), DLco and 6MWD was correlated with PGE2 and 8-isoprostane values in BALF by a linear regression analysis. For this evaluation IPF and ILD were taken as one group. Unfortunately, there were no significant correlations between each eicosanoid and the course of FVC, DLco or 6 MWD over time.

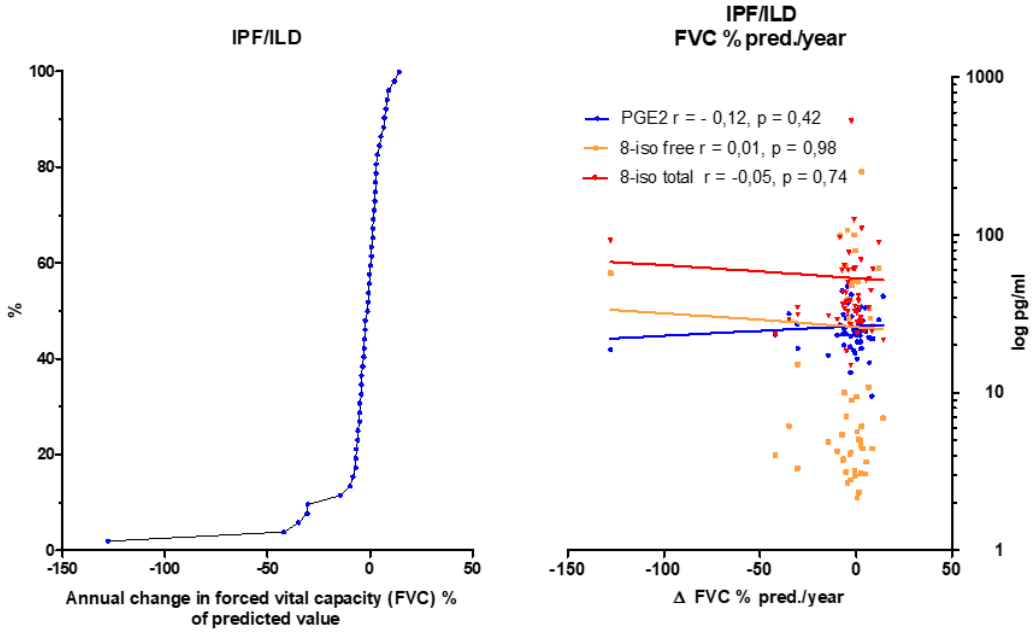

Figure 16. Correlation of the annual change in FVC (% pred.) in IPF and ILD patients and PGE2, total and free 8-isoprostane values in BALF. Left panel: The annual change of the FVC is summarized in the cumulative frequency diagram. Right panel: Correlation of FVC (% pred.) to PGE2 and 8-isoprostane. Abbreviations: FVC- forced vital capacity, PGE2- prostaglandine E2, r -correlation coefficient according to Spearman, 8-iso- 8 isoprostane, % pred.- percent of predicted value. If data was not available from all patients, the number (s) is specified.

There was no significant difference in FeNO values between IPF, non-IPF ILDs and healthy subjects, although some individual patients showed highly elevated FeNO. On the basis of the FeNO signal, it was neither possible to differentiate between the kind of disease nor to detect exacerbations. In addition, there was no correlation between FeNO

values and lung function. The investigation of the eicosanoids in EBCs was challenging (PGE2) or unreliable (8-isoprostane), but worked out well in BALF. A significant increase of free 8-isoprostane was observed in BALF, but not in EBCs, of patients with IPF, HP and sarcoidosis, possibly indicating severity of oxidative stress. Our results displayed, that FeNO-measurements are not of diagnostic benefit in different ILDs including IPF. The same holds true for PGE2 and 8-isoprostane in EBC by ELISA [10]. Therefore, none of these markers turned out to have any diagnostic or prognostic potential.

There is a high unmet clinical need to improve screening and to increase specifity of earlier ILD detection by addition of reliable, non-invasive screening tests. Because eNoses have been reported to identify patients affected by different types of respiratory diseases, they therefore might help to establish an early ILD diagnosis and to predict prognosis and response to the treatment [125]. Thus, as an easy to handle, non-invasive diagnostic tool, they could represent an important aid during the diagnostic process. To the best of our knowledge there are yet no explorative studies published, in which eNoses have been used for ILD diagnosis.

Therefore, in our further research, "Exploring the ability of electronic nose technology to recognize interstitial lung diseases (ILD) by non-invasive breath screening of exhaled volatile compounds (VOC): a pilot study from the European IPF Registry (eurIPFreg) and Biobank" (attachment 6), we aimed to investigate the diagnostic accuracy of an Aeonose® to distinguish different ILDs on the basis of VOC patterns in subjects with ILD as well as healthy controls (HC) [126]. The study cohort consisted of 174 ILD patients, 23 COPD and 33 controls from the University of Giessen and Marburg Lung Center (UGMLC) sites in Giessen and Greifenstein, who were recruited in the eurIPFreg and eurIPFbank.

All participants provided an exhaled-breath sample by in- and exhaling for 5 min, using a nose clamp, through the Aeonose®. The definition of "healthy" was self-reported and meant absence of known chronic diseases. After the initial training phase, signatures of VOC of ILD patients were captured using the Aeonose® and compared to HC in prospective correlation analyses. Additionally, we performed cross-analyses between the ILD groups to deeper validate the ability of the eNose to detect disease-specific patterns.

To evaluate VOC signatures, a software program called Aethena was used for pre-processing, data compression and neural networking [127]. To interpret the Aeonose®

data, the following parameters were measured: Area under Curve (AUC), sensitivity, specificity, as well as Matthews's correlation coefficient (MCC). The MCC is a measure of the quality of binary classifications and is generally regarded as a balanced measure which can be used even if the classes are of very different sizes. In essence, the MCC is a correlation between the observed and predicted binary classification, where a value of +1 represents a perfect condition, 0 no better than random prediction and -1 indicates total disagreement between prediction and observation [128]. Comparisons between groups were performed using ROC-Analysis.

In a first approach, the VOC patterns of IPF patients were directly compared to HC after a training (calibration) phase. The Aeonose® was able to differentiate IPF-patients (n=51) vs. HC (33), showing a sensitivity of 0.88 and a specificity of 0.85, an AUC of 0.95 and MC of 0.73. Figure 17 displays the data.

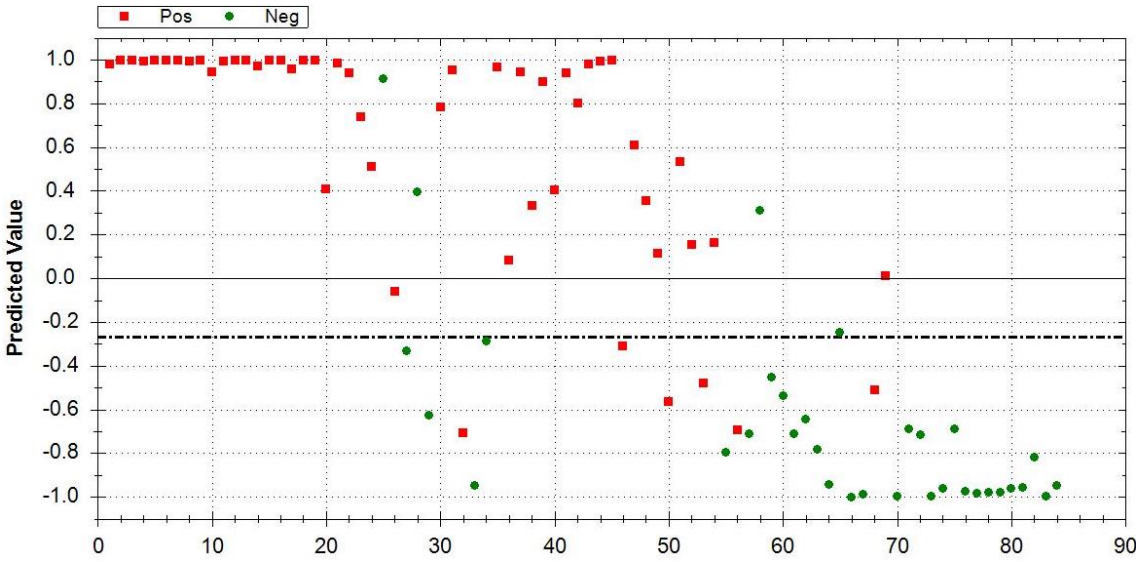

Figure 17. Direct comparison between IPF (n=51, red squares) and healthy controls (HC; n=33, green dots) by Aeonose®. Abbreviations: IPF area 0-1: Red squares indicate correctly recognized IPF patients; green dots denote false positive patients. HC area 0--1: Green dots represent correctly identified healthy controls, red squares mark false negative results. The dotted line is inserted for values around the threshold where there is doubt about which side it tends to, and, hence, reflecting an area of uncertainty.

By directly comparing patients with CTD-ILD (n=25) vs. HC (n=33), an AUC of 0.90, MC of 0.69, sensitivity of 0.84 and specificity of 0.85 were encountered. Figure 18 shows the ability of Aeonose® to identify CTD-ILD patients in direct comparison with HC.

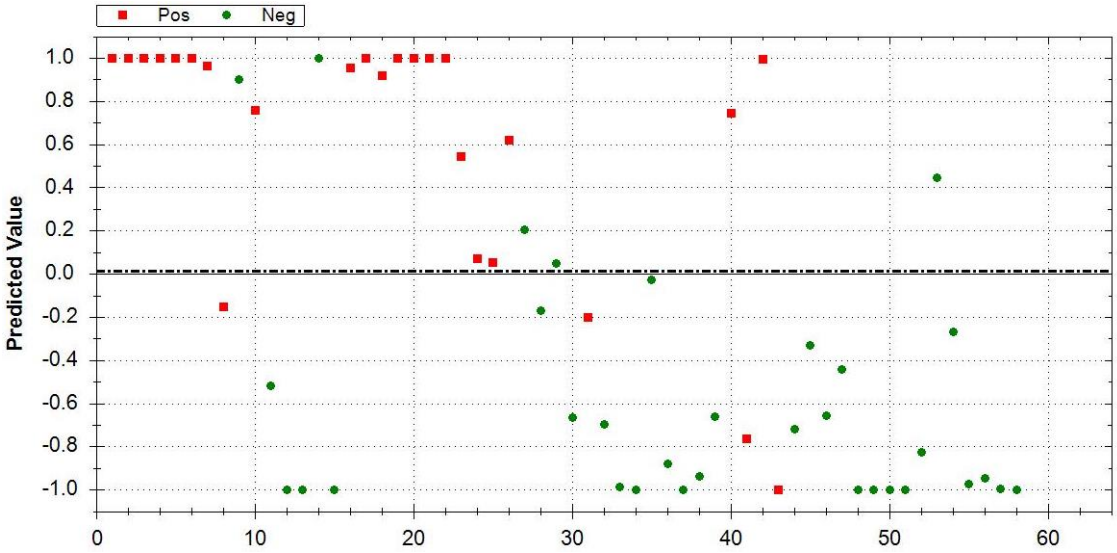

Figure 18. Direct comparison between CTD-ILD (n=25, red squares) and HC (n=33, green dots) by Aeonose®. Abbreviations: CTD-ILD area 0-1: Red squares indicate correctly recognized CTD-ILD patients; green dots denote false positive patients. HC area 0- -1: Green dots represent correctly identified healthy controls, red squares mark false negative results.

In a further direct comparison between cryptogenic organizing pneumonitis (COP, n=28) vs. HC (n=33), an AUC of 0.89 and MC of 0.67 were obtained. Sensitivity was 0.86 and specificity 0.82. Figure 19 summarizes the data.

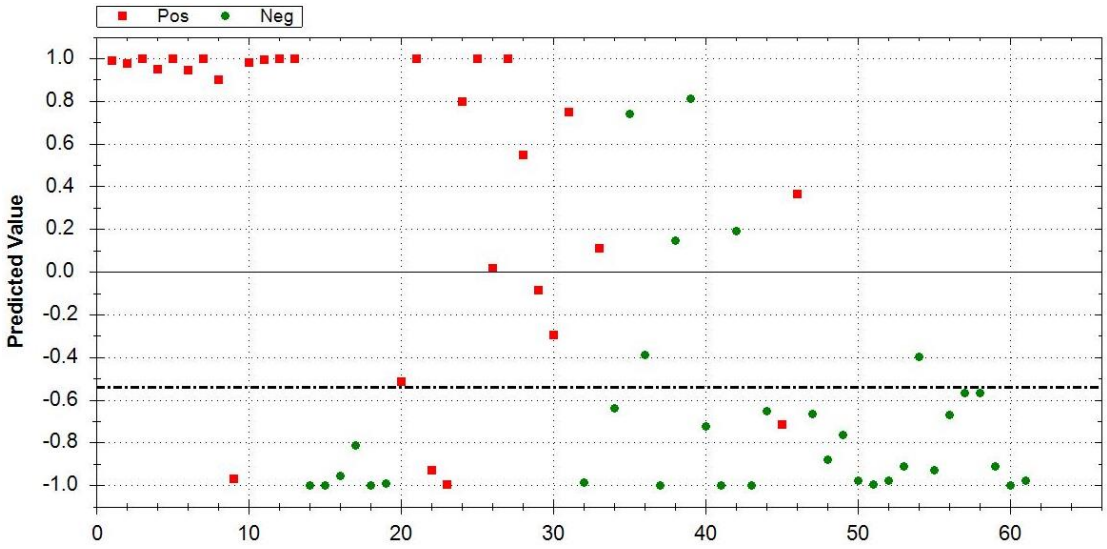

Figure 19. Direct comparison between COP (n=28, red squares) vs. HC (n=33, green dots). Abbreviations: COP area 0-1: Red squares indicate correctly recognized COP patients; green dots denote false positive patients. HC area 0- -1: Green dots represent correctly identified healthy controls, red squares mark false negative results. The dotted line is inserted for values around the threshold where there is doubt about which side it tends to, and, hence, reflecting an area of uncertainty.

The direct comparison analyses forwarded promising and interesting results, with AUC as well as sensitivity and specificity values suitable for a potential use of the Aeonose® as a diagnostic test. As, however, we had not checked the performance of the Aeonose® in an independent, second control cohort, COPD patients were taken into the analysis (n=23). When comparing COPD and HC, an AUC of 0.91, a MC of 0.73, a sensitivity of 0.86 and a specificity of 0.88 were obtained. In direct assessment between COP (n=28) and COPD, an AUC of 0.77, a MC of 0.46, a sensitivity of 0.75 and a specificity of 0.71 were obtained. In the analysis of CTD-ILD (n=25) vs. COPD (n=23), Aeonose® forwarded an AUC of 0.85, a sensitivity of 0.88, a specificity of 0.71, and a MC of 0.61.

To further validate the ability of eNose to recognize the disease-specific VOC pattern, we compared breath patterns of ILD groups to each other instead of applying a case-control study design. Following this approach, however, the sensitivity and specificity showed a relevant drop. Although the device was previously trained in disease-specific pattern recognition using two control cohorts (HC and COPD), Aeonose® was only party able to distinguish the groups correctly. The ILD subgroup comparison revealed a lower ability of disease-specific pattern recognition, as compared to direct analysis. In the group analysis between IPF (n=51) vs. COP (n=28), an AUC of 0.82, a sensitivity of 0.84, a specificity of 0.64 and a MC of 0.49 were obtained. Figure 20 displays the results.

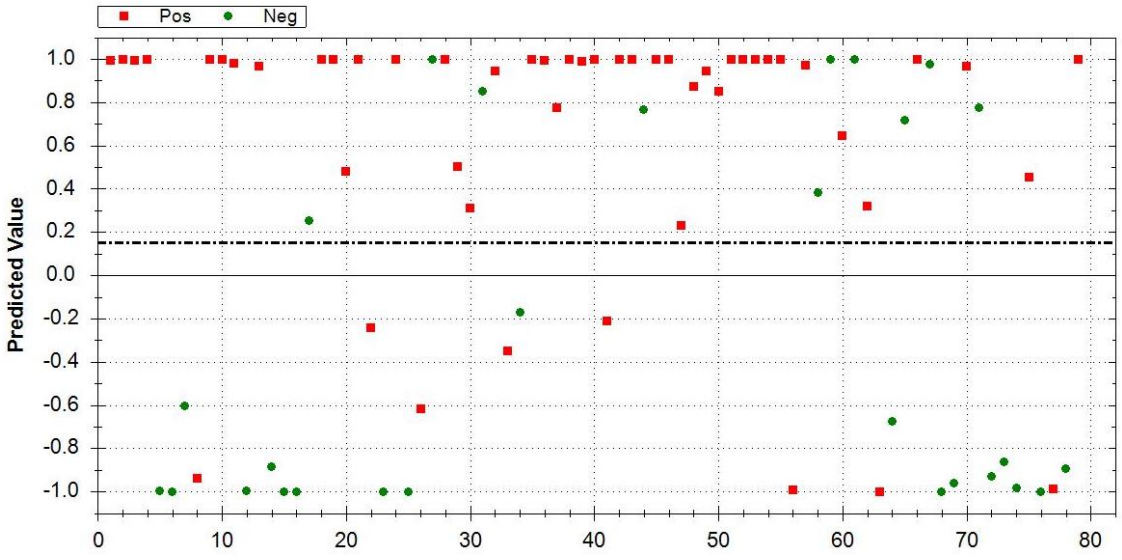

Figure 20. Direct comparison between IPF (n=51, red squares) vs. COP (n=28, green dots). Abbreviations: IPF area 0-1: Red squares indicate correctly recognized IPF patients; green dots denote false positive patients. COP area 0- -1: Green dots represent correctly identified COP patients, red squares mark false negative results. The dotted line is inserted around the threshold for uncertain cases and included two patients.

In the analysis between COP (n=28) vs. CTD-ILD (n=25), an AUC of 0.75, a sensitivity of 0.82, a specificity of 0.56 and a MC of 0.40 were obtained. Between IPF (n=51) vs. CTD-ILD (n=25), AUC 0.84, a sensitivity 0.86, and a specificity 0.68 and MC of 0.55 were obtained. Figure 21 displays the results.

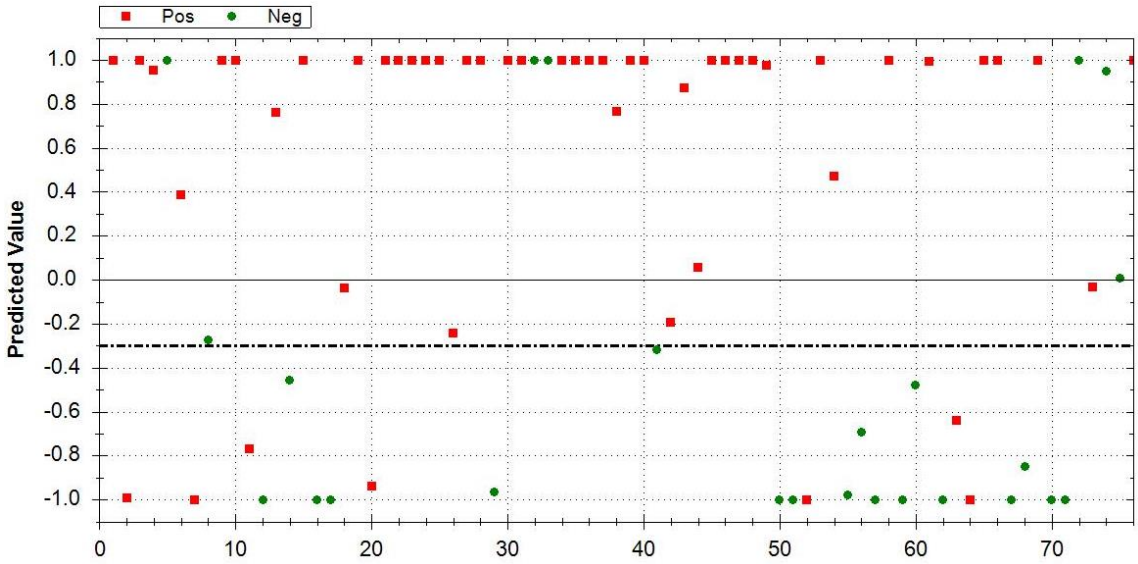

Figure 21. Direct comparison between IPF (n=51, red squares) vs. CTD-ILD (n=25, green dots). Abbreviations: IPF area 0-1: Red squares indicate correctly recognized IPF patients; green dots denote false positive patients. CT-ILD area 0- -1: Green dots represent correctly identified CTD-ILD, red squares mark false negative results. The dotted line is inserted around the threshold for uncertain cases and included two patients.

The validation of eNose was continued with two other groups, LC and patients with COPD, where VOC signatures were collected by Aeonose® in 42 incident and 78 prevalent LC patients, of them 29 LC patients in complete remission (LC CR), 33 healthy controls (HC) and 23 COPD patients, as presented in our publication "Recognition of breathprints of lung cancer and chronic obstructive pulmonary disease using electronic nose Aeonose®" (attachment 7) [129]. In this study we assessed if LC- specific VOC patterns can be established by the eNose Aeonose® to safely detect LC and to distinguish this entity from pulmonary comorbidities such as COPD. Indeed, our initial results reflecting a direct comparison of LC to HC, following a case-control design (established by previous studies), were quite promising. The established algorithm of Aeonose® signature allowed safe separation of LC and HC, showing an AUC of 0.92, sensitivity of 0.84 and a specificity of 0.97. Figure 22 shows the results.

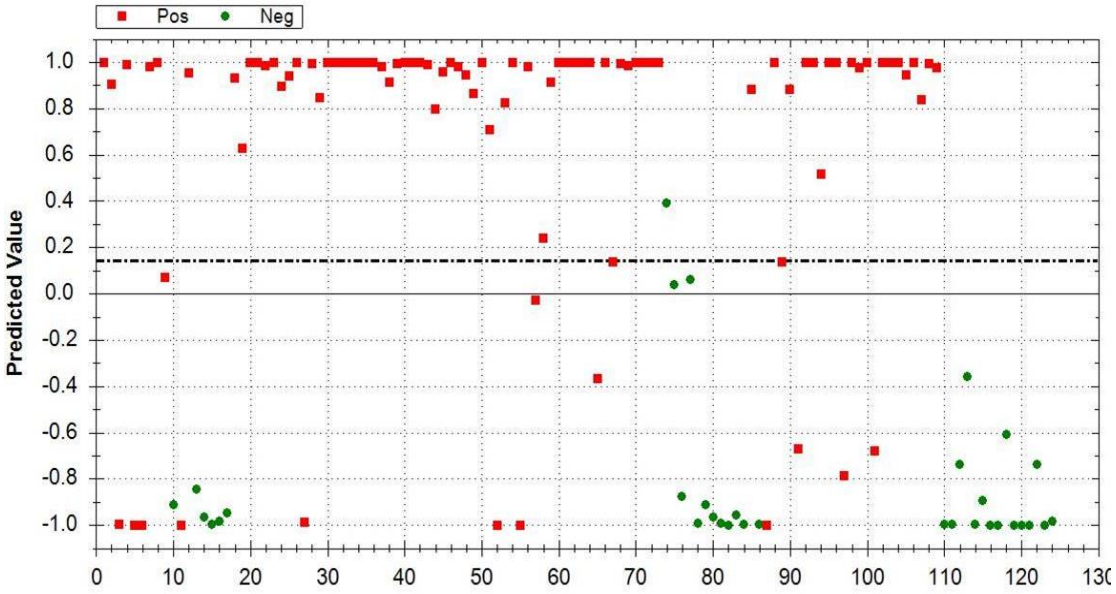

Figure 22. Direct comparison of LC cases (n=91, red squares) vs HC (n=33, green dots) by Aenose®. Abbreviations: LC area 0-1: Red squares indicate correctly recognized LC patients; green dots denote false positive patients. HC area 0- -1: Green dots represent correctly identified healthy controls, red squares mark false negative results. As previously described, the dotted line is inserted for values around the threshold where there is doubt about which side it tends to, and, hence, reflecting an area of uncertainty.

When tested in a blinded fashion (as shown in Figure 23), the device recognized 19 out of 29 LC CR patients (=65.5%) as LC-positive, of which only five developed recurrent LC later on (after 18.6 months±9.02; mean value ± SD).

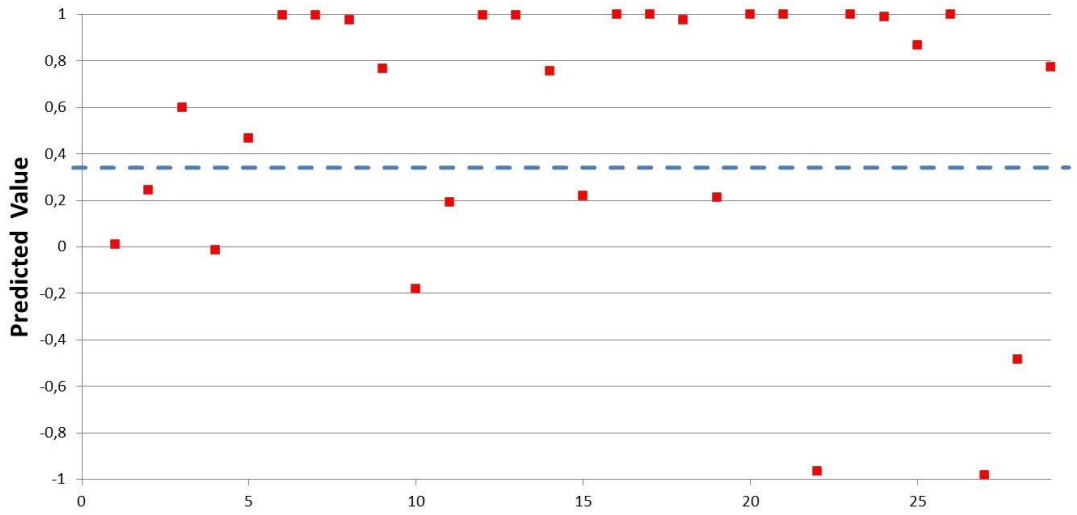

Figure 23. LC CR group (n=29, red squares) as blind samples, being identified mostly as VOC positive for LC. In this group are five LC relapsed patients. Abbreviations: LC

area 0-1: 19 LC CR cases in this area are recognized as VOC positive for LC. HC area 0- -1: Ten cases in this area are LC CR patients, being recognized as VOC negative for LC. The dotted line is inserted around the threshold for uncertain cases and included two patients.

Unfortunately, the algorithm also recognized 11 of 24 COPD patients as being LC positive (with only one of the 24 COPD patients developing LC 56 months after the measurement). The data presented in Figure 24.

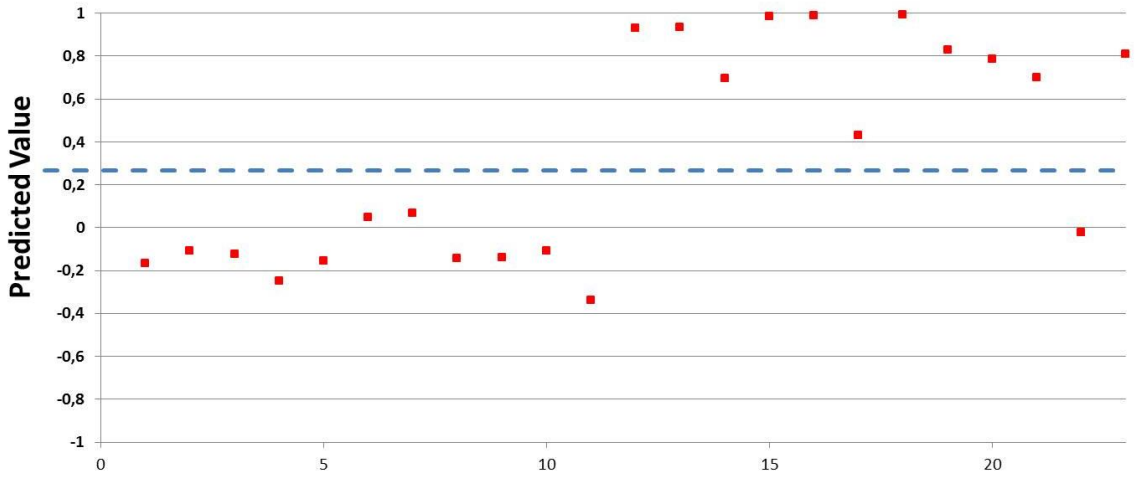

Figure 24. COPD group (n=23, red squares) as blind samples, being identified partly as VOC positive for LC. In this group there is one COPD patient, who developed LC over time. Abbreviations: LC area 0-1: 11 COPD cases are in this area, being recognized as VOC positive for LC. HC area 0- -1: 12 red squares in this area are COPD patients, being recognized as VOC negative for LC. The dotted line is inserted around the threshold for uncertain cases.

4. Discussion.

In the frame of the European IPF Network “Natural course, Pathomechanisms and Novel Treatment Options in Idiopathic Pulmonary Fibrosis” (eurIPFnet), the multicenter, European-wide IPF registry (eurIPFreg) and biobank (eurIPFbank) were launched in 2009 [116]. The primary purpose of eurIPFreg was to characterize the natural course of IPF, and to explore novel capture diagnostic and treatment strategies. This is in line with a previous note, according to which improved survival of IPF depends on better understanding of the epidemiology of the disease, its diagnostic spectrum and outcomes from emerging therapies [130]. Furthermore, a real-life data registry significantly complements data from randomised controlled trials, typically comprising a wide range of severity, progression and co-morbidities.

The results of this research extend beyond baseline findings and include clinical characteristics, survival outcomes, and psychosocial impairments of our large European ILD cohort together with profound analyses of familial cases. Furthermore, we assessed novel methods in ILD diagnosis, evaluated the uselfullness of electronic noses and exhalative breath biomarkers in ILD diagnosis. With regard to demographics, the data obtained in our IPF cohort were similar to those reported in large randomized clinical trials and other registries [20, 131]. Pulmonary function tests in our cohort revealed a marked restrictive disease, with the FVC and DLco being somewhat similar to the observations in the ASCEND study[74].

The prevalence and impact of comorbidities on the clinical course of IPF remains unclear and is the subject of our upcoming studies. From all reported co-morbidities, cardiovascular were most common, followed by reflux disease, sleep disorders, hyperlipidemia, diabetes and pulmonary hypertension. This appears to be also consistent with other registries[132].

As the latest IPF guidelines were released in 2018, the eurIPFreg comprises patients initially diagnosed and treated according to the ATS/ERS Consensus Statement of 2000, as well as those diagnosed and treated according to the ATS/ERS/JRS/ALAT IPF guidelines of 2011 [13, 98, 99]. Hence, the eurIPFreg is a useful tool in order to explore changes in the diagnostic and clinical practice in IPF in the last years. The diversity of our European IPF cohort may reflect not only the variability in the natural course of the disease, but also changes in the clinical management of the patients, especially when comparing our results to historic IPF cohorts. Thus, the decline in the usage of

prednisolone and other immunosuppressive medication reflects the implementation of recent IPF guidelines, and the knowledge arising from the PANTHER-IPF trial, according to which these therapeutic strategies are rather harmful than helpful in IPF[133]. The use of prednisolone is nowadays restricted mostly to the therapy of severe exacerbations.

Our study reflects the heterogeneity of the natural course of IPF in a ”real world” clinical setting, ranging from slow progression, with long periods of stable disease, to a rapid progressive fibrosis, with successive lung function impairment and death within few months. Our registry also reflects the steady increase of the usage of antifibrotic drugs. In October 2016, for example, 27 patients received nintedanib and 224 patients received pirfenidone. Our results showed an improved survival in patients on anti-fibrotic medication (p=0.001), in accordance with randomized controlled trials, such as CAPACITY, ASCEND as well as open-label extension study (RECAP) [134, 135]. Interestingly, the effect site with regard to the treatment effect was similar between our registry data and the data from the previous phase III studies. Prognostic indicators for IPF patients under antifibrotic treatment have yet to be established in the future, using epidemiological data and taking into account a larger numbers of patients with definite outcome data and longer follow up periods.

Familial IIP cases in our study were estimated by the presence of at least one direct relative (genetic mother / father, children, full siblings), which suffered or died from IPF / NSIP (evidence level A) or other ILD (evidence level B). The majority of studies indicated that f-IPF does not differ clinically or histologically from its sporadic form. The only difference being observed was an earlier age at onset of familial cases [29, 38, 39]. The mean age at first diagnosis of the s-IPF is known to range at 66-68 years; in case of f-IPF, a significantly lower age (55-62 years) has been described and has been reconfirmed in our study [29, 136].

In the present study, we found 23% of all IPF and 14% of all IIP patients to have a familial background. Our data therefore seem to fit to the data reported by Loyd et al [43] as well as other study groups and suggest that a familial background is rather common and not a rarity. Loyd at al commented that nine out of 47 (19%) pulmonary fibrosis patients who underwent lung transplantation at their study center had at least one relative with pulmonary fibrosis [43]. Marshall et al. investigated 21 IPF families with 57 affected members and found out that 0.5-2.2% of IPF cases were familial [137]. They estimated the prevalence to be even higher, with only about half responding to the questionnaire.

By analyzing pedigree charts of f-IPF, we observed mostly autosomal dominant inheritance pathway (16 of 25 pedigrees) with variable penetrance, which has been confirmed by other authors as well [38, 138]. However, a coexistence of different modes or more complex patterns of inheritance, autosomal recessive inheritance, or heterogeneity of hereditary traits cannot be ruled out as well [33, 44, 139]. Our results showed in f-IPF patients definite UIP pattern in HRCT in 66.7% and possible UIP in 33.3% of the cases. Of note, there were nine families with multiple cases of bronchial carcinoma. This phenomenon has been linked by Wang et al to a surfactant protein A2 mutation [51] and opens the question if the observed high rate of pulmonary malignancies reported in earlier autopsy studies is largely based on these familial cases or if it rather represents a more common “complication” of IPF in general [140]. An unusual presence of diverse respiratory diseases in four families was also observed.

Within the f-IPF families, the age at onset of disease and of death was found to come down with each generation, hence reflecting the phenomenon of anticipation. The affected family members of youngest generation died on average at the age of 66 years, as compared to 71 years in the parental generation. The grandparent generation was first diagnosed on average at the age of 72 and died on average at age 79, as shown in Table 5. The mortality was linked to the age of diagnosis (p=0.013).

In genetics, anticipation is a phenomenon whereby a genetic disorder is passed on to the next generation, but the symptoms of the disease become apparent at an earlier age with each age group. In some cases, an increase of severity of symptoms was also noted [141]. The proportion of IPF cases between the youngest and the previous generation seems similar in this study (40 vs. 38%). It should be noted that more patients of the younger generation might appear in the future, as they have not yet reached the classic age of onset. Therefore, the anticipation of familial IPF can be assumed. In light of the progressive shortening of telomers in successive generations of affected families in those with Terc/Tert mutations, such anticipation phenomenon could be easily explained by the underlying pathomechanisms [142].

Chibbar at al showed in the analysis of a large family with many IPF patients within several generations the phenomenon of anticipation in the f-IPF [143]. Ravaglia showed a significant difference in onset of f-IPF between the younger and the older generation of 58 vs. 74 years [144]. One possible explanation for this phenomenon could be the increasing telomere shortening over consecutive generations [141]. In addition, the

known susceptibility genes lead to premature lung aging because of deteriorated regeneration ability of the lung [138].

Scholand et al showed that f-IPF patients have a significantly increased risk of death vs. s-IPF among the first- through third-degree relatives ($p<0.001$) [145]. The evaluation of the third (grandparents) generation was challenging due to sparse data. The difference in the first year of diagnosis of the second (parents) generation was nevertheless significant (58 vs. 66 years, $p=0.013$). Some pedigree charts display that IPF manifests in the younger generation over a decade earlier vs. parents' generation. It must be taken into account that the younger generation might be more aware of the disease and could therefore be diagnosed earlier. Nevertheless, the younger generation tends to die earlier (66 vs. 71 years of age, $p=0.131$). Despite the lack of significance, this difference of five years on average is considerable, as the younger generation had access to novel therapies, unlike the older generations.

Eighteen of the 25 affected relatives in our study had been diagnosed by HRCT (including four patients who also underwent a VATS). Another difference to previous studies is that, in addition to the patients, their healthy relatives were invited for a medical checkup, in order to actively search for an evidence for a diffuse parenchymal lung disease. In particular, young, close relatives and people with mild respiratory symptoms volunteered gladly to take part in this study. This group of family members is precisely the cohort that may have previously unrecognized IPF/IIP; and so the number of false negatives within the families could also be kept to a minimum. Such early detection may have a significant impact on the life of these patients: prevention of respiratory infections, prevention of exposure to a fumes and gases known to contribute to disease progression (cigarette smoke, outdoor pollution) and early initiation of anti-fibrotic treatment may be mentioned as immediate consequences of such approach and justify a more rigorous screening for concerned family members on clinical grounds.

Whether one needs to conduct genetic screening for routine purpose is a matter of debate and will be subject of position papers in the future: here, the advantage of identifying a disease-causing mutation and the potential to identify family members who even do not show any visible disease at the time of testing contrasts with the burden of knowing that one could develop the disease in an unpredictable time span. We therefore tend to offer clinical screening, with a special emphasis on lung auscultation and screening for velcro rales, as well as lung function and DLco tests, to the families of our

IPF patients, and not routine genetic testing. For research purpose, however, sequencing should be done and will also be done in our cohort.

In ILDs, HRQoL as patient-reported outcome measures gains increasing relevance. However, existing HRQoL instruments are not yet sufficiently validated as clinically meaningful endpoints. Therefore, the utilisation of validated HRQoL instruments is strongly recommended for marketing-authorisation application of novel treatments. The SF-36 Questionnaire is a generic instrument which is frequently used in clinical trials in IPF as a secondary endpoint. Such instruments are designed to measure overall health states and allow comparisons across patients with different diseases and the general population. Evaluating the validity of these generic instruments in specific diseases is indispensable and is also needed for the SF-36 in IPF.

Currently, two studies provide psychometric characteristics of the SF-36 in IPF based on longitudinal data. It is our knowledge that only these studies analysed if the SF-36 can detect changes or stability over time of HRQoL, which is essential as an endpoint in clinical trials. Swigris et al. provide disease specific minimally important differences (MID), which are obligatory to evaluate changes in HRQoL over time. Therefore, patients would benefit from further longitudinal analysis based on multicentre-data and in a real-world setting. By assessing psychometric characteristics of the SF-36 in IPF (acceptance and feasibility; discrimination ability; construct and criterion validity, and internal consistency; responsiveness and test-retest- reliability), we intended to evaluate disease specific MIDs, using real-world data from eurIPFreg from patients in different disease stages and ethnical backgrounds. In evaluation of psychosocial impairments, SF-36 appeared to be a valid instrument to measure HRQoL in IPF and, hence, can be used in RCTs or individual monitoring in this disease.

UILD has been the topic of some previous publications. The inconsistent terminology and definitions across studies resulted in different prevalence data and reflect the absence of established diagnostic criteria [146]. In our study, the uILD cohort represented ~15% of the total ILD cohort, and ~26% of the IIP cohort. The study by Ryerson et al. reported a percentage of uILD of 10% of the ILD cohort [147]. Our uILD cohort was on average 68 years old; also, 71% of the patients were current or previous smokers and 29% of the patients received LTOT. In the study by Ryerson et al. uILD patients had a comparable mean age (68 years), smoking history (63.6% current / former smokers), or LTOT usage (21.9%)[147].

In 16% of cases, uILD was diagnosed due to insufficient amount of lung tissue in the biopsy, and this was exclusively observed in patients undergoing transbronchial forceps biopsies, but in none of the patients receiving cryobiopsy. Cryobiopsies represent an important adjunct for the diagnosis of ILD, enhancing the diagnostic confidence of the treating clinicians in absence of VATS [148]. In our uILD cohort, recruited between 2009-2019, seven patients underwent a cryobiopsy. Meanwhile, this minimal-invasive technique has become our new standard for most ILD cases and has almost completely replaced transbronchial forceps biopsy [3]. In a Cox regression analysis for survival we could show that smoking, FVC % pred. at baseline, FVC decline ≥10% p.a., and a DLco ≤55% pred. at baseline are significantly associated with progressive disease and fatal outcome.

Without any doubt, the process of ILD diagnosis would also profit from other, novel non-invasive principles aside of imaging. A combination of several, blood based biomarkers may be useful to identify comprehensive individual signatures in ILD patients, leading to a more personalized medicine [11]. In addition, comprehensive metabolomic analysis could allow the tracking of metabolic pathways, and to monitor the efficiency of therapeutic interventions [149]. Several molecules related to epithelial cell injury, matrix remodeling and immune regulation have been discussed as promising candidates.

Moreover, exhaled breath-based methods have been studied in the past decades for their applicability in the assessment of airway inflammation and as possible diagnostic tools in several inflammatory lung diseases, e.g. Asthma or COPD. Here, a large number of biomarkers in breath have been examined as possible indicators of inflammation, to diagnose and monitor the diseases as well as to evaluate the response to treatment [150]. Aiming to investigate if FeNO or exhaled eisocanoids could be of diagnostic help in ILD's and other chronic lung diseases, we also performed correlation analyses of FeNO, PGE2 or 8-isoprostane values with PFT parameters to investigate the prognostic relevance of these markers and their relation to medication. Unfortunately, we did not observe any meaningful or significant difference between FeNO or eicosanoid values of the different cohorts as well as controls. Yet, for reasons not understood, some patients showed highly elevated FeNO values. Re-evaluation of these cases did not forward any particular explanation (e.g. exacerbation).

Previous studies have shown that measurement of FeNO appears to be a quantitative, non-invasive, simple and safe method of measuring airway inflammation and to assess activity of airway disease, especially in asthma [151]. In our study, FeNO measurement

and result interpretation were done according to an official ATS guideline [152]. When interpreting FeNO measurements, individual factors should always be considered. In adults, a positive correlation was observed between the FeNO level and age of the patients [153]. We also detected decreased FeNO values in active smokers, which is in line with previous reports; e.g. Lu at al compared FeNO levels between ex-smokers and current smokers in COPD and reported that FeNO levels in ex-smokers were higher than those in current smokers [154].

The effect of steroids on the FeNO values had been discussed differently in the literature and seems to depend on the underlying disease and its pathomechanism [82]. Lehtimäki et al reported the FeNO values of IPF and HP patients appear to be responsive to steroids [155]. However, no significant change in steroid intake across all groups was observed in this work. Our study showed that steroid-negative COPD patients display slightly higher FeNO values than those treated with steroids.

With regard to changes in FeNO in IPF, Saleh et al showed that NO values might be upregulated in IPF patients, with a significant increase in early to intermediate stage of the disease, suggestive of a valuable role of constitutively produced NO in pulmonary homeostasis and of a protective effect on lung architecture. In his study, IPF patients showed strong expression of nitrotyrosine and NOS was seen in macrophages, neutrophils, and alveolar epithelium, especially in the early to intermediate stage of IPF [156]. The active stage of IPF was associated with increased inflammatory and alveolar expression of nitrotyrosine and NOS, where increased production of NO and peroxynitrite seemed to contribute to the oxidative damage [156].

In another study, Cameli et al. compared FeNO values in IIP patients (22 IPF, 8 NSIP) and healthy controls (n=30) at different flow rates. At 50 ml/s IPF patients showed similarly high levels (22.3 ± 8.4 ppb; MW ± SD) as those reported by Guilleminault, but were lower as compared to Schildge et al. (27.6 ± 16.3 ppb; MW ± SD) [157, 158]. Again, FeNO measurements in the Cameli and the Guilleminault studies were performed with the same device (Hypair FeNO, Medisoft). Absolute FeNO values have been reported to depend on the underlying method of NO measurement of the device. To this end, lower values of FeNO have been noted when using the chemiluminescence method (NIOX Mino, Aerocrine) versus the electrochemical method (Hypair FeNO, Medisoft) [124].

With regard to the eisocanoid measurements, it had been previously suggested that PGE2 is down-regulated in IPF patients, thus favoring the development of pulmonary

fibrosis [159, 160]. In IPF, reduction in PGE2 level is assumed to result from a defective COX2 pathway [161]. Despite the many references in the literature, no significant PGE2-level difference between the IPF, ILD and the healthy controls could be found in BALF in our study. Compared to the other ILD subgroups, IPF did not appear to have the lowest PGE2 values in the EBC. Free and total 8-isoprostane are known markers for oxidative stress-induced lipid peroxidation. Elevated levels of free 8-isoprostane have been used as markers of oxidative stress in asthma, COPD, pulmonary sarcoidosis, ILD and IPF [151, 162]. Our mean value of free 8-isoprostane of 31.07 pg/ml in BALF in IPF is not that much different as compared to theirs, but our healthy subjects showed clearly higher 8-isoprostane values.

In conclusion, it was neither possible to differentiate between the kind of disease, to detect exacerbation or to correlate FeNO or eicosanoid values with lung function parameters DLco or 6MWD. In addition, analysis of arachidonic acid derivates in EBC was challenging (PEG2) or impossible (8-isoprostane), whereas it worked out well in BALF. Some group-specific, significant differences were observed with regard to free 8-isoprostane in BALF, the meaning of which appears questionable in light of the unchanged total 8-isoprostane values and the missing correlation to progression of disease.

Exhaled breath analysis by means of eNose technology has been of great scientific interest over the last few years and is a rapidly emerging field of medicine. However, despite all efforts, eNoses appear not to be ready to be implemented as medical diagnostic tool. However, there are no known studies describing specific VOC patterns in fibrotic lung diseases. Drawn against this background, we examined if ILD–specific VOC patterns can be clearly recognized by the Aeonose® and distinguished from pulmonary comorbidities such as COPD. Without a doubt, our initial results reflecting the direct comparison of different ILD groups to HC and following a case-control design as established in previous studies, were quite appealing. In this regard it appeared as if the Aeonose® clearly has a potential in recognizing ILD patients with acceptable sensitivity and specificity.

Nonetheless, knowing about the risk of bias due to the case-control design, as reviewed by Leopold et al, we extended our assessment from direct comparison to a cross-correlation analysis, by comparing subgroups within themselves [163]. Here, although being previously trained in disease-specific pattern recognition, the Aeonose® algorithm

performed less effective and was not able to distinguish the patient categories correctly. Instead, reduced MC, sensitivity and specificity values were encountered under these conditions. Based on our results, we speculate that the further development of the eNose based, non-invasive diagnosis of ILDs and LC recognition may require a different approach: rather than comparing different clinical phenotypes in a dichotomous fashion, one should consider use of artificial intelligence-based algorithms, and also of unsupervised clustering of data, e.g. by the principle component analysis currently employed in single cell omics. Such approach should allow a more reliable and safe differentiation of COPD or IPF (being frequent comorbidities) related signatures versus those of the LC.

5. Summary

For many years, the challenge of translating the pathogenic mechanisms of IPF or other ILDs with the goal of developing novel targets for therapeutic intervention lied within academic groups working rather separately. The establishment of IPF scientific networks in both the USA and European Union (EU), supported by pharmaceutical and biotech companies, significantly improved IPF research. Still, the access to patient material from large patient cohorts and biobanks for a long time remained a major challenge for IPF researchers.

Based on funding through the 7th Framework Programme, the EU supported the European IPF Network, aiming to decipher the natural course and molecular pathogenic mechanisms of IPF and to develop future novel therapeutic strategies. As a part of this network, in November 2009, a European-wide, internet-based patient registry (eurIPFreg) and biobank (eurIPFbank) were launched, allowing translational research of the natural course in IPF and other ILDs, evaluating its dependence on environmental factors, comorbidities, progression factors, as well as response to therapy, and also enabling access to IPF biomaterials for investigators involved in IPF research.

In this research, we report detailed clinical characteristics of a large European IPF cohort with outcome data extending up to 10 years. Our patients are diverse in age, impairment of lung function, therapeutic regimes and co-morbidities. In this regard, the diversity of the eurIPFreg- cohort may reflect not only the variety in natural course of the disease, but also changes in clinical management of the patients, especially when comparing our results to historic IPF data and data from controlled clinical phase III trials. Thus, the decline in the usage of prednisolone and other immunosuppressive medication reflects the implementation of recent IPF guidelines, and the knowledge arising from the PANTHER-IPF trial, according to which these therapeutic strategies are rather harmful than helpful in IPF. Our registry data also reflect the steady increase in the use of cryobiopsy for diagnosis and of usage of antifibrotic drugs.

However, our data indicate that IPF still has a high mortality rate and that survival times are quite heterogenous, reflecting both, the heterogeneous natural course of IPF in a clinical setting, ranging from stable disease to a rapid progressive fibrosis, and the change in the pharmacological approach to IPF subjects.

Familial IPF (f-IPF) patients showed an earlier onset and more aggressive natural course of the disease as compared to sporadic form (s-IPF). The most likely mode of inheritance

in familial IPF cases seemed to be autosomal dominant with variable penetrance. We also observed that the disease seems to affect consecutive generations at a younger age (phenomenon of anticipation).

The most important known prognostic factors in uILD remain the decline in lung function and smoking. The use of Fleischner diagnostic criteria allows further differentiation and accurate diagnosis together with new therapeutic options for patients, who currently have no established approach to therapy.

The results of our biomarker study do not support previous ILD studies, according to which NO is an important marker for the assessment of disease severity. To our understanding, FeNO or PGE2 or 8-isoprostane measurements in BALF do not offer as diagnostic or prognostic marker, nor do they indicate acute exacerbations.

The algorithm developed in our eNose study to differentiate ILD or LC from controls and COPD patients using the Aeonose® resulted in good sensitivity and specificity in separating two conditions in a case-control approach. Unfortunately, when comparing the different ILD entities directly with each other, the performance of the Aeonose was markedly weaker and does not offer for routine use. Still, we believe that VOC signatures, once being adequately clustered and annotated to the underlying pulmonary phenotype, may be used for rapid and safe recognition of different ILD or LC entities. We suggest that artificial intelligence or principle component analysis- based studies of a much broader data set of patients with LC and interstitial lung diseases may allow safe differentiation in the future.

The presented data reflect changes in the diagnostic and therapeutic approach in IPF in the last ten years, supporting the important role of large real-world data registries to document and scrutinize changes in IPF management. Furthermore, a real-life data registry significantly complements data from randomised controlled trials, typically comprising a wide range of severity, progression and co-morbidities. Prognostic indicators for IPF patients under antifibrotic treatment have yet to be established in the future, using epidemiological data and taking into account a larger numbers of patients with definite outcome data and longer follow up periods.

6. Zusammenfassung

Im Laufe der letzten Jahre bestand eine große Herausforderung in dem verbesserten Verstehen des Pathomechanismus der IPF oder anderen ILDs um die Entwicklung der neuen therapeutischen Konzepte zu ermöglichen. Die Einrichtung wissenschaftlicher IPF-Netzwerke in den USA und in der EU, und der vermehrte Einsatz von Pharma- und Biotech-Unternehmen, führten diesbezüglich zu einer deutlichen Verbesserung. Der Zugang zu Patientenmaterial aus großen Patientenkohorten und Biobanken war für IPF-Forscher jedoch lange Zeit eine große Herausforderung.

Basierend auf der Finanzierung durch das 7. Rahmenprogramm unterstützte die EU das europäische IPF-Netzwerk mit der Aufgabe, den natürlichen Verlauf und die molekularpathogenen Mechanismen von IPF zu entschlüsseln, sowie zukünftige Therapiestrategien zu entwickeln. Als Teil dieses Netzwerks wurden im November 2009 ein europaweites internetbasiertes Patientenregister (eurIPFreg) und eine Biobank (eurIPFbank) implementiert um eine translationale Forschung des natürlichen Verlaufs von IPF und anderen ILDs zu ermöglichen, die Abhängigkeit von ILDs von Umweltfaktoren, Komorbiditäten, und Progressionsfaktoren zu beschreiben, sowie das Ansprechen auf die Therapie detaillierter zu erforschen.

In dieser Studie berichteten wir ausführlich über die klinischen Charakteristika einer großen europäischen IPF-Kohorte, inklusiv Outcome-Daten für einen Zeitraum bis zu 7-10 Jahren. Unsere Patienten zeigen sich in Bezug auf Alter, Ausmaß der Beeinträchtigung der Lungenfunktion, Therapieregime, Komorbiditäten und Progression der Erkrankung sehr heterogen. In dieser Hinsicht spiegelt die Heterogenität der eurIPFreg-Kohorte möglicherweise nicht nur die Vielfalt des natürlichen Krankheitsverlaufs wider, sondern auch Veränderungen im klinischen Management der Patienten. Dies werde insbesondere beim Vergleich unserer Ergebnisse mit historischen IPF-Daten vor Einführung der Antifibrotika im Jahr 2011 deutlich. Der Rückgang des Einsatzes von Prednisolon und anderen immunsuppressiven Medikamenten und die stetige Zunahme der Verordnung von Antifibrotika reflektiert somit die Umsetzung der aktuellen ATS/ERS Leitlinien und die Erkenntnisse aus der PANTHER-IPF-Studie.

Die Ergebnisse zeigen jedoch, dass die IPF immer noch eine hohe Sterblichkeit aufweist und die Überlebenszeiten recht heterogen sind. Dies spiegelt sowohl den variablen natürlichen Verlauf von IPF in einem klinischen Umfeld wider, der von einer stabilen

Erkrankung bis zu einer schnell fortschreitenden Fibrose reicht, als auch die Veränderung der Medikation für IPF-Patienten.

Patienten mit familiar gehäuft auftretender IPF (f-IPF) zeigten im Vergleich zur sporadischen Form (s-IPF) einen früher einsetzenden und aggressiveren natürlichen Krankheitsverlauf. Die wahrscheinlichste Art der Vererbung in familiären IPF-Fällen schien autosomal-dominant mit variabler Penetranz zu sein. Wir beobachteten auch, dass die Krankheit im jüngeren Alter mehrere aufeinanderfolgende Generationen betrifft. Die wichtigsten prognostischen Faktoren in uILD bleiben der Abfall der Lungenfunktion und das Rauchen. Die Anwendung von Fleischner-Diagnosekriterien ermöglicht eine weitere Differenzierung und genaue Diagnose sowie neue therapeutische Möglichkeiten für Patienten.

Die Ergebnisse unserer Biomarker-Studie stützen frühere ILD-Studien nicht, wonach NO ein wichtiger Marker für die Beurteilung der Schwere der Erkrankung zu sein schien. Nach unserem Verständnis haben FeNO- oder PGE2- oder 8-Isoprostan-Messungen in der BALF weder einen diagnostischen oder prognostischen Nutzen als Biomarker noch weisen sie auf eine Exazerbation hin.

Der in unserer eNose-Studie entwickelte Algorithmus zur Unterscheidung der LC und ILD von Kontroll- und COPD-Patienten unter Verwendung der Aeonose® ergab eine gute Sensitivität und Spezifität bei der Trennung der ILDs in einer Fall-Kontroll-Studie. Leider war die Leistung der Aeonose beim direkten Vergleich der verschiedenen LC und ILD-Einheiten deutlich geringer und bietet aktuell keine Möglichkeit für den routinemäßigen Einsatz. Wir sind jedoch der Ansicht, dass VOC-Signaturen, sobald sie korrekt gruppiert und mit Merkmalen zum zugrunde liegenden Krankheitsphänotyp versehen sind, zur schnellen und sicheren Erkennung verschiedener LC und ILD-Entitäten verwendet werden können.

Die in diesem Werk vorgestellten Daten spiegeln Änderungen im diagnostischen und therapeutischen Ansatz bei ILD/IPF in den letzten zehn Jahren wider und unterstützen die wichtige Rolle großer „real-world data“ Datenregister, um Änderungen im IPF-Krankheitsverlauf und Management zu erforschen. Prognoseindikatoren für IPF-Patienten unter antifibrotischer Behandlung, die epidemiologischen Daten verwenden und eine größere Anzahl von Patienten mit eindeutigen Outcome-Daten und längeren Nachbeobachtungszeiträumen berücksichtigen, müssen in Zukunft noch ermittelt werden.

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8. Abkürzungsverzeichnis / Abbreviations

% pred.	percent of predicted value
6 MWD	six-minute walking distance
ANA	anti-nuclear antibodies
AROIP	area right of inflexion point
ATS/ ERS	American Thoracic Society/ European Respiratory Society
AUC	area under the curve
BALF	bronchoalveolar lavage fluid
BGA	blood gas analysis
CaNO	alveolar NO concentration
CD	cause of death
CI	confidence intervall
colon Ca	colon carcinoma
COP	cryptogenic organizing pneumonia
COPD	chronic obstructive pulmonary disease
CPFE	combined pulmonary fibrosis and emphysema
CTD-ILD	connective tissue disease-associated interstitial lung disease
CTGF	connective tissue growth factor
DD	differential diagnosis
def	definitive
DIP	Desquamative interstitial pneumonia
DLco	diffusing capacity or transfer factor of the lung for carbon monoxide
DPLD	diffuse parenchymal lung disease

EBC	exhaled breath condensate
EBC	exhaled breath condensate
ELISA	Enzyme Linked Immunosorbent Assay
EMA	European Medicines Agency
ENA	extractable nuclear antibodies
eNose	electronic nose
EU	European U
eurIPFbank	European IPF Biobank
eurIPFreg	European IPF Registry
FD	initial diagnosis year / age at first diagnosis in years
FDA	Food and Drug Administration
FeNO	fractionated exhaled nitrogen monoxide
FEV1	forced expiratory volume
FGF	fibroblast growth factor
f-IPF	familial idiopathic pulmonary fibrosis
FVC	forced vital capacity
FVC	forced vital capacity
GHP	general health perceptions
Heart Dis	heart disease
HC	healthy controls
HP	hypersensitivity pneumonitis
HRCT	high resolution computed tomography
HU	Hounsfield units

IIP	interstitial idiopathic pneumonia
ILD	Interstitial lung diseases
ILD	interstitial lung disease
IPF	idiopathic pulmonary fibrosis
Joint Dis	joint disease
LC	Lung cancer
LTOT	long term oxygen treatment
LTX	lung transplantation
Mamma Ca	breast cancer
MCC	Matthews correlation coefficient
MDT	multidisciplinary team discussion
MHI	mental health
MLA	mean lung attenuation
MMP	matrix metalloproteinase
MOF	multi organ failure
MTX	methotrexate
nBNP	n-pro brain natriuretic peptide
NSAID	non-steroidal anti-inflammatory drugs
NSIP	non-specific interstitial pneumonia
NYHA	New York Heart Association
PAIN	physical pain
pCO2	partial pressure of carbon dioxide
PDGF	platelet- derived growth factor

PFI	physical functioning
PFT	pulmonary function tests
PGE2	prostaglandin E 2
PH	pulmonary hypertension
pO2	partial pressure of oxygen
PPI	proton pump inhibitors
py	pack years
RA	rheumatoid arthritis
radiol.	radiological
RB-ILD	respiratory bronchiolitis-associated ILD
recrt	recurrent
RF	rheumatoid factor
ROLEM	emotional role functioning
ROLPH	physical role functioning
RV	residual volume
SaO2	oxygen saturation
SOCIAL	social role functioning
SP-A	surfactant-protein A
sPAP	dystolic pulmonary arterial pressure
TLC	total lung capacity
UGMLC	Universities of Giessen and Marburg Lung Center
uILD	unclassifiable interstitial lung disease
UIP	usual interstitial pneumonia

VC	vital capacity
VEGF	vascular endothelial growth factor
VITAL	vitality
VOC‘s	volatile organic compounds
WHO	World Health Organization

9. **Patienteninformation zum eurIPFreg / Informed content of the eurIPFreg.**

Patienteninformation und Einwilligungserklärung zum Forschungsvorhaben „Europäisches IPF Register“ (*eurIPFreg*)

Sehr geehrte Patientin, sehr geehrter Patient,

bei Ihnen oder bei einem direkten Verwandten wurde eine Idiopathische Pulmonale Fibrose (IPF), eine Nicht-Spezifische Interstitielle Pneumonie (NSIP) oder eine andere Erkrankung aus dem Formenkreis der Idiopathischen Interstitiellen Pneumonien diagnostiziert oder Sie gehören einer Gruppe von Patienten mit Lungenerkrankungen oder anderen Erkrankungen an, die wir aus Gründen des Vergleichs mit Patienten mit Idiopathischen Interstitiellen Pneumonien gerne in unser Register und in unsere Biobank mit aufnehmen möchten. Wir möchten Sie um Ihre Einwilligung zur Teilnahme an einem Forschungsvorhaben bitten, über dessen Ziele und dessen Ablauf wir Sie in der nachfolgenden Patienteninformation informieren wollen. Eine solche Teilnahme ist freiwillig, Sie werden in diese Studie also nur dann einbezogen, wenn Sie Ihre Einwilligung erklären. Um Sie über das Vorhaben und über die etwaigen Vorteile und Risiken Ihrer Teilnahme zu informieren, wird der verantwortliche Arzt ein ausführliches Gespräch mit Ihnen führen. Vor diesem Gespräch **möchten wir Sie bitten, die nachfolgenden Ausführungen zu lesen**. Sie können sich dadurch bereits einen eingehenden Überblick verschaffen. Bitte fragen Sie Ihren Arzt, wenn Sie etwas nicht verstehen oder wenn Sie zusätzlich etwas wissen möchten.

Version 1.6 vom 20.03.2013

Patienteninformation und Einwilligungserklärung zum Forschungsvorhaben Europäisches IPF Register (eurIPFreg)

Patienteninformation

Patient/in (Name, Vorname):_____

geb. am: _____

in: _____

Zusammenfassende Darstellung des Vorhabens (Kurzversion)

Um den natürlichen Verlauf, die Risikofaktoren und die Gründe für das Entstehen der IPF und anderen Formen der Idiopathischen Interstitiellen Pneumonie besser erforschen zu können, werden wir im Falle Ihrer Zustimmung sowohl Angaben zu Ihrem Beschwerdebild und Ihrer Lebensqualität, klinische Daten als auch die von Ihnen entnommenen Biomaterialien zentral speichern und analysieren. Der Umgang mit den Ihnen entstammenden Daten und Biomaterialien ist hierbei vertraglich geregelt. Das Leitungsgremium des Europäischen IPF Registers in Zusammenarbeit mit einem Ethikgremium wertet die im Register gespeicherten Daten unter wissenschaftlichen Gesichtspunkten aus und wird Ergebnisse dieser Auswertungen, ohne jeglichen Bezug zu Ihrer Person, veröffentlichen. Ihre Daten sind durch die vorgesehenen Maßnahmen gesichert, d.h. aufgrund der Organisation der Datenbank und des Umgangs mit Ihren Biomaterialien ist eine Identifikation Ihrer Person durch Unbefugte nicht möglich. Im nachfolgenden ist unser Vorhaben nochmals im Detail erläutert.

Wer führt die Studie durch?

Das Europäische IPF Register (eurIPFreg) ist eine Registerstudie, die im Rahmen des Europäischen IPF Netzwerks (eurIPFnet) 2009 ins Leben gerufen wurde. Das IPF Netzwerk bestand von 2008 bis 2011 als ein von der Europäischen Kommission im Rahmen des 7. Rahmenprogramms finanzierter Forschungsverbund. Das Europäische IPF Register, dem derzeit mehrere universitäre und klinische Partner in Europa angehören, besteht dauerhaft weiter. Der Koordinator dieses Registers ist Prof. Dr. Andreas Günther, der unter folgender Adresse erreichbar ist:

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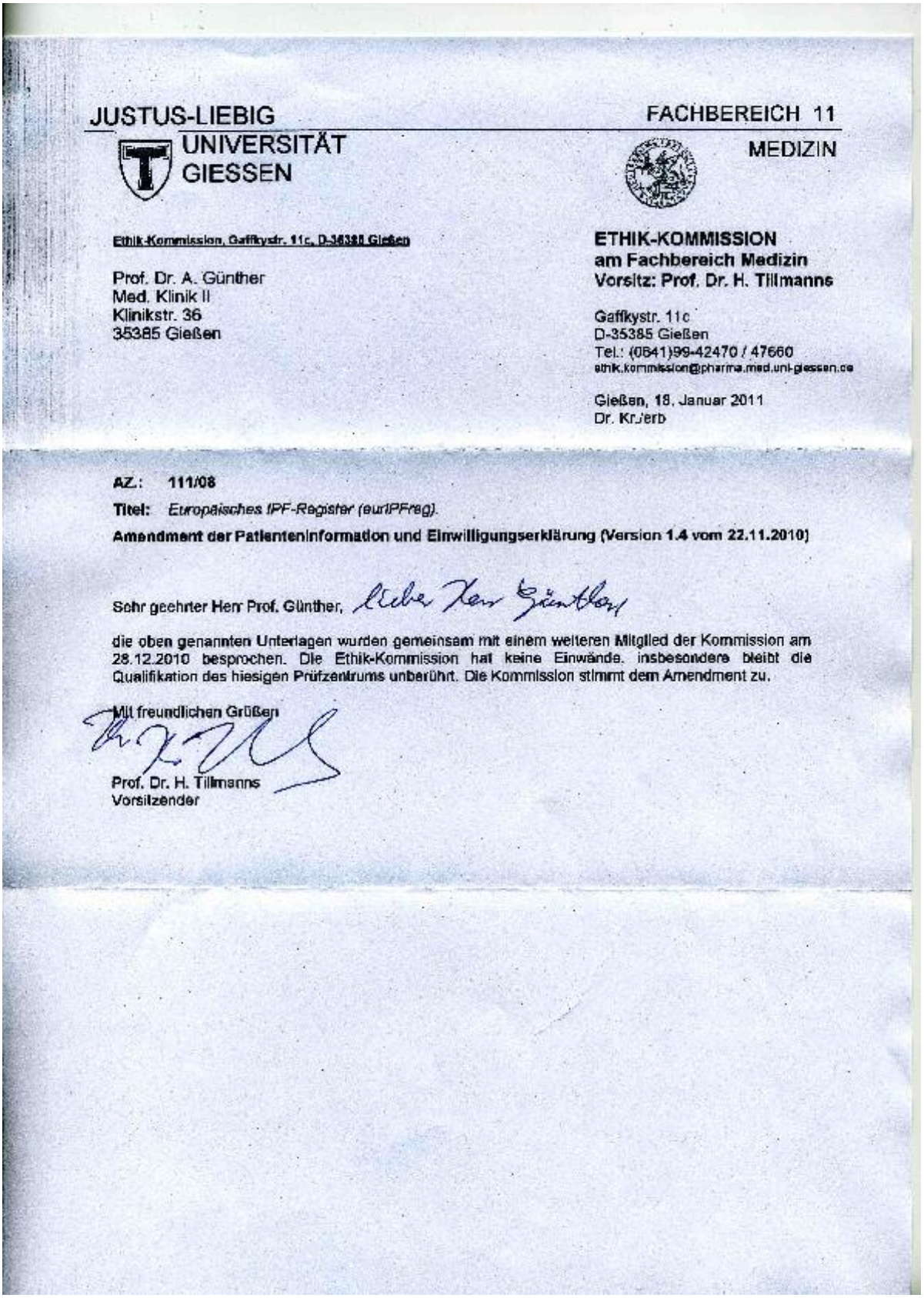
Version 1.6 vom 20.03.2013

10. Aktenzeichen des Ethikvotums / Ethics committee



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Gießen, 18. Januar 2011
Dr. Krüerb

AZ.: 111/08

Titel: *Europäisches IPF-Register (eurIPFreg).*

Amendment der Patienteninformation und Einwilligungserklärung (Version 1.4 vom 22.11.2010)

Sehr geehrter Herr Prof. Günther, liebe Herr Günther

die oben genannten Unterlagen wurden gemeinsam mit einem weiteren Mitglied der Kommission am 28.12.2010 besprochen. Die Ethik-Kommission hat keine Einwände, insbesondere bleibt die Qualifikation des hiesigen Prüfzentrums unberührt. Die Kommission stimmt dem Amendment zu.

Mit freundlichen Grüßen

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Gießen, 20. März 2013
Dr. Kr/

AZ.: 111/08

Titel: *Europäisches IPF-Register (eurIPFreg).*

Amendment No. 2 zur Einverständniserklärung (Version 1.6 vom 15.02.2013)

Korrigierte Version vom 20.03.13

Sehr geehrter Herr Prof. Günther, Lieber Herr Günther,

die oben genannten Unterlagen wurden im vereinfachten Verfahren durch den Vorsitzenden Prof. Tillmanns gemeinsam mit dem Leiter der Geschäftsstelle geprüft. Nach Eingang der korrigierten Patienteninformation Version 20.3.13 hat die Ethik-Kommission keine Einwände und stimmt dem Amendment zu.

Mit freundlichen Grüßen

Prof. Dr. H. Tillmanns
Vorsitzender

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12. Publikationsverzeichnis / List of publications

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14. Ehrenwörtliche Erklärung zur Habilitationsschrift / Statement on the habilitation thesis

„Hiermit erkläre ich, dass ich die vorliegende Arbeit bzw. die mir zuzuordnenden Teile im Rahmen einer kumulativen Habilitationsschrift, selbstständig und ohne zulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nichtveröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Ich versichere, dass ich für die nach §2 (3) der Habilitationsordnung angeführten bereits veröffentlichten Originalarbeiten als Erst- oder Seniorautor fungiere, da ich den größten Teil der Daten selbst erhoben habe, für das Design der Arbeiten verantwortlich bin und die Manuskripte maßgeblich gestaltet habe. Für alle von mir erwähnten Untersuchungen habe ich die in der „Satzung der Justus-Liebig-Universität zur Sicherung guter wissenschaftlicher Praxis“ niedergelegten Grundsätze befolgt. Ich versichere, dass alle an der Finanzierung der Arbeiten beteiligten Geldgeber in den jeweiligen Publikationen genannt worden sind. Ich versichere außerdem, dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Weise einer anderen Prüfungsbehörde vorgelegt wurde oder Gegenstand eines anderen Prüfungsverfahrens war. Mit der Überprüfung meiner Arbeit durch eine Plagiatserkennungssoftware bzw. ein internetbasiertes Softwareprogramm erkläre ich mich einverstanden.“

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Ort, Datum Unterschrift

15. Ausgewählte Publikationen / Attached Publications

1. Guenther, A.; **Krauss, E.;** Tello, S.; Wagner, J.; Paul, B.; Kuhn, S.; Maurer, O.; Heinemann, S.; Costabel, U.; Barbero, M.A.N.; et al. The European IPF registry (eurIPFreg): baseline characteristics and survival of patients with idiopathic pulmonary fibrosis. Respir. Res. 2018, 19, 141, doi:10.1186/s12931-018-0845-5.

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