Lung transplantation in idiopathic pulmonary fibrosis: old & new concepts
Liesbeth ten Klooster

Thesis University of Utrecht
ISBN: 978-9-46233-36-4
Cover design & lay-out: Alexander van der Linden
Print: Gildeprint, Enschede

Publication of this thesis was financially supported by:
Boehringer Ingelheim, Therabel,Teva NL, Longfibrose patiëntenvereniging,
Chiesi en Bayer.

Lung transplantation in idiopathic pulmonary fibrosis: old & new concepts

Longtransplantatie bij idiopathische pulmonale fibrose:
oude & nieuwe concepten

(met een samenvatting in het Nederlands)

Proefschrift ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 20 september 2016 des middags te 4.15 uur

door

Liesbeth ten Klooster

geboren op 24 maart 1982 te Noordoostpolder

Promotor:
Prof.dr. J.C. Grutters

Copromotoren:
Dr. C.H.M. van Moorsel
Dr. J.M. Kwakkel-van Erp

Paranimfen:
Drs. S. Boerman
Drs. L.E.M. Langenberg

Contents

CHAPTER ONE

GENERAL INTRODUCTION

Introduction

'Interstitial lung diseases' (ILD) is a collective term for more than hundred pulmonary diseases affecting the alveoli and the space in between, called the interstitium. The traditional classification of ILD distinguish between ILD with known and unknown etiology (1, 2), see figure 1. The most common ILD are idiopathic pulmonary fibrosis (IPF), sarcoidosis, extrinsic allergic alveolitis (EAA) and ILD in the context of connective tissue disease. Most of these diseases are not common, some of them even ultra-rare and are referred to as 'orphan diseases'.

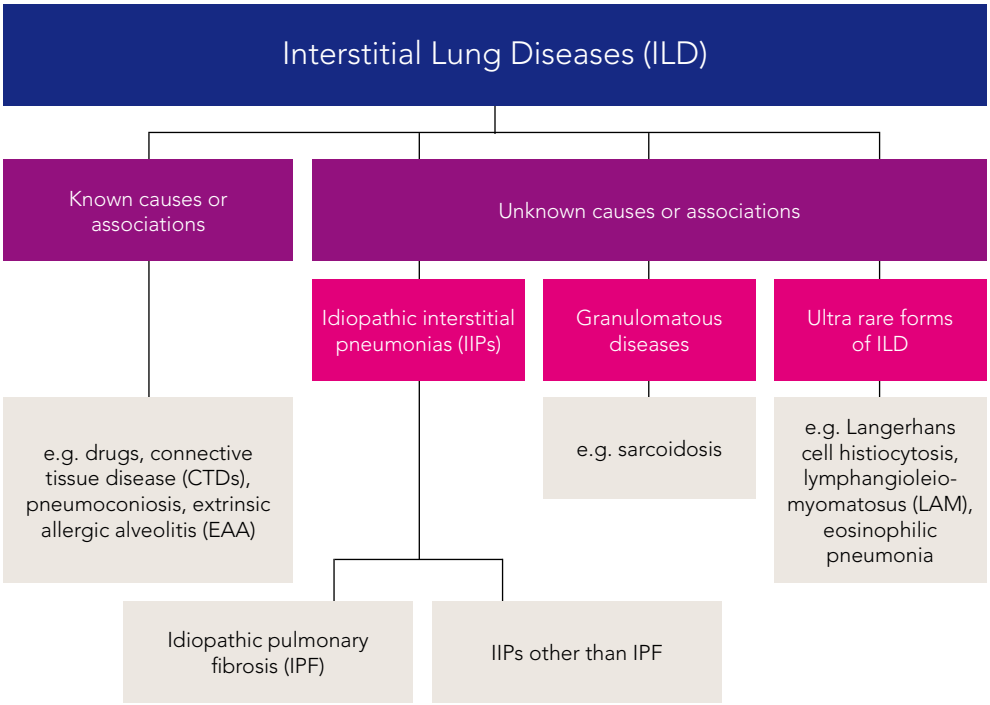


Figure 1: Classification of interstitial lung diseases (ILD).

In 2002 the American Thoracic Society/European Respiratory Society (ATS/ERS) published a classification of IIPs to define specific entities and to provide standardized terminology and diagnostic criteria (2). Since 2002 many publications have provided new information and therefore this statement was updated in 2013 (3).

One of the important changes since 2002 was that major IIPs (including IPF, nonspecific interstitial pneumonia (NSIP), respiratory bronchiolitis-interstitial lung diseases (RB-ILD), desquamative interstitial pneumonia (DIP) and cryptogenic organizing pneumonia (COP)) were distinguished from very rare IIPs (lymphoid interstitial pneumonia (LIP) and pleuroparenchymal fibroelastosis (PPFE)) and unclassifiable cases (inconclusive clinical, radiologic, and pathologic findings and/or major discordance between these findings). The most common form of IIP is IPF (2, 4, 5).

Idiopathic pulmonary fibrosis

IPF is a progressive and irreversible lung disease, occurs in middle-aged and elderly people, and has limited treatment options (6). IPF is characterised by a radiologic or histopathological pattern of usual interstitial pneumonia (UIP) (5-7). It is recommended that the diagnosis of IPF is set through a multidisciplinary discussion between pulmonologists, radiologists, and pathologists experienced in the diagnosis of ILD. The definition of IPF requires the exclusion of other IIPs and ILD associated with environmental exposure, medication, or systemic disease, the presence of an UIP pattern on HRCT in patients not subjected to surgical lung biopsy, and/or specific combinations of HRCT and histological features in patients subjected to surgical lung biopsy (2, 5).

Epidemiology of IPF

The incidence of IPF appears to rise over the years and is estimated between 4.3 – 16.3 per 100,000 persons per year (8-10). The incidence seems to have increased in the period of 1991 – 2003 (8). The prevalence is estimated at 2 – 42.7 cases per 100,000 (9-14). The wide range of the estimated prevalence is probably due to the previous lack of an uniform definition used in identifying cases of IPF and differences in study designs and populations. It is not well known if the incidence and prevalence of IPF is influenced by geographic, ethnic, cultural or racial factors.

Clinical features and diagnostic tools

Patients with IPF often present with a slowly progressive exertional dyspnea and cough for more than 3 months. On chest auscultation bibasilar inspiratory crackles are usually heard and finger clubbing can be found (15, 16).
The incidence of IPF increases with age and most presentations tend to occur in the sixth and seventh decades of life (6, 8). There is a significant higher predominance of the disease in men (1.5 to 1.7:1) (6, 10). The diagnosis of IPF is based on a combination of clinical, radiologic and histologic findings.

Radiology

High-resolution computed tomography (HRCT) is an essential element in the diagnosis of IPF (table 1, figure 1). UIP on the HRCT is characterised by the presence of reticular opacities and often associated with traction bronchiectasis. Honeycombing is a common feature and is manifested as clustered cystic airspaces which are usual localised subpleural. The distribution pattern of UIP is characteristically basal and peripheral, though often patchy. Several studies demonstrated a high accuracy of UIP pattern on the HRCT for the presence of UIP pattern on surgical lung biopsy (17, 18).

UIP pattern (all four features)	**Possible UIP pattern (all three features)**	**Inconsistent with UIP pattern (any of the seven features)**
Subpleural, basal predominance	Subpleural, basal predominance	Upper or mid-lung predominance
Reticular abnormality	Reticular abnormality	Peribronchovascular predominance
Honeycombing with or without traction bronchiectasis	Absence of features listed as inconsistent with UIP pattern (see third column)	Extensive groundglass abnormality (extent > reticular abnormality)
Absence of features listed as inconsistent with UIP pattern (see third column)		Profuse micronodules (bilateral, predominantly, upper lobes)
		Discrete cysts (multiple, bilateral, away from areas of honeycombing)
		Diffuse mosaic attenuation/air-trapping (bilateral, in three or more lobes)
		Consolidation in bronchopulmonary segment(s)/lobe(s)

Table 1: High-resolution computed tomography criteria for *usual interstitial pneumonia 'UIP'* pattern (5).

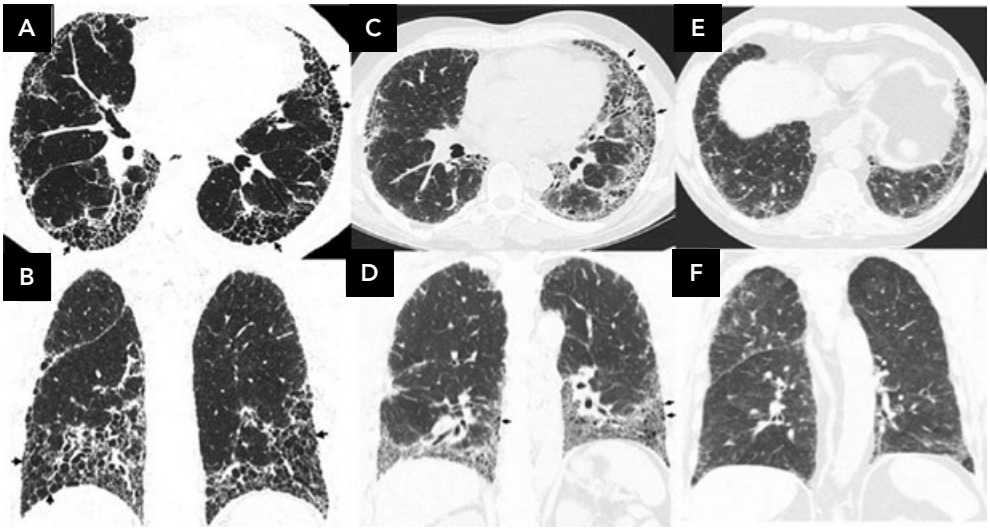


Figure 2: High-resolution computed tomography (HRCT) demonstrating usual interstitial pneumonia (UIP) pattern and possible UIP pattern (5).
(A and B) UIP pattern with extensive honeycombing: axial and coronal HRCT images show basal predominant, peripheral predominant reticular abnormality with multiple layers of honeycombing (arrows). (C and D) UIP pattern with less severe honeycombing: axial and coronal CT images show basal predominant, peripheral predominant reticular abnormality with subpleural honeycombing (arrows). (E and F) Possible UIP pattern: axial and coronal images show peripheral predominant, basal predominant reticular abnormality with a moderate amount of ground glass abnormality, but without honeycombing.

Histology

The main histopathologic feature of UIP is a heterogeneous appearance of subpleural and paraseptal fibrosis and honeycombing with areas of less affected or normal parenchyma. The fibrotic areas consist of dense collagen alternating with subepithelial foci of proliferating fibroblasts and myofibroblasts, which are called fibroblast foci. Inflammation is usually mild and consists of a patchy lymphoplasmacytic interstitial infiltrate. The criteria for histopathological UIP pattern are listed in table 2 (5).

UIP pattern (all four criteria)	**Probable UIP pattern**	**Possible UIP pattern (all three criteria)**	**Not UIP pattern (any of the six criteria)**
Evidence of marked fibrosis/architectural distortion, ± honeycombing in a predominantly subpleural/paraseptal distribution	Evidence of marked fibrosis/ architectural distortion, ± honeycombing	Patchy or diffuse involvement of lung parenchyma by fibrosis, with or without interstitial inflammation	Hyaline membranes*
Presence of patchy involvement of lung parenchyma by fibrosis	Absence of either patchy involvement or fibroblastic foci, but not both	Absence of other criteria for UIP **(see UIP Pattern column)**	Organizing pneumonia*†
Presence of fibroblast foci	Absence of features against a diagnosis of UIP suggesting an alternate diagnosis (see fourth column)	Absence of features against a diagnosis of UIP suggesting an alternate diagnosis **(see fourth column)**	Granulomas†
Absence of features against a diagnosis of UIP suggesting an alternate diagnosis **(see fourth column)**	OR		Marked interstitial inflammatory cell infiltrate away from honeycombing
	Honeycomb changes only‡		Predominant airway centered changes
			Other features suggestive of an alternative diagnosis

Table 2: Histopathological criteria for UIP pattern.

** Can be associated with acute exacerbation of idiopathic pulmonary fibrosis;*

†An isolated or occasional granuloma and/or mild component of organizing pneumonia pattern may rarely be coexisting in lung biopsies with an otherwise UIP pattern;

‡ This scenario usually represents end-stage fibrotic lung disease where honeycombed segments have been sampled but where a UIP pattern might be present in other areas. Such areas are usually represented by overt honeycombing on HRCT and can be avoided by pre-operative targeting of biopsy sites away from these areas using HRCT.

Bronchoalveolar lavage (BAL)

Bronchoalveolar lavage (BAL) is a relatively well-tolerated diagnostic procedure in ILD. Analysis of cell differentials and morphologic features can be helpful in differentiating between ILD, but also to exclude infection or malignancy (5). Absence of BAL lymphocytosis supports the diagnosis of IPF (19, 20). Furthermore, previous studies have demonstrated that granulocytosis or neutrophilia in BAL are an important diagnostic and prognostic factor in IPF (21, 22).

A study of retrospective data from a renown ILD-centre in Germany suggested that in 8% of patients with an HRCT pattern consistent with UIP BAL findings suggested an alternative diagnosis (23). Nevertheless, official guidelines currently recommend that BAL cellular analysis should not be performed routinely in the diagnostic work up of the majority of IPF patients (5).

Etiology and pathogenesis of IPF

Recent findings suggest that IPF results from multiple factors that eventually lead to interstitial lung fibrosis. It is thought that complex relationships between genetic predispositions, environmental exposures and lung infections promote repetitive lung injury, which drives the fibroproliferative response that cause IPF. Several potential risk factors for the development of IPF have been described. The most important risk factor is cigarette smoking and is strongly associated with IPF, especially in patients with a smoking history of more than 20 packyears (24, 25). Other potential risk factors are environmental exposures (for example metal and wood dust, farming, raising birds, hair dressing, stone cutting/polishing), microbial agents (chronic viral infections with Epstein-Barr virus, cytomegalovirus or human herpesvirus), gastroesophageal reflux and diabetes mellitus (26-32).
Initially, it was thought that (chronic) inflammation plays a pivotal role in most ILD and finally evolves to pulmonary fibrosis. However, the hypothesis of the pathogenesis of IPF has changed considerably over the past 10-15 years (33). After redefinition of IPF as a distinct condition characterised by an UIP pattern (2, 4) and the observed adverse effects of anti-inflammatory therapy such as corticosteroids and/or azathioprine (5, 34), it is now believed that the formation of fibrosis in IPF is primarily driven by repetitive micro injury of the alveolar epithelium and aberrant wound-healing responses (6). Nevertheless, inflammation might still play a role as a possible 'trigger' in the onset and/or progressiveness of the disease in a subgroup of patients (35). Therefore, it is suggested that at least 2 cellular routes –the inflammatory pathway and the epithelial pathway– could lead to pulmonary fibrosis.
The alveolar epithelium consist of 90% of type I alveolar epithelial cells (AECs) which provide a surface for gas exchange and a few type II AECs (6). Type II AECs are multifunctional and secrete surfactant, function as antigen presenting cells and are progenitor cells than can regenerate the alveolar epithelium after injury.

A proposed mechanism in the pathogenesis of IPF is that an ageing-related susceptible lung is subjected to repetitive microinjuries (i.e., viruses, cigarette smoke, microaspiration) resulting in the death of type I and II AECs (6). The areas of lung injury causes capillary leakage of proteins, like fibrinogen and fibronectin, into the interstitial and alveolar spaces, which leads to the formation of a wound clot.
It is thought that highly active AECs lead to a dysregulated repair process that seems to be perpetually turned on even in the absence of the primary stimulus (34). These abnormally activated AECs induce the migration of fibroblasts and fibrocytes to the sites where the microinjuries are occurring (36). Moreover, the activated AECs secrete and activate the latent transforming growth factor- β (TGF-β) (37, 38). TGF-β is a versatile cytokine, which promotes the forming of the extracellular matrix and the differentiation of fibroblasts to myofibroblasts (39). All these processes lead to the formation of fibroblast foci. In this hypercoagulable milieu degradation of the extracellular matrix is not possible and fibrogenesis is continuously stimulated. Finally, this abnormal process of lung repair finally leads to irreversible, progressive lung remodelling with the formation of honeycomb cysts (6).

Natural course and prognosis

The natural history of IPF is characterised by an irreversible decline in pulmonary function and eventually leads to death from respiratory failure or complicating comorbidities. Median survival from the time of diagnosis is poor with 3 – 4 years, although its clinical course might greatly vary (5, 6, 40, 41). Many patients have a slowly progressive clinical course over a period of years, whereas in 10 – 15% of patients the course of the disease is much more rapid, leading to death from respiratory failure in few months. It has been noted that male cigarette smokers have an accelerated clinical course with shortened survival (42). Finally, a minority of patients present relative stability over long periods, punctuated by episodes of rapid acute deterioration, either fatal or leading to a step down in pulmonary function (5, 40). Some of these episodes are idiopathic, as they do not recognize an identifiable cause (such as infections, left heart failure, pulmonary embolism, etc.), and are referred to as acute exacerbations of IPF (AE-IPF).
The incidence of AE-IPF is still unclear, varying from 5 to 15% per year in retrospective studies on the placebo arm populations enrolled in clinical trials

(43-45) and might increase with time, reaching 20% at 3 years from diagnosis (46). The natural course of IPF is illustrated by figure 3.

The prognosis of IPF seems to be influenced by the presence of several comorbidities, like emphysema and secondary arterial pulmonary hypertension, both commonly present in IPF patients and associated with poor survival (47, 48). Lung cancer is also frequently associated with IPF and has a significant impact on survival (49). Also gastro-oesophageal reflux disease has been proven an important factor both in pathogenesis and progression of the disease (50).

A deeper understanding of the mechanisms responsible for an accelerated course of the disease and the identification of biomarkers of progression could lead to a better stratification of the disease, essential for delivering individualised therapeutic strategies and timing of referral and listing for lung transplantation (LTX).

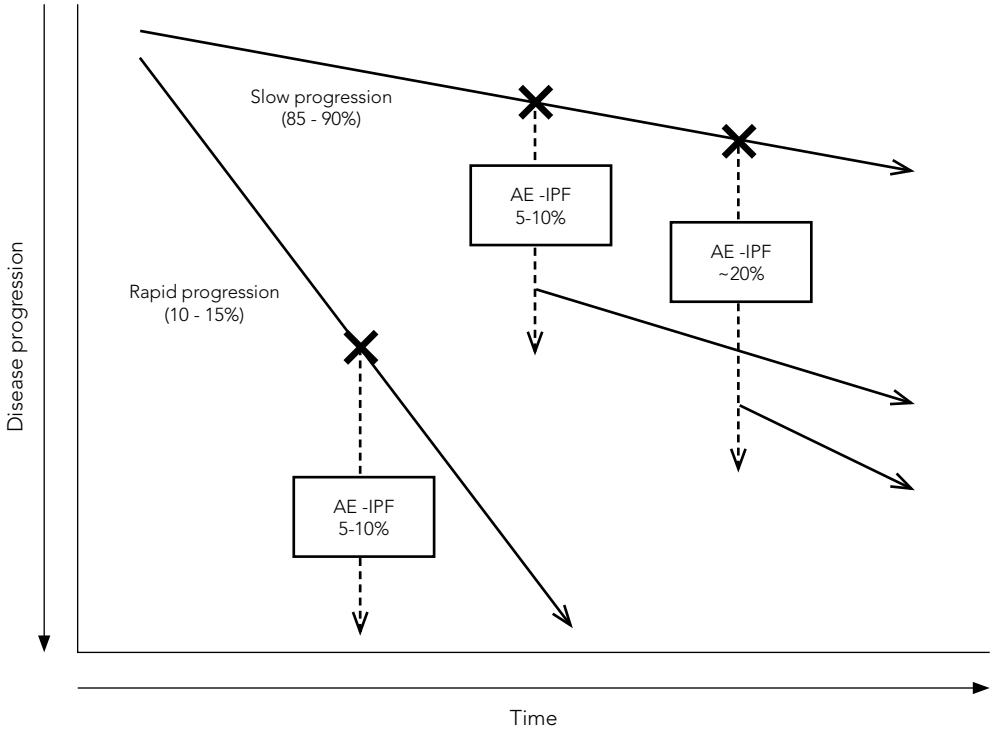


Figure 3: Natural course of idiopathic pulmonary fibrosis
The heterogeneous natural history pattern in patients with IPF. Most patients follow a relatively slow clinical and functional decline after diagnosis. About 10 – 15% of IPF patients have a more accelerated decline after diagnosis, mostly male-smokers. In 5 – 10% of IPF patients an episode of acute worsening (acute exacerbation of IPF, AE-IPF) leads to a step down in pulmonary function or might be fatal. The incidence of AE-IPF might increase in time, reaching 20% after 3 years of diagnosis.

Genetic background

In a substantive proportion of cases of IPF the disease is familial. Two to nineteen percent of IPF patients have been reported to have at least one first-degree family member with some form of IIP (51). Familial cases appeared to be younger, but otherwise are not distinguishable from the sporadic form of IPF. Recent studies indicates that mutations in the genes encoding the lung surfactant proteins C and A2 (*SFTPC* and *SFTPA2*, respectively), telomerase (*TERT* and *TERC*) and proteins of lamellar bodies (*ABCA3* and *HPS1*) may cause IPF through different biological pathways.

Mutations in surfactant proteins (SFTPC and SFTPA2)

The expression of the SP-C gene is restricted to type II AEC and a mutation in the SP-C gene may lead to accumulation of the pro-SP-C protein and subsequent vulnerability to apoptosis of these cells and/or decreased SP-C expression leading to less protection of the alveolar epithelial layer to damaging stimuli from the environment. The potential causal relation was first shown in a family with IIP, where candidate gene sequencing detected a heterozygous mutation in *SFTPC* (52).

Nowadays, a number of IIP families with surfactant mutations have been described and they are especially characterised by the concurrent occurrence of interstitial lung disease in both adults and children (53-56). Mutations in *SFTPC* causing accumulation of pro-SP-C can cause an increase in endoplasmic reticulum (ER) stress and activate the unfolded protein response in AEC type II, leading to an increased apoptotic response, but can also induce epithelial-to-mesenchymal transition in lung epithelial cells, depending on the position of the mutation in the gene. Of note, the most common *SFTPC* mutation is I73T and causes dysregulated proteostasis but not a significant elevation of ER stress (57).

After the discovery of mutations in *SFTPC*, analysis of large kindreds with familial pulmonary fibrosis also revealed mutations in the gene encoding SP-A (58, 59). The family members presented with adult early-onset fibrosis pulmonary fibrosis or lung cancer with features of bronchioloalveolar carcinoma (BAC) (59). Surfactant protein A2 is a C-type lectin expressed by AEC type II, but also by Clara cells and submucosal glands (60). The SP-A2 mutations create a protein that is not excreted but retained in the ER and induces ER stress, similar as seen in *SFTPC* mutants (58). Although ER stress is linked to tumorigenesis (61), lung cancer in patients with *SFTPC* mutations has not been reported. The high incidence of lung cancer in patients with a *SFTPA2* mutation should therefore have another explanation, e.g. involvement of Clara cells (62).

Mutations in telomerase (TERT and TERC)

Telomeres shorten with each cell division and finally induce a DNA-damage response that leads to apoptosis of the cell or cell-cycle arrest. In other words, the length of telomeres limits the replicative capacity of tissues and therefore has been implicated in age-related diseases like IPF (63).

Former studies demonstrated that not only patients with familial fibrosis, but also sporadic IPF patients have significantly shorter telomeres compared to age-related healthy controls (63-65). Telomerase is a specialised enzyme that adds telomere repeats *(TTAGGG)* to the end of chromosomes. The most genetic mutations have been found in two components, telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC). Mutations in TERT and TERC are a risk factor for pulmonary fibrosis underlying the inheritance in 8-15% of familial cases (63, 64). IPF is inherited as an autosomal trait with age-dependent penetrance. Heterogeneous mutations lead to haploinsufficiency that results in an overall minor decrease in telomerase activity. More importantly, this decreased in telomerase activity seems to cause a significant reduction of telomere length over generations, a phenomenon called genetic anticipation. Therefore, in contrast to surfactant mutations, concurrent occurrence of interstitial lung disease in adults and children is not seen in *TERT* mutation carriers. It is thought that this phenomenon leading to critical short telomere length confers the main risk factor for occurrence of disease and not only the presence of the mutation.

Mutations in lamellar bodies (ABCA3 and HPS1)

Pulmonary surfactant is processed and transported by lamellar bodies. Lamellar bodies are secretory organelles unique to type II AEC. The proteins of the membrane of the lamellar bodies are encoded by the gene *ABCA3* and are expressed in the lung (66). Recently, compound heterozygous or homozygous mutations in *ABCA3* were described in adult IIP (67, 68), however, recessive mutations in *ABCA3* are the most common genetic cause of lethal surfactant deficiency in neonates or chronic ILD in children (69, 70).
Dysfunctional lamellar bodies in AEC type II were also identified as a cause of pulmonary fibrosis in Hermansky-Pudlak syndrome (HPS). Nine genes (*HPS1, AP3B1, and HPS3 to HPS9*) are associated with the syndrome, resulting in subphenotypes HPS-1 to HPS-9, however, a recent study defined a mutation in AP3D1 which is associated with immunodeficiency and seizures as a new type of Hermansky-Pudlak (HPS10) (71, 72). HPS-1 is the most frequent subphenotype of HPS. HPS-1, together with HPS-4, is a subunit of the lysosomal complex BLOC-3. Loss of either subunit results in destabilisation of the remaining subunits, consequently resulting in clinical features of HPS (73).

Pharmacologic therapies

In the last 10 years major progression has been made in the clinical trials in IPF, especially in mild to moderate IPF (74). Primarily mild to moderate disease, because most studies excluded patients with severe functional impairment (FVC < 50% of predicted), which is still a substantial group of patients in everyday clinical practice. In 2011 the ATS/ERS/JRS/ALAT published a new guideline on management of IPF and provided evidence-based recommendations for treatment (5). However, since 2011 new and important evidence for the treatment of IPF has become available and therefore this guideline has been updated recently (75).

They formulated a strong recommendation against the use of anticoagulation (warfarin), the combination of prednisone + azathioprine + *N*-acetylcysteine, ambrisentan and imatinib and a conditional recommendation against the use of sildenafil, bosentan and *N*-acetylcysteine monotherapy (75). In comparison with the guideline published in 2011 the recommendations for the use of nintedanib and pirfenidone have been changed and the use of these drugs is now the cornerstone in the medical treatment of IPF.

Nintedanib (previously known a molecule BIBF 1120) is a small molecule that inhibits multiple receptor tyrosine kinases and nonreceptor tyrosine kinases, especially the platelet-derived growth factor (PDGF) receptor, the fibroblast growth factor (FGF) receptor and the vascular endothelial growth factor (VEGF) receptor. The PDGF-, FGF- and VEGF-receptor are all believed to be involved in the pathogenesis of IPF.

Furthermore, binding of nintedanib to these receptors blocks the intracellular signalling, which is essential for the proliferation, migration and transformation of fibroblasts. Nintedanib was evaluated in three RCTs, published in 2 reports (45, 76). They reported a potential benefit of nintedanib on disease progression measured by rate of FVC decline, however, no significant effect on overall mortality was seen.

Pirfenidone is an oral antifibrotic drug, and although its mechanism of action is not fully understood, *in vitro* studies and animals models of pulmonary fibrosis suggest that pirfenidone exerts anti-fibrotic, anti-inflammatory, and antioxidant properties through downregulation of key profibrotic growth factors, including TGF-β (77).

Pooled results of two Japanese trials and the CAPACITY study group and the ASCEND study group suggested improved mortality with pirfenidone (43, 78-80).
Antiacid treatment is also recommended therapy in patients with IPF. Gastroesophageal reflux (GER) has been observed in up to 90% of patients with IPF (30) and might be a risk factor for aspiration and microaspiration, which can cause pneumonitis, a mechanism that is suggested to cause or to aggravate IPF. Combined analysis of 3 RCTs showed a significantly smaller decrease in FVC during the study period for those receiving antiacid treatment at baseline (50).
In the future long-term studies are warranted to determine the safety and efficacy of treatment options for IPF in terms of survival benefit. Importantly, the majority of IPF patients are older than 60 years and they manifest an increasing number of comorbidities that need attention and treatment. Furthermore, as long as there is no curative treatment, adequate palliative care for patients with IPF at the end of life is of great importance and future studies on this subject remain needed.

Lung Transplantation

Lung transplantation (LTX) is a treatment option for patients who fail to respond to medical therapy and progress to advanced lung disease. Although both nintedanib and pirfenidone are available and have been shown to slow disease progression, LTX is the only treatment with proven survival benefit in IPF (81, 82).
LTX was first performed in 1963 by James Hardy and colleagues at the University of Mississippi Medical Center (83). Multiple attempts at LTX failed because of rejection and problems with anastomotic bronchial healing. Another 20 years passed before meaningful survival was achieved and only after the invention of the heart-lung machine and the development of immunosuppressive drugs, lungs could be transplanted with a reasonable chance of success.
In the Netherlands, the first single LTX was performed in 1989 and one year later the first bilateral LTX in St. Antonius Hospital in Nieuwegein. From 1991 – 2001 the University Medical Centre (UMC) in Groningen was assigned to perform LTX in the Netherlands. However, due to an increasing demand authorisation for another LTX centre was granted to Heart Lung Center Utrecht (UMC Utrecht and St. Antonius Hospital Nieuwegein) and Erasmus Medical Centre in Rotterdam in 2001 (84).

Criteria for referral and listing of IPF for LTX

International guidelines for LTX candidates have been published to provide guidance to medical doctors (85). According to these guidelines all patients with a histopathological or radiographic pattern of UIP should be referred for LTX, regardless of their lung function.

Listing on the waiting list is recommended when one of the following criteria is met: a) decline in forced vital capacity (FVC) ≥ 10% during 6 months of follow up; b) decline of diffusing capacity for carbon monoxide (DLco) ≥ 15% during 6 months follow up; c) desaturation to 88% or distance < 250m on six-minute-walk test or > 50m decline in the six-minute-walk test over a 6 months period; d) pulmonary hypertension on right heart catheterisation or 2-dimensional echocardiography; e) hospitalisation because of respiratory decline, pneumothorax, or acute exacerbation (85).

Allocation system

Since the start of the LTX program in the Netherlands the allocation system was based on waiting period. In 2001 a new allocation system was introduced, which made it possible to list patients with a diminished life expectancy as 'high-urgent' to increase their chance of receiving a transplant. In May 2005 the United Network for Organ Sharing (UNOS) implemented the Lung Allocation Score (LAS) in the United States. The LAS has been utilized in Germany since December 2011 and the Netherlands followed in April 2014. The international exchange of donor lungs between all Eurotransplant countries is now based upon LAS.

The LAS is an effort to identify the best candidates for transplantation. The score is calculated using various measures of a patient's health that estimate survival probability and projected duration of survival with or without a lung transplant. LAS scores range from 0 – 100 and patients with higher score, reflecting greater predicted survival benefit, get priority. Implementation of the LAS resulted in an increased number of IPF patients receiving a lung transplant and IPF became the most common diagnosis group to receive a lung transplant in the US in 2007 (86).

Lung transplantation in IPF patients

From the 8,528 IPF patients transplanted between 1990 and 2011 and reported to the International Society of Heart & Lung Transplantation, median survival was 4.5 years (Adult Lung Transplantation Statistics. [www.ishlt.org/registries/slides.asp?slides-heartLungRegistry). The post-transplant survival for IPF patients of 4.5 years was significantly lower compared to patients with pre-transplant diagnosis of cystic fibrosis (CF: 7,8 years) and chronic obstructive pulmonary disease (COPD; 5,4 years).

Currently, it is unclear whether bilateral LTX or single LTX should be standard of care in patients with IPF. There are no randomized controlled trials to address this question, the only evidence are observational studies that assessed the survival of patients with IPF after LTX, accepting bilateral LTX versus single LTX (87-93). The ATS/ERS/JRS/ALAT did not make a recommendation regarding single versus bilateral LTX in patients with IPF, but acknowledged that additional evidence is needed to properly address this question (75). Furthermore, shortage of organs is a worldwide problem and this should be considered in the decision to give bilateral LTX to a single patient rather than give a single LTX to two patients.

Bridging to transplantation

'Bridge to lung transplantation' refers to strategies to manage with artificial support an acutely decompensating patient until a suitable organ is available. Mechanical ventilation today has been the most commonly used bridging strategy to LTX, but these ventilated patients are at risk for ventilator-induced lung injury and ventilator-associated pneumonia (94, 95).

More than three decades ago, extracorporeal life support (ECLS) or extracorporeal membrane oxygenation (ECMO) was introduced for the first time to manage patients on the lung transplant waiting list who were dying of acute respiratory failure refractory to mechanical ventilation (96).

However, the first clinical experiences were discouraging with a high mortality rate and many complications associated with the application of ECLS (96). Thanks to improvements in technology, safety profile and manageability of ECLS strategies (97), ECMO has been reintroduced as an option for patients with severe respiratory failure awaiting lung transplant (96, 98, 99). Recent study demonstrated that ECMO can be a lifesaving option for patients with ILD and acute respiratory failure provided they are candidates for LTX (100).

Aim of the thesis

Taking into account the poor prognosis and limited treatment options, including LTX for only a selected number of patients, and the unpredictable clinical course of IPF and often delay in referral of patients to a tertiary ILD centre, the aim of this thesis was to evaluate the results of LTX in IPF patients in the Netherlands and to identify factors that might help to improve the outcome of LTX in IPF patients in the future.

The first part of this thesis provides a historical overview of all IPF patients referred for LTX since the start of the transplant program in the Netherlands and evaluation of the waiting list mortality of IPF patients. We aimed to identify different clinical factors associated with mortality on the waiting list. Secondly, we evaluated the survival outcomes after LTX and compared the outcomes of single and bilateral LTX in IPF patients.

Another aim was to identify new prognostic biomarkers in patient's blood or lung lavage fluids that might help to optimise scoring models like the LAS and to guide clinicians in the complex matter of referral and listing for LTX. This thesis evaluates the potential of IgA in serum as a prognostic biomarker in patients with IPF.

Furthermore, this thesis focuses on different genetic mutations identified in familial and sporadic IIPs. We aimed to investigate patients with pulmonary fibrosis and genetic mutations involved in surfactant homeostasis, telomere maintenance and lamellar bodies in order to define the clinical relevance of the genetic contribution to the disease. Furthermore, we investigate the prevalence, clinical characteristics and prognosis in these patients and discuss the potential consequences for LTX.

Outline of the thesis

Chapter 2 provides a retrospective overview of all ILD patients referred for LTX since the start of transplant program in 1989 in the Netherlands. It focus on trajectory and clinical data in IPF patients and describe waiting list mortality.
Chapter 3 evaluates survival after LTX in IPF patients and compares survival outcomes after single and bilateral LTX. Furthermore, it demonstrate potential clinical predictors of waiting list mortality in IPF patients.
Chapter 4 analyses the potential of immunoglobulin A as a prognostic biomarker in IPF patients and confirms the results in a duplication cohort.
Chapter 5 describes the clinical characteristics, prognosis of sporadic and familial IIP patients with a *TERT* mutation in comparison with sporadic IPF patients. Furthermore, it evaluates immunoglobulin A serum levels in *TERT* mutation carriers as a potential prognostic biomarker of disease.
Chapter 6 describes the clinical course of a *TERT* mutation carrier after LTX and describes the possible consequences of the genetic mutation for LTX.
Chapter 7 describes *SFTPA2* mutations in familial and sporadic idiopathic interstitial pneumonias and discuss the possible consequences of the genetic mutation for LTX.
Chapter 8 is a cohort study to investigate the occurrence of Hermansky Pudlak Syndrome (HPS) in IPF patients and describes the possible consequences of HPS for LTX.

References

(1) Crystal RG, Gadek JE, Ferrans VJ, Fulmer JD, Line BR, Hunninghake GW. Interstitial lung disease: current concepts of pathogenesis, staging and therapy. *Am J Med* 1981;70:542-568.

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

(3) Travis WD, Costabel U, Hansell DM, King TE,Jr, Lynch DA, Nicholson AG, Ryerson CJ, Ryu JH, Selman M, Wells AU, Behr J, Bouros D, Brown KK, Colby TV, Collard HR, Cordeiro CR, Cottin V, Crestani B, Drent M, Dudden RF, Egan J, Flaherty K, Hogaboam C, Inoue Y, Johkoh T, Kim DS, Kitaichi M, Loyd J, Martinez FJ, Myers J, Protzko S, Raghu G, Richeldi L, Sverzellati N, Swigris J, Valeyre D, ATS/ERS Committee on Idiopathic Interstitial Pneumonias. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013;188:733-748.

(4) American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med* 2000;161:646-664.

(5) Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE,Jr, Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schunemann HJ, ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788-824.

(6) King TE,Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet* 2011;378:1949-1961.

(7) Visscher DW, Myers JL. Histologic spectrum of idiopathic interstitial pneumonias. *Proc Am Thorac Soc* 2006; 3:322-329.

(8) Gribbin J, Hubbard RB, Le Jeune I, Smith CJ, West J, Tata LJ. Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. *Thorax* 2006;61:980-985.

(9) Coultas DB, Zumwalt RE, Black WC, Sobonya RE. The epidemiology of interstitial lung diseases. *Am J Respir Crit Care Med* 1994;150:967-972.

(10) Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2006;174:810-816.

(11) Scott J, Johnston I, Britton J. What causes cryptogenic fibrosing alveolitis? A case-control study of environmental exposure to dust. *BMJ* 1990;301:1015-1017.

(12) Iwai K, Mori T, Yamada N, Yamaguchi M, Hosoda Y. Idiopathic pulmonary fibrosis. Epidemiologic approaches to occupational exposure. *Am J Respir Crit Care Med* 1994;150:670-675.

(13) von Plessen C, Grinde O, Gulsvik A. Incidence and prevalence of cryptogenic fibrosing alveolitis in a Norwegian community. *Respir Med* 2003;97:428-435.

(14) Karakatsani A, Papakosta D, Rapti A, Antoniou KM, Dimadi M, Markopoulou A, Latsi P, Polychronopoulos V, Birba G, Ch L, Bouros D, Hellenic Interstitial Lung Diseases Group. Epidemiology of interstitial lung diseases in Greece. *Respir Med* 2009;103:1122-1129.

(15) Douglas WW, Ryu JH, Schroeder DR. Idiopathic pulmonary fibrosis: Impact of oxygen and colchicine, prednisone, or no therapy on survival. *Am J Respir Crit Care Med* 2000;161:1172-1178.
(16) King TE,Jr, Tooze JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am J Respir Crit Care Med* 2001;164:1171-1181.

(17) Hunninghake GW, Zimmerman MB, Schwartz DA, King TE,Jr, Lynch J, Hegele R, Waldron J, Colby T, Muller N, Lynch D, Galvin J, Gross B, Hogg J, Toews G, Helmers R, Cooper JA,Jr, Baughman R, Strange C, Millard M. Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2001;164:193-196.

(18) Raghu G, Mageto YN, Lockhart D, Schmidt RA, Wood DE, Godwin JD. The accuracy of the clinical diagnosis of new-onset idiopathic pulmonary fibrosis and other interstitial lung disease: A prospective study. *Chest* 1999;116:1168-1174.

(19) Nagai S, Handa T, Ito Y, Takeuchi M, Izumi T. Bronchoalveolar lavage in idiopathic interstitial lung diseases. *Semin Respir Crit Care Med* 2007;28:496-503.

(20) Nagao T, Nagai S, Kitaichi M, Hayashi M, Shigematsu M, Tsutsumi T, Satake N, Izumi T. Usual interstitial pneumonia: idiopathic pulmonary fibrosis versus collagen vascular diseases. *Respiration* 2001;68:151-159.

(21) Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE,Jr. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. *Chest* 2008;133:226-232.

(22) Tabuena RP, Nagai S, Tsutsumi T, Handa T, Minoru T, Mikuniya T, Shigematsu M, Hamada K, Izumi T, Mishima M. Cell profiles of bronchoalveolar lavage fluid as prognosticators of idiopathic pulmonary fibrosis/usual interstitial pneumonia among Japanese Patients. *Respiration* 2005;72:490-498.

(23) Ohshimo S, Bonella F, Cui A, Beume M, Kohno N, Guzman J, Costabel U. Significance of bronchoalveolar lavage for the diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;179:1043-1047.

(24) Baumgartner KB, Samet JM, Stidley CA, Colby TV, Waldron JA. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1997;155:242-248.

(25) Taskar VS, Coultas DB. Is idiopathic pulmonary fibrosis an environmental disease? *Proc Am Thorac Soc* 2006;3:293-298.

(26) Hubbard R, Lewis S, Richards K, Johnston I, Britton J. Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. *Lancet* 1996;347:284-289.

(27) Kitamura H, Ichinose S, Hosoya T, Ando T, Ikushima S, Oritsu M, Takemura T. Inhalation of inorganic particles as a risk factor for idiopathic pulmonary fibrosis--elemental microanalysis of pulmonary lymph nodes obtained at autopsy cases. *Pathol Res Pract* 2007;203:575-585.

(28) Egan JJ, Stewart JP, Hasleton PS, Arrand JR, Carroll KB, Woodcock AA. Epstein-Barr virus replication within pulmonary epithelial cells in cryptogenic fibrosing alveolitis. *Thorax* 1995;50:1234-1239.

(29) Meliconi R, Andreone P, Fasano L, Galli S, Pacilli A, Miniero R, Fabbri M, Solforosi L, Bernardi M. Incidence of hepatitis C virus infection in Italian patients with idiopathic pulmonary fibrosis. *Thorax* 1996;51:315-317.

(30) Tobin RW, Pope CE,2nd, Pellegrini CA, Emond MJ, Sillery J, Raghu G. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1998;158:1804-1808.

(31) D'Ovidio F, Singer LG, Hadjiliadis D, Pierre A, Waddell TK, de Perrot M, Hutcheon M, Miller L, Darling G, Keshavjee S. Prevalence of gastroesophageal reflux in end-stage lung disease candidates for lung transplant. *Ann Thorac Surg* 2005;80:1254-1260.

(32) Gribbin J, Hubbard R, Smith C. Role of diabetes mellitus and gastro-oesophageal reflux in the aetiology of idiopathic pulmonary fibrosis. *Respir Med* 2009;103:927-931.

(33) Noble PW, Homer RJ. Back to the future: historical perspective on the pathogenesis of idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2005;33:113-120.
(34) Selman M, King TE, Pardo A, American Thoracic Society, European Respiratory Society, American College of Chest Physicians. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med* 2001;134:136-151.

(35) Gilani SR, Vuga LJ, Lindell KO, Gibson KF, Xue J, Kaminski N, Valentine VG, Lindsay EK, George MP, Steele C, Duncan SR. CD28 down-regulation on circulating CD4 T-cells is associated with poor prognoses of patients with idiopathic pulmonary fibrosis. *PLoS One* 2010;5:e8959.

(36) Andersson-Sjoland A, de Alba CG, Nihlberg K, Becerril C, Ramirez R, Pardo A, Westergren-Thorsson G, Selman M. Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. *Int J Biochem Cell Biol* 2008;40:2129-2140.

(37) Selman M, Pardo A. Role of epithelial cells in idiopathic pulmonary fibrosis: from innocent targets to serial killers. *Proc Am Thorac Soc* 2006;3:364-372.

(38) Khalil N, O'Connor RN, Unruh HW, Warren PW, Flanders KC, Kemp A, Bereznay OH, Greenberg AH. Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 1991;5:155-162.

(39) Massague J, Blain SW, Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell* 2000;103:295-309.

(40) Ley B, Collard HR, King TE, Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;183:431-440.

(41) Sgalla G, Biffi A, Richeldi L. Idiopathic pulmonary fibrosis: Diagnosis, epidemiology and natural history. *Respirology* 2016;21:427-437.

(42) Selman M, Carrillo G, Estrada A, Mejia M, Becerril C, Cisneros J, Gaxiola M, Perez-Padilla R, Navarro C, Richards T, Dauber J, King TE,Jr, Pardo A, Kaminski N. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. *PLoS One* 2007;2:e482.

(43) Azuma A, Nukiwa T, Tsuboi E, Suga M, Abe S, Nakata K, Taguchi Y, Nagai S, Itoh H, Ohi M, Sato A, Kudoh S. Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2005;171:1040-1047.

(44) Idiopathic Pulmonary Fibrosis Clinical Research Network, Martinez FJ, de Andrade JA, Anstrom KJ, King TE,Jr, Raghu G. Randomized trial of acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2093-2101.

(45) Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, Cottin V, Flaherty KR, Hansell DM, Inoue Y, Kim DS, Kolb M, Nicholson AG, Noble PW, Selman M, Taniguchi H, Brun M, Le Maulf F, Girard M, Stowasser S, Schlenker-Herceg R, Disse B, Collard HR, INPULSIS Trial Investigators. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2071-2082.

(46) Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. *Eur Respir J* 2011;37:356-363.

(47) Cottin V. The impact of emphysema in pulmonary fibrosis. *Eur Respir Rev* 2013;22:153-157.

(48) Corte TJ, Wort SJ, Wells AU. Pulmonary hypertension in idiopathic pulmonary fibrosis: a review. *Sarcoidosis Vasc Diffuse Lung Dis* 2009;26:7-19.

(49) Tomassetti S, Gurioli C, Ryu JH, Decker PA, Ravaglia C, Tantalocco P, Buccioli M, Piciucchi S, Sverzellati N, Dubini A, Gavelli G, Chilosi M, Poletti V. The impact of lung cancer on survival of idiopathic pulmonary fibrosis. *Chest* 2015;147:157-164.

(50) Lee JS, Collard HR, Anstrom KJ, Martinez FJ, Noth I, Roberts RS, Yow E, Raghu G, IPFnet Investigators. Anti-acid treatment and disease progression in idiopathic pulmonary fibrosis: an analysis of data from three randomised controlled trials. *Lancet Respir Med* 2013;1:369-376.
(51) Loyd JE. Pulmonary fibrosis in families. *Am J Respir Cell Mol Biol* 2003;29:S47-50.

(52) Nogee LM, Dunbar AE,3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med* 2001;344:573-579.

(53) van Moorsel CH, van Oosterhout MF, Barlo NP, de Jong PA, van der Vis JJ, Ruven HJ, van Es HW, van den Bosch JM, Grutters JC. Surfactant protein C mutations are the basis of a significant portion of adult familial pulmonary fibrosis in a dutch cohort. *Am J Respir Crit Care Med* 2010;182:1419-1425.

(54) Lawson WE, Grant SW, Ambrosini V, Womble KE, Dawson EP, Lane KB, Markin C, Renzoni E, Lympany P, Thomas AQ, Roldan J, Scott TA, Blackwell TS, Phillips JA,3rd, Loyd JE, du Bois RM. Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. *Thorax* 2004;59:977-980.

(55) Markart P, Ruppert C, Wygrecka M, Schmidt R, Korfei M, Harbach H, Theruvath I, Pison U, Seeger W, Guenther A, Witt H. Surfactant protein C mutations in sporadic forms of idiopathic interstitial pneumonias. *Eur Respir J* 2007;29:134-137.

(56) Hamvas A, Nogee LM, White FV, Schuler P, Hackett BP, Huddleston CB, Mendeloff EN, Hsu FF, Wert SE, Gonzales LW, Beers MF, Ballard PL. Progressive lung disease and surfactant dysfunction with a deletion in surfactant protein C gene. *Am J Respir Cell Mol Biol* 2004;30:771-776.

(57) Cameron HS, Somaschini M, Carrera P, Hamvas A, Whitsett JA, Wert SE, Deutsch G, Nogee LM. A common mutation in the surfactant protein C gene associated with lung disease. *J Pediatr* 2005;146:370-375.

(58) Maitra M, Wang Y, Gerard RD, Mendelson CR, Garcia CK. Surfactant protein A2 mutations associated with pulmonary fibrosis lead to protein instability and endoplasmic reticulum stress. *J Biol Chem* 2010;285:22103-22113.

(59) Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, DiMaio JM, Kinch LN, Grishin NV, Garcia CK. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am J Hum Genet* 2009;84:52-59.

(60) Madsen J, Tornoe I, Nielsen O, Koch C, Steinhilber W, Holmskov U. Expression and localization of lung surfactant protein A in human tissues. *Am J Respir Cell Mol Biol* 2003;29:591-597.

(61) Zhu B, Ferry CH, Markell LK, Blazanin N, Glick AB, Gonzalez FJ, Peters JM. The nuclear receptor peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) promotes oncogene-induced cellular senescence through repression of endoplasmic reticulum stress. *J Biol Chem* 2014;289:20102-20119.

(62) Yousem SA, Beasley MB. Bronchioloalveolar carcinoma: a review of current concepts and evolving issues. *Arch Pathol Lab Med* 2007;131:1027-1032.

(63) Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA,3rd, Lansdorp PM, Greider CW, Loyd JE. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 2007;356:1317-1326.

(64) Cronkhite JT, Xing C, Raghu G, Chin KM, Torres F, Rosenblatt RL, Garcia CK. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008;178:729-737.

(65) Diaz de Leon A, Cronkhite JT, Katzenstein AL, Godwin JD, Raghu G, Glazer CS, Rosenblatt RL, Girod CE, Garrity ER, Xing C, Garcia CK. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. *PLoS One* 2010;5:e10680.

(66) Mulugeta S, Maguire JA, Newitt JL, Russo SJ, Kotorashvili A, Beers MF. Misfolded BRICHOS SP-C mutant proteins induce apoptosis via caspase-4- and cytochrome c-related mechanisms. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L720-9.

(67) Campo I, Zorzetto M, Mariani F, Kadija Z, Morbini P, Dore R, Kaltenborn E, Frixel S, Zarbock R, Liebisch G, Hegermann J, Wrede C, Griese M, Luisetti M. A large kindred of pulmonary fibrosis associated with a novel ABCA3 gene variant. *Respir Res* 2014;15:43-9921-15-43.
(68) Epaud R, Delestrain C, Louha M, Simon S, Fanen P, Tazi A. Combined pulmonary fibrosis and emphysema syndrome associated with ABCA3 mutations. *Eur Respir J* 2014;43:638-641.

(69) Shulenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M. ABCA3 gene mutations in newborns with fatal surfactant deficiency. *N Engl J Med* 2004;350:1296-1303.

(70) Deutsch GH, Young LR, Deterding RR, Fan LL, Dell SD, Bean JA, Brody AS, Nogee LM, Trapnell BC, Langston C, Pathology Cooperative Group, Albright EA, Askin FB, Baker P, Chou PM, Cool CM, Coventry SC, Cutz E, Davis MM, Dishop MK, Galambos C, Patterson K, Travis WD, Wert SE, White FV, ChILD Research Co-operative. Diffuse lung disease in young children: application of a novel classification scheme. *Am J Respir Crit Care Med* 2007;176:1120-1128.

(71) Cullinane AR, Curry JA, Carmona-Rivera C, Summers CG, Ciccone C, Cardillo ND, Dorward H, Hess RA, White JG, Adams D, Huizing M, Gahl WA. A BLOC-1 mutation screen reveals that PLDN is mutated in Hermansky-Pudlak Syndrome type 9. *Am J Hum Genet* 2011;88:778-787.

(72) Ammann S, Schulz A, Krageloh-Mann I, Dieckmann NM, Niethammer K, Fuchs S, Eckl KM, Plank R, Werner R, Altmuller J, Thiele H, Nurnberg P, Bank J, Strauss A, von Bernuth H, Zur Stadt U, Grieve S, Griffiths GM, Lehmberg K, Hennies HC, Ehl S. Mutations in AP3D1 associated with immunodeficiency and seizures define a new type of Hermansky-Pudlak syndrome. *Blood* 2016;127:997-1006.

(73) Wei AH, Li W. Hermansky-Pudlak syndrome: pigmentary and non-pigmentary defects and their pathogenesis. *Pigment Cell Melanoma Res* 2013;26:176-192.

(74) Myllarniemi M, Kaarteenaho R. Pharmacological treatment of idiopathic pulmonary fibrosis - preclinical and clinical studies of pirfenidone, nintedanib, and N-acetylcysteine. *Eur Clin Respir J* 2015;2:10.3402/ecrj.v2.26385. eCollection 2015.

(75) Raghu G, Rochwerg B, Zhang Y, Garcia CA, Azuma A, Behr J, Brozek JL, Collard HR, Cunningham W, Homma S, Johkoh T, Martinez FJ, Myers J, Protzko SL, Richeldi L, Rind D, Selman M, Theodore A, Wells AU, Hoogsteden H, Schunemann HJ, American Thoracic Society, European Respiratory society, Japanese Respiratory Society, Latin American Thoracic Association. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. *Am J Respir Crit Care Med* 2015;192:e3-19.

(76) Richeldi L, Costabel U, Selman M, Kim DS, Hansell DM, Nicholson AG, Brown KK, Flaherty KR, Noble PW, Raghu G, Brun M, Gupta A, Juhel N, Kluglich M, du Bois RM. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. *N Engl J Med* 2011;365:1079-1087.

(77) Oku H, Shimizu T, Kawabata T, Nagira M, Hikita I, Ueyama A, Matsushima S, Torii M, Arimura A. Antifibrotic action of pirfenidone and prednisolone: different effects on pulmonary cytokines and growth factors in bleomycin-induced murine pulmonary fibrosis. *Eur J Pharmacol* 2008;590:400-408.

(78) Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, Suga M, Taguchi Y, Takahashi H, Nakata K, Sato A, Takeuchi M, Raghu G, Kudoh S, Nukiwa T, Pirfenidone Clinical Study Group in Japan. Pirfenidone in idiopathic pulmonary fibrosis. *Eur Respir J* 2010;35:821-829.

(79) Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, King TE,Jr, Lancaster L, Sahn SA, Szwarcberg J, Valeyre D, du Bois RM, CAPACITY Study Group. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. *Lancet* 2011;377:1760-1769.

(80) King TE,Jr, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, Gorina E, Hopkins PM, Kardatzke D, Lancaster L, Lederer DJ, Nathan SD, Pereira CA, Sahn SA, Sussman R, Swigris JJ, Noble PW, ASCEND Study Group. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2083-2092.

(81) Hosenpud JD, Bennett LE, Keck BM, Edwards EB, Novick RJ. Effect of diagnosis on survival benefit of lung transplantation for end-stage lung disease. *Lancet* 1998;351:24-27.

(82) Thabut G, Mal H, Castier Y, Groussard O, Brugiere O, Marrash-Chahla R, Leseche G, Fournier M. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. *J Thorac Cardiovasc Surg* 2003;126:469-475.

(83) HARDY JD, WEBB WR, DALTON ML,Jr, WALKER GR,Jr. Lung Homotransplantation in Man. *JAMA* 1963;186:1065-1074.

(84) Geertsma A, Ten Vergert EM, de Boer WJ, van der Bij W. The need for lung transplantation in The Netherlands. *Transpl Int* 1997;10:457-461.

(85) Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, Lederer DJ, Mulligan MJ, Patterson GA, Singer LG, Snell GI, Verleden GM, Zamora MR, Glanville AR. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* 2015;34:1-15.

(86) Egan TM, Murray S, Bustami RT, Shearon TH, McCullough KP, Edwards LB, Coke MA, Garrity ER, Sweet SC, Heiney DA, Grover FL. Development of the new lung allocation system in the United States. *Am J Transplant* 2006;6:1212-1227.

(87) Force SD, Kilgo P, Neujahr DC, Pelaez A, Pickens A, Fernandez FG, Miller DL, Lawrence C. Bilateral lung transplantation offers better long-term survival, compared with single-lung transplantation, for younger patients with idiopathic pulmonary fibrosis. *Ann Thorac Surg* 2011;91:244-249.

(88) Mason DP, Brizzio ME, Alster JM, McNeill AM, Murthy SC, Budev MM, Mehta AC, Minai OA, Pettersson GB, Blackstone EH. Lung transplantation for idiopathic pulmonary fibrosis. *Ann Thorac Surg* 2007;84:1121-1128.

(89) Neurohr C, Huppmann P, Thum D, Leuschner W, von Wulffen W, Meis T, Leuchte H, Baumgartner R, Zimmermann G, Hatz R, Czerner S, Frey L, Ueberfuhr P, Bittmann I, Behr J, Munich Lung Transplant Group. Potential functional and survival benefit of double over single lung transplantation for selected patients with idiopathic pulmonary fibrosis. *Transpl Int* 2010;23:887-896.

(90) Algar FJ, Espinosa D, Moreno P, Illana J, Cerezo F, Alvarez A, Baamonde C, Redel J, Vaquero JM, Santos F, Salvatierra A. Results of lung transplantation in idiopathic pulmonary fibrosis patients. *Transplant Proc* 2010;42:3211-3213.

(91) Keating D, Levvey B, Kotsimbos T, Whitford H, Westall G, Williams T, Snell G. Lung transplantation in pulmonary fibrosis: challenging early outcomes counterbalanced by surprisingly good outcomes beyond 15 years. *Transplant Proc* 2009;41:289-291.

(92) Thabut G, Christie JD, Ravaud P, Castier Y, Dauriat G, Jebrak G, Fournier M, Leseche G, Porcher R, Mal H. Survival after bilateral versus single-lung transplantation for idiopathic pulmonary fibrosis. *Ann Intern Med* 2009;151:767-774.

(93) De Oliveira NC, Osaki S, Maloney J, Cornwell RD, Meyer KC. Lung transplant for interstitial lung disease: outcomes for single versus bilateral lung transplantation. *Interact Cardiovasc Thorac Surg* 2012;14:263-267.

(94) Gottlieb J, Warnecke G, Hadem J, Dierich M, Wiesner O, Fuhner T, Strueber M, Haverich A, Welte T. Outcome of critically ill lung transplant candidates on invasive respiratory support. *Intensive Care Med* 2012;38:968-975.

(95) de Perrot M, Granton JT, McRae K, Cypel M, Pierre A, Waddell TK, Yasufuku K, Hutcheon M, Chaparro C, Singer L, Keshavjee S. Impact of extracorporeal life support on outcome in patients with idiopathic pulmonary arterial hypertension awaiting lung transplantation. *J Heart Lung Transplant* 2011;30:997-1002.

(96) Diaz-Guzman E, Hoopes CW, Zwischenberger JB. The evolution of extracorporeal life support as a bridge to lung transplantation. *ASAIO J* 2013;59:3-10.

(97) Riley JB, Scott PD, Schears GJ. Update on safety equipment for extracorporeal life support (ECLS) circuits. *Semin Cardiothorac Vasc Anesth* 2009;13:138-145.

(98) George TJ, Beaty CA, Kilic A, Shah PD, Merlo CA, Shah AS. Outcomes and temporal trends among high-risk patients after lung transplantation in the United States. *J Heart Lung Transplant* 2012;31:1182-1191.

(99) Javidfar J, Bacchetta M. Bridge to lung transplantation with extracorporeal membrane oxygenation support. *Curr Opin Organ Transplant* 2012;17:496-502.

(100) Trudzinski FC, Kaestner F, Schafers HJ, Fahndrich S, Seiler F, Bohmer P, Linn O, Kaiser R, Haake H, Langer F, Bals R, Wilkens H, Lepper PM. Outcome of Patients with Interstitial Lung Disease Treated with Extracorporeal Membrane Oxygenation for Acute Respiratory Failure. *Am J Respir Crit Care Med* 2016;193:527-533.

CHAPTER TWO

High mortality in patients with idiopathic pulmonary fibrosis on the Dutch lung transplant waiting list

ten Klooster L, van Moorsel CH, van Hal PT, van den Blink B, Nossent GD, Verschuuren EA, Kwakkel-van Erp JM, van de Graaf EA, van Kessel DA, Grutters JC

Ned Tijdschr Geneesk. 2012;156(2): A3752. Dutch.

ABSTRACT

Objective

To describe patients diagnosed with idiopathic pulmonary fibrosis (IPF) referred for lung transplantation and to evaluate the current listing criteria for lung transplantation in the Netherlands.

Design

Retrospective study.

Methods

All patients diagnosed with an interstitial lung disease and referred for lung transplantation from September 1989 - June 2010 were included in this study. Patients diagnosed with IPF according to the American Thoracic Society - European Respiratory Society criteria were included. Clinical data at the time of screening for lung transplantation and survival data were collected.

Results

In total 289 IPF patients were referred for lung transplantation. After a first evaluation for contraindications and screening programme, 90 patients were listed for lung transplantation. During the waiting period, 30 patients (33%) died, 7 were taken off the list due to newly developed comorbidities or excessive physical deterioration, 51 underwent transplantation and 2 were still on the waiting list at the end of the study. At the time of screening, the mean FVC was 51% of predicted (SD: 19.0) and the mean diffusing capacity was 27% of predicted (SD: 9.3).

Conclusion

One-third of the IPF patients died on the waiting list. A mean diffusing capacity of 27% of predicted at the time of screening is considerably lower than recommended in the international listing criteria for lung transplantation. This study, therefore, shows that the timing of screening IPF patients for lung transplantation can be improved in the Netherland.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive and fibrosing interstitial disease with a median survival of 2,5-5 years (1-3). IPF is the most common and severe form of interstitial lung diseases (ILD) and is characterised by diffuse remodelling that leads to architectural changes of the lung parenchyma resulting in a progressive decline in lung function. The pathogenesis of IPF is not clear, but it is hypothesized that normal regeneration of damaged alveolar epithelium fails to occur and proliferation of fibroblasts causes irreversible scarring of the lungs (4). IPF is a multidisciplinary diagnosis based on radiographic and histopathological pattern of 'usual interstitial pneumonia' (UIP) (5, 6). There is no medicinal treatment to improve the prognosis in IPF patients; the only proven medical therapy to prolong the patient's life is lung transplantation (LTX) (7, 8).
In the Netherlands the first LTX was performed in 1989. From 1991-2001 the University Medical Centre (UMC) in Groningen was assigned to perform LTX. However, due to an increasing waiting list authorisation for another LTX programme was enhanced to UMC Utrecht, St Antonius Hospital Nieuwegein and Erasmus Medical Centre in Rotterdam in 2001 (9). In that same year a new allocation system was introduced which made it possible to list patients with a diminished life expectancy as 'high-urgency' (HU) to increase their possibility of LTX.
Timing of referral for LTX is important for survival and quality of life. International guidelines for selecting LTX candidates have been published to provide guidance to the medical doctor (10). According to these guidelines all patients with a histopathological or radiographic pattern of UIP should be referred for LTX. Listing on the lung transplant waiting list is recommended when one of the following criteria is met: a) diffusing capacity for carbon monoxide (DLco) < 39% of predicted; b) 10% or more decline in forced vital capacity (FVC) in the last six months of follow up; c) decline of oxygen saturation below 88% during the six-minute walking distance (6-MWD) or d) the presence of honeycombing on the high resolution CT-scan (10). However, these guidelines are not evaluated for the Dutch practice.

The aim of this article is to describe trajectory and clinical data of ILD patients referred for LTX in the Netherlands since the start of the lung transplant program in 1989. Furthermore, we focused on IPF patients and the degree of failure in these patients. The aim was to provide better insights and an incentive to improve referral of IPF patients for lung transplantation in the Netherlands.

Methods

Patient selection

Data was retrospectively collected from all patients enlisted for LTX with a diagnosis of ILD from September 1989 – June 2010. To collect clinical data at time of screening for LTX and survival data we used the different databases in St. Antonius Hospital, UMC Utrecht, UMC Groningen and Erasmus MC Rotterdam. IPF patients were included when the diagnosis criteria based on the American Thoracic Society (ATS) and European Respiratory Society (ERS) guideline were met (5, 11).

Statistical analyses

Univariate group comparisons of continuous and categorical variables were performed using two-sample *t* test and χ^2 tests or Fisherman's test when appropriate. Survival estimates were calculated with the Kaplan-Meier method and patients who received LTX or were removed from the waiting list for other reasons were censored. Analyses were performed using Statistical Package for the Social Sciences version 21.0 (SPSS Inc., New York, VS). All tests were evaluated at the 0.05 alpha level and *p*-values were 2-sided.

Results

Patients characteristics

Altogether 122 IPF patients were screened for LTX: 56 patients from UMC Groningen, 44 patients from UMC Utrecht / St. Antonius Hospital and 22 patients from Erasmus MC. Seventy-five percent were men and the mean age at screening was 53.9 years (SD: 8.5). The majority of the patients (71%) received oxygen therapy, of which 88% used oxygen 24 hours per day. At time of screening 102 patients (84%) were treated with prednisolone with a mean dose of 23 mg per day (SD: 15.3).

Sixty-six of the patients had a smoking history with a median of 18.6 pack years (range 1 – 75). Pulmonary function tests demonstrated a severe restrictive pulmonary function with a mean total lung capacity (TLC) of 53% of predicted (SD: 16.9) and a mean diffusing capacity for carbon monoxide (DL_{CO}) of 25% of predicted (SD: 9.3). The patient characteristics at time of screening are demonstrated in table 1.

Clinical characteristics	**N= 122**
Sex (male); n (%)	92 (75)
Age (yrs.); mean (SD)	53.9 (8.5)
Body Mass Index; mean (SD)	26.3 (3.5)
Smoking status; n (%) Non-smoker Ex-smoker	 42 (34.4) 80 (65.6)
Oxygen therapy; n (%)	86 (70.5)
Corticosteroids; n (%)	102 (83.6)
6-minute walking test (m); mean (SD)	294 (181.6)
Pulmonary function test; % of predicted (SD) FEV_1 FVC TLC DL_{CO}	 53.3 (17.8) 51.1 (19) 52.5 (16.9) 27.1 (9.3)

Table 1: Patients characteristics of 122 patients with idiopathic pulmonary fibrosis at time of screening for lung transplantation
Abbreviations: FEV_1= forced expirated volume in 1 second; FVC= forced vital capacity; TLC= total lung capacity; DL_{CO} = diffusing capacity of carbon monoxide

Trajectory of registration to transplantation

In the period from September 1989 – June 2010 688 patients diagnosed with ILD were referred for LTX, of which 289 (42%) were diagnosed with IPF (figure 1). During the complete transplant trajectory 71 IPF patients died (25% of the 289 IPF patients referred for LTX). The flowchart of all referred ILD patients is depicted in figure 2.
Thirty-six IPF patients died before they were screened for LTX. In addition, 3 patients followed the screening programme in Belgium and 122 patients were rejected for screening due to contraindications (figure 2).

Reasons for rejection were a history of cardiovascular disease (27%), comorbidities (16%), mild disease (13%) and other, less common reasons such as history of malignancy and BMI higher than 30 kg/m^2.
Five IPF patients died during the screening programme and 27 were rejected after screening because of contraindications like newly diagnosed coronary artery disease. During the study period 122 IPF patients were screened for LTX and 6 patients were scheduled for screening. Finally, 90 IPF patients were listed for LTX. Due to deterioration 31 IPF patients (34%) placed on the waiting list were converted from transplantable to 'high urgency'. Eventually, 51 patients (57%) received a LTX of which 45% were listed as 'high urgency'.
Furthermore, we noticed some significant differences between IPF patients and the ILD not IPF patients. First during the referral period 13% of the IPF patients died versus 5% of the ILD not IPF patients ($p < 0.001$). Secondly, after the referral period only 44% of the IPF patients were included in the screenings program compared to 54% of the ILD not IPF patients ($p = 0.007$).

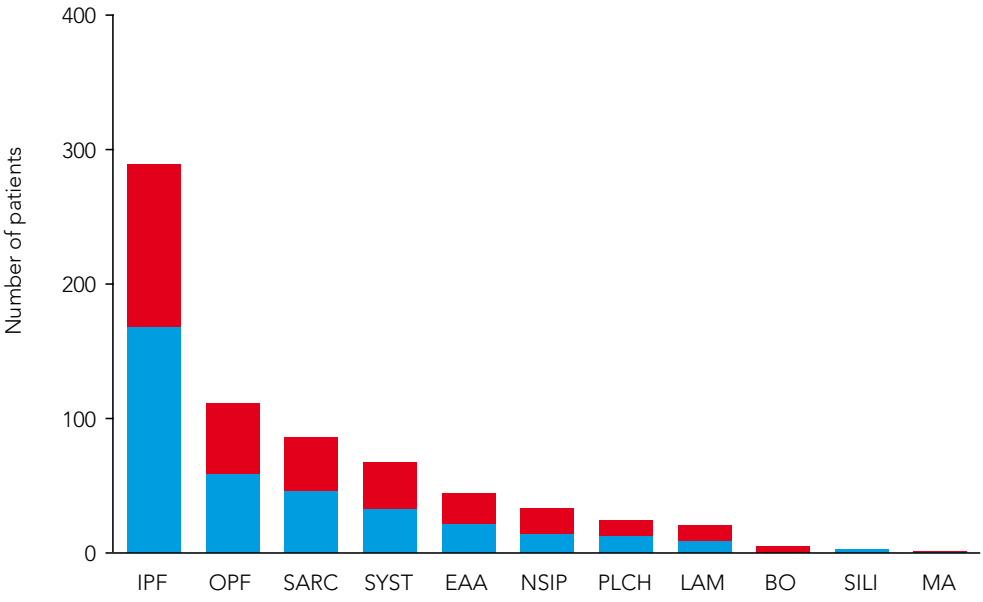


Figure 1: Number of patients with an interstitial lung disease referred for lung transplantation from September 1989 – June 2010 in the Netherlands. For every diagnosis is specified who is (red) and who is not (blue) screened for lung transplantation.
Abbreviations: IPF = idiopathic pulmonary fibrosis; OPF = pulmonary fibrosis not IPF; SARC = sarcoidosis; SYST = systemic diseases; EAA = extrinsic alveolar alveolitis; NSIP = nonspecific interstitial pneumonia; PLCH = pulmonary Langerhans cell histiocytosis; LAM = lymphangioleiomyomatosis; BO = bronchiolitis obliterans; SILI = silicosis; MA = alveolar microlithiasis

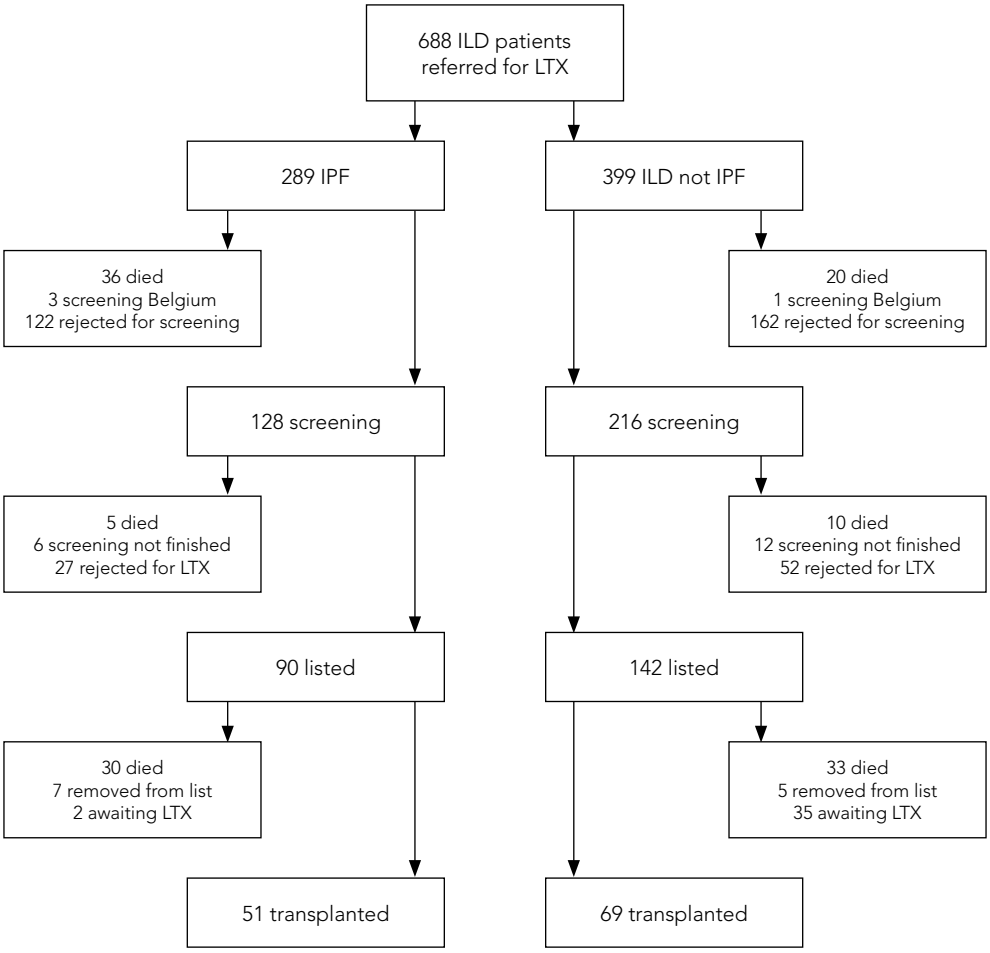


Figure 2: Flowchart of 688 patients with interstitial lung disease referred for lung transplantation

Mortality on the waiting list

Ninety IPF patients were listed for LTX and their survival on the waiting list is demonstrated in figure 3. Median survival time on the waiting list was 22 months. Awaiting LTX 30 IPF patients (33%) died. Of the 30 patients who died awaiting LTX, 25% died within 1 month, 50% after 3 months and 75% after 13.5 months after listing. The median time to death on the waiting list was 3 months (range 0 – 53 months).

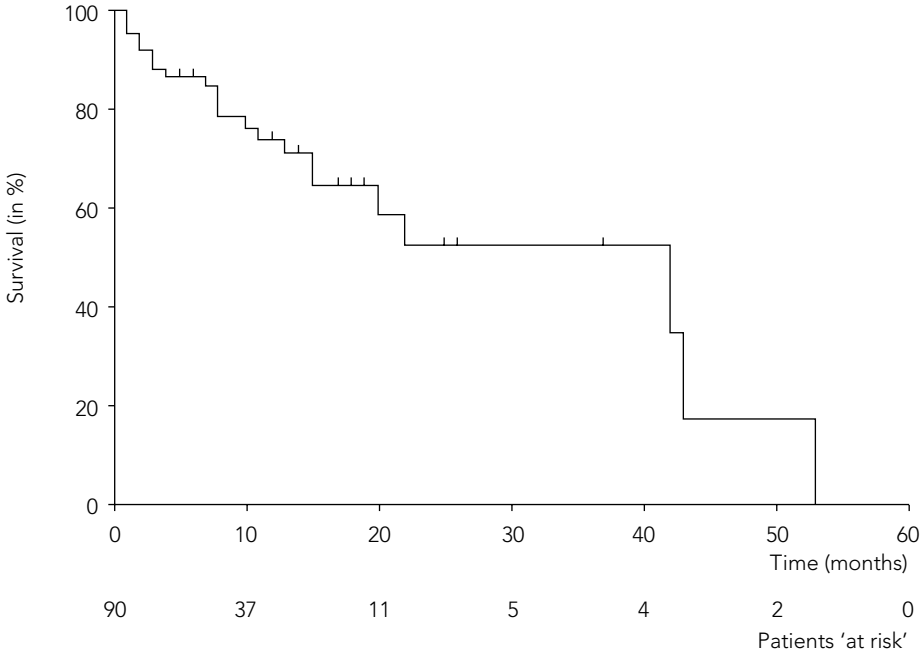


Figure 3: Survival curve of 90 patients with idiopathic pulmonary fibrosis listed for lung transplantation from September 1989 – June 2010 in the Netherlands. Patients who received LTX or were removed from the waiting list for other reasons were censored. Median survival was 22 months.

Discussion

This study provides an overview of all ILD patients, with an emphasis on IPF patients, registered for LTX in the Netherlands from September 1989 to June 2010. All IPF patients should be considered for LTX because of the devastating and lethal course and lack of therapeutic options. The patients screened for LTX were mostly men (75%), according to previously reported data about IPF cohorts (3, 12). Previously reported data about a Dutch IPF cohort showed a mean age of 61.9 years at time of diagnosis (3), however the mean age at time of screening for LTX was 53.9 years. This demonstrates that mostly relatively young IPF patients were screened for LTX. An adequate explanation could be that younger patients are more often a suitable candidate than older patients, and older age (> 65 years) is related to worse outcome after LTX (13). Ideally, the patient is listed for LTX if the life expectancy is greatly diminished.

Adequate timing of listing is difficult because of the variable course of IPF. Most IPF patients have a slowly progressive disease course, other patients experience periods of stable disease and others have a rapid accelerated clinical decline. Approximately 5 – 10% of IPF patients suffer from an acute exacerbations which are often fatal (14-17).

The international guidelines recommend to refer all patients with a histological or radiographic pattern of 'usual interstitial pneumonia' for LTX (13). This implies that, if there are no evident contraindications, candidacy for LTX should be discussed with the patient in an early stage of the disease.

However, this study demonstrated that most IPF patients referred for LTX have advanced disease. This is mainly reflected by a severe pulmonary function at time of screening, particularly a mean DL_{CO} of only 27% of predicted. Moreover, this could very well be an overestimation, since approximately 40% of the screened IPF patients were not able to perform diffusion test due to their poor clinical condition. Thirty percent of all IPF patients listed for LTX died waiting for a suitable organ, 25% in the first month and 50% in the first 3 months of enlistment. We analysed the waiting list mortalities for patients diagnosed with chronic obstructive pulmonary disease (COPD) or cystic fibrosis (CF). These analyses showed a waiting list mortality of 16% in COPD patients and 14% in CF patients (data not shown).

The high waiting list mortality can be explained by difficulties in timing of referral due to the variable and unpredictable course of IPF. Furthermore, the allocation system before 2001 and the scarcity of lung donors had also a significant role in the high waiting list mortality.

In the Netherlands approximately 60 LTX were performed annually. At the end of 2010 over 200 patients were listed for LTX, which is an increase of 16% compared to the preceding year. Due to an increasing demand for LTX, the mean waiting list time is longer than 3 years.

In the Netherlands donor registration occurs on a 'permission-based' system. Only the donor, or legal partner or family can give permission for organ donation. Belgium and France use a different system based on active objection, where everyone is an organ donor except for persons who object in writing. In these countries there are significant less issues concerning LTX and the mean waiting time in Belgium was 190 days. This could stimulate the discussion in the Netherlands about the preferred system of donor registration.

The use of donation after cardiac death (DCD) donors seems to be a possibility to enlarge the donor pool (18). Studies demonstrated similar short-term survival outcomes after LTX of donation after brain death (DBD) versus DCD donors. Increased awareness of the utility of lungs of DCD donors, better insights in donor management system and better communication between donor hospital and transplant team can potentially increase the use of DCD donors (19-21).
A life expectancy of less than a few weeks is one of the criteria to be listed as HU. Of all transplanted IPF patients 45% received a 'high-urgency' (HU) status. After implementation of the new allocation system in 2001 waiting list mortality was decreased from 42% to 30% (data not shown).
The first years after implementing the HU-status, patients older than 60 years could not receive a HU-status. In October 2011 age was no longer a strict contraindication, however, critical clinical judgement of patients older than 60 is still recommended. Furthermore, due to donor scarcity the HU-waiting list can easily become an 'alternative' waiting list and patients on the 'normal' waiting list could be bypassed. In 2010 more than 50% of all LTX was performed for HU patients.
In May 2005 the Lung Allocation Score (LAS) is introduced in the United States. The main reason for introducing the LAS was to decrease waiting list mortality. The LAS is a numerical value and takes into account various measures of a patient's health in order to direct donated organs towards the patients who would best benefit from LTX. After introducing the LAS in the United States the waiting period was reduced with 54% (mean waiting period reduced of more than 2 years to 134 days) (22). The introduction of the LAS caused an increase of LTX in IPF patients. Nevertheless, after implementation of the LAS system, 1 year survival has not been changed if corrected for age and diagnosis (23, 24). In the Netherlands the distribution of organ donors is regulated by Eurotransplant and does not use criteria favouring specific patient groups yet. Nowadays the possibility for implementing the LAS or similar scoring system in the Netherlands is being investigated.
This study demonstrated a mean DL_{CO} of 27% at the time of screening, which is much lower compared to the DL_{CO} of 39% recommended by the international guidelines (13). These results suggest that IPF patients should be referred for screening in an earlier stage of the disease. Early listing of IPF patients could be beneficial according to waiting list mortality.

Conclusion

The results of this study plead for a better knowledge according to the referral criteria for LTX in IPF patients. An option to increase understanding is to formulate Dutch guidelines with specific criteria for referral for LTX per (ILD) diagnosis taking into account the Dutch situation. Hopefully this will lead to early referral of IPF patients to the transplant centres.
Future research is needed to optimise international guidelines according to the Dutch situation and to investigate probable prognostic biomarkers in IPF patients for guidance and timing of IPF patients in the transplant programme.

References

(1) Caminati A, Harari S. IPF: New insight in diagnosis and prognosis. *Respir Med* 2010;104 Suppl 1:S2-10.

(2) Bjoraker JA, Ryu JH, Edwin MK, Myers JL, Tazelaar HD, Schroeder DR, Offord KP. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1998;157:199-203.

(3) Barlo NP, van Moorsel CH, van den Bosch JM, van de Graaf EA, Kwakkel-van Erp JM, Grutters JC. Idiopathic pulmonary fibrosis; description of a Dutch cohort. *Ned Tijdschr Geneeskd* 2009;153:B425.

(4) Harari S, Caminati A. IPF: new insight on pathogenesis and treatment. *Allergy* 2010;65:537-553.

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

(6) Hunninghake GW, Zimmerman MB, Schwartz DA, King TE,Jr, Lynch J, Hegele R, Waldron J, Colby T, Muller N, Lynch D, Galvin J, Gross B, Hogg J, Toews G, Helmers R, Cooper JA,Jr, Baughman R, Strange C, Millard M. Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2001;164:193-196.

(7) Thabut G, Mal H, Castier Y, Groussard O, Brugiere O, Marrash-Chahla R, Leseche G, Fournier M. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. *J Thorac Cardiovasc Surg* 2003;126:469-475.

(8) Hosenpud JD, Bennett LE, Keck BM, Edwards EB, Novick RJ. Effect of diagnosis on survival benefit of lung transplantation for end-stage lung disease. *Lancet* 1998;351:24-27.

(9) Geertsma A, Ten Vergert EM, de Boer WJ, van der Bij W. The need for lung transplantation in The Netherlands. *Transpl Int* 1997;10:457-461.

(10) Orens JB, Estenne M, Arcasoy S, Conte JV, Corris P, Egan JJ, Egan T, Keshavjee S, Knoop C, Kotloff R, Martinez FJ, Nathan S, Palmer S, Patterson A, Singer L, Snell G, Studer S, Vachiery JL, Glanville AR, Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. International guidelines for the selection of lung transplant candidates: 2006 update--a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* 2006;25:745-755.

(11) Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE,Jr, Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schunemann HJ, ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788-824.

(12) Demedts M, Wells AU, Anto JM, Costabel U, Hubbard R, Cullinan P, Slabbynck H, Rizzato G, Poletti V, Verbeken EK, Thomeer MJ, Kokkarinen J, Dalphin JC, Taylor AN. Interstitial lung diseases: an epidemiological overview. *Eur Respir J Suppl* 2001;32:2s-16s.

(13) Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Dobbels F, Kirk R, Rahmel AO, Stehlik J, Hertz MI, International Society of Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report-2012. *J Heart Lung Transplant* 2012;31:1073-1086.

(14) Selman M, Carrillo G, Estrada A, Mejia M, Becerril C, Cisneros J, Gaxiola M, Perez-Padilla R, Navarro C, Richards T, Dauber J, King TE,Jr, Pardo A, Kaminski N. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. *PLoS One* 2007;2:e482.

(15) Hyzy R, Huang S, Myers J, Flaherty K, Martinez F. Acute exacerbation of idiopathic pulmonary fibrosis. *Chest* 2007;132:1652-1658.

(16) Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, King TE,Jr, Lasky JA, Loyd JE, Noth I, Olman MA, Raghu G, Roman J, Ryu JH, Zisman DA, Hunninghake GW, Colby TV, Egan JJ, Hansell DM, Johkoh T, Kaminski N, Kim DS, Kondoh Y, Lynch DA, Muller-Quernheim J, Myers JL, Nicholson AG, Selman M, Toews GB, Wells AU, Martinez FJ, Idiopathic Pulmonary Fibrosis Clinical Research Network Investigators. Acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2007;176:636-643.

(17) Ley B, Collard HR, King TE,Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;183:431-440.

(18) Nijkamp DM, van der Bij W, Verschuuren EA, Heemskerk MB, de Buijzer E, Erasmus ME. Non-heart-beating lung donation: how big is the pool? *J Heart Lung Transplant* 2008;27:1040-1042.

(19) De Vleeschauwer S, Van Raemdonck D, Vanaudenaerde B, Vos R, Meers C, Wauters S, Coosemans W, Decaluwe H, De Leyn P, Nafteux P, Dupont L, Lerut T, Verleden G. Early outcome after lung transplantation from non-heart-beating donors is comparable to heart-beating donors. *J Heart Lung Transplant* 2009;28:380-387.

(20) Dark JH. Lung transplantation from the non-heart beating donor. *Transplantation* 2008;86:200-201.

(21) Van De Wauwer C, Verschuuren EA, van der Bij W, Nossent GD, Erasmus ME. The use of non-heart-beating lung donors category III can increase the donor pool. *Eur J Cardiothorac Surg* 2011;39:e175-80; discussion e180.

(22) Eberlein M, Garrity ER, Orens JB. Lung allocation in the United States. *Clin Chest Med* 2011;32:213-222.

(23) Gries CJ, Mulligan MS, Edelman JD, Raghu G, Curtis JR, Goss CH. Lung allocation score for lung transplantation: impact on disease severity and survival. *Chest* 2007;132:1954-1961.

(24) Merlo CA, Weiss ES, Orens JB, Borja MC, Diener-West M, Conte JV, Shah AS. Impact of U.S. Lung Allocation Score on survival after lung transplantation. *J Heart Lung Transplant* 2009;28:769-775.

CHAPTER THREE

Ten years survival in patients with idiopathic pulmonary fibrosis after lung transplantation

Liesbeth ten Klooster, George D. Nossent, Johanna M. Kwakkel-van Erp, Diana A. van Kessel, Erik J. Oudijk, Ed A. van de Graaf, Bart Luijk, Rogier A. Hoek, Bernt van den Blink, Peter Th. van Hal, Erik A. Verschuuren, Wim van der Bij, Coline H. van Moorsel, Jan C. Grutters

Lung 2015 Dec; 193(6): 919-26

ABSTRACT

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive and lethal fibrosing lung disease with a median survival of approximately 3 years after diagnosis. The only medical option to improve survival in IPF is lung transplantation (LTX).
The purpose of this study was to evaluate trajectory data of IPF patients listed for LTX and to investigate the survival after LTX.

Methods and Results

Data were retrospectively collected from September 1989 until July 2011 of all IPF patients registered for lung transplantation in the Netherlands. Patients were included after revision of the diagnosis based on the criteria set by the ATS/ERS/JRS/ALAT. Trajectory data, clinical data at time of screening and donor data were collected. In total 98 IPF patients were listed for lung transplantation. During the waiting list period 30% of the patients died. Mean pulmonary artery pressure, six-minute walking distance and the use of supplemental oxygen were significant predictors of mortality on the waiting list. Fifty-two patients received LTX with a median overall survival after transplantation of 10 years.

Conclusions

This study demonstrated a 10-year survival time after LTX in IPF. Furthermore, our study demonstrated a significantly better survival after bilateral LTX in IPF as compared to single LTX although bilateral LTX patients were significantly younger.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive, irreversible and life-threatening fibrosing lung disease of unknown etiology. The diagnosis of IPF is associated with histopathologic or radiologic pattern of *usual interstitial pneumonia* (UIP) and requires the exclusion of other forms of interstitial lung diseases caused by environmental exposure, medication, or systemic disease (1). The clinical course of IPF is heterogeneous and carries a poor prognosis with a median survival of approximately 3 years after diagnosis (1, 2). Treatment options are limited and although clinical trials showed that some pharmacologic agents may be beneficial, life-prolonging medicinal therapy does not exist for IPF (3). Currently, the only therapeutic option to improve survival in IPF patients is lung transplantation (LTX) (4, 5).

LTX is standard of care for selected patients with advanced lung diseases of non-malignant etiologies (6). Over the years post-LTX survival has steadily improved and the International Society for Heart and Lung Transplantation (ISHLT) reports an overall median survival rate for adult lung recipients of 5.5 years at present and in IPF a median survival rate of 4.1 years (7).

Successful outcome after LTX depends on careful candidate selection, timing of listing and choice of procedure. Appropriate timing of LTX is challenging in IPF patients because of clinical variations in disease progression, which varies from a slowly prolonged course to a more rapid pattern of progression, including unpredictable acute exacerbations (8, 9). Historically, single lung transplantation (SLT) was almost uniformly utilized in IPF patients.

As in other disease indications, bilateral lung transplantation (BLT) is nowadays becoming the procedure of choice. This preferred application of BLT might be related to better long-term outcomes as compared with SLT (6, 7). However, IPF patients listed for BLT seem to have an increased risk of pretransplant mortality and evaluation of transplant outcomes might not be based solely on post-transplant survival, but also account for the impact of the choice of procedure (10). Up till now, the preferential use of either SLT or BLT in IPF patients remains controversial (6).

The aims of this study were to evaluate clinical data of IPF patients listed for LTX, to identify predictors of waiting list mortality and evaluate survival after LTX. Furthermore, we investigated whether survival after LTX was related to the type of LTX, i.e. either SLT or BLT. Preliminary results of this study have been reported in abstract form.

Materials and Methods

We performed a retrospective cohort study and collected data of IPF patients listed for lung transplantation in the Netherlands from the start of the lung transplant program in September 1989 until July 2011. Clinical data at time of screening were collected. Donor data were provided by the Dutch Transplantation Foundation (*NTS, Nederlandse Transplantatie Stichting*). Informed consent was given by participants and all patients records were anonymized and de-identified prior to analysis. The research was conducted using appropriate ethical guidelines and approved by the Medical-ethical Committee VCMO (*Verenigde Commissies Mensgebonden Onderzoek*) of St. Antonius Hospital in Nieuwegein.

Study population

The LTX program in The Netherlands started in 1989. Because of the growing demand, the LTX program was extended in 2001 to 2 other medical centres. Lung allocation was essentially based on waiting time. In that same year it became possible to list patients as *high urgent* (HU) for better waiting list regulation. The criteria set by Eurotransplant for HU listing are described in table 1 and are not disease-specific criteria. Each centre independently collects clinical data in separate databases. Before 2001, the immunosuppressive regimen consisted of antithymocyte globulin induction therapy followed by maintenance therapy with cyclosporin A, azathioprine and prednisolone. Since 2001, the regimen changed to induction therapy with basiliximab followed by maintenance therapy with tacrolimus, mycophenolate mofetil and prednisolone. The University Medical Center of Groningen used in their maintenance regimen azathioprine instead of mycophenolate mofetil.

We searched the databases for patients diagnosed with IPF or idiopathic interstitial pneumonia. Diagnoses were reviewed and patients were included when criteria set by the ATS/ERS/JRS/ALAT were met (1). In only a few patients the radiologic or histological diagnosis was questionable and an experienced clinician

set the diagnosis. Patients with a first-degree family member with pulmonary fibrosis were classified as familial IPF (FPF). In three patients with a non-classifiable diagnosis on HRCT it was decided not to perform a biopsy, because they had a first-degree family member with pulmonary fibrosis. Time of clinical presentation was defined as the date the patient visited the pulmonologist for the first time and presented with clinical complaints associated with IPF, such as dry cough or dyspnea. Time of diagnosis was defined as the date of first multidisciplinary meeting between the pulmonologist, radiologist and pathologist.

Eurotransplant HU criteria

Taken up in an intensive care unit of the transplant centre
Acute life threatening situation
Imminent need for controlled ventilation in spite of optimal conservative therapy
OR
Assisted / controlled ventilation due to respiratory insufficiency

Table 1: Overview of High Urgency criteria set by Eurotransplant

Statistical analysis

Univariate group comparisons of continuous and categorical variables were performed using two-sample *t* test and χ^2 tests or Fisherman's test when appropriate. Survival estimates were calculated with the Kaplan-Meier method. For survival calculation after LTX the patients were censored when they were still alive at the end of the study. Comparison of survival in different groups was done with the log-rank test. Cox regression analysis was used to test the effect of the variables on overall survival and differences between SLT and BLT. Missing data were assumed to be missing at random and replaced by mean imputation so that the whole sample could be analyzed and to avoid selection bias that might occur when cases with missing variables of interest would be deleted.

Analyses were performed using Statistical Package for the Social Sciences version 21.0 (SPSS Inc., New York, VS). All tests were evaluated at the 0.05 alpha level and *p*-values were 2-sided.

Results

Since the start of the LTX program in 1989 until 1st of July 2011 98 IPF patients were registered for LTX. The mean age (SD) of the patients screened for LTX was 53 (8) years and 74% were men. Eighteen percent indicated an affected first-degree family member and were classified as familial IPF. Sixty-seven percent were former smoker with a median of 19 pack years. At the time of screening the mean FVC% was 52 ± 17% and mean diffusing capacity for carbon monoxide expressed as a percentage of the predicted value (DL_{CO}%) was 28 ± 10%. Patients' characteristics are presented in table 2.

	Data available (n)	**All patients listed for LTX (n = 98)**	**Alive awaiting LTX (n = 69)**	**Died awaiting LTX (n = 29)**	***p*-value**
Age, yr. at screening (+/- SD)	98	53 (8)	53 (8)	53 (9)	0.652
Age, yr. at transplantation (+/- SD)	52	53 (8)	53 (8)	-	-
Male (%)	98	72 (74)	51 (74)	21 (72)	0.878
Body mass index, kg/m^2 (+/- SD)	98	26 (4)	27 (3)	26 (5)	0.186
Smoking, never (%)	95	31 (33)	18 (27)	13 (46)	0.064
Smoking, former (%)	95	64 (67)	49 (73)	15 (54)	0.064
Cigarette packyears[a] (range)	95	19 (1 – 75)	14 (1 – 45)	12 (0 – 75)	0.651
Use of oxygen (%)	97	69 (71)	46 (68)	23 (79)	0.246

Continued	**Data available (n)**	**All patients listed for LTX (n = 98)**	**Alive awaiting LTX (n = 69)**	**Died awaiting LTX (n = 29)**	***p*-value**
Steroid treatment (%)	95	81	58 (85)	23 (85)	1.000
Pulmonary hypertension (%)	91	26 (29)	15 (23)	11 (41)	0.095
Mean PAP, mmHg (+/- SD)	91	23 (10)	22 (7)	26 (14)	0.039*
6-minute walking test, m (+/- SD)	88	303 (153)	315 (152)	272 (156)	0.230
FVC, % predicted (+/-SD)	94	52 (17)	53 (17)	48 (16)	0.187
VC, % predicted (+/-SD)	97	53 (17)	54 (17)	50 (16)	0.211
TLC, % predicted (+/-SD)	78	54 (14)	55 (15)	51 (11)	0.247
DL_{CO}, % predicted (+/-SD)	69	28 (10)	29 (10)	26 (8)	0.298

Table 2: Patients characteristics of 98 IPF patients at time of screening for lung transplantation
Abbreviations: FVC = forced vital capacity, VC = vital capacity, TLC = total lung capacity, DLCO = diffusing capacity for carbon monoxide, PAP = pulmonary artery pressure.
a Pack-year data are shown for former smokers only.

Of the 98 IPF patients listed for LTX, 9 were removed from the list: 2 patients were transferred to the transplant program in Belgium, 3 patients developed a malignancy, 2 developed increasingly poor physical condition, and 2 patients had a stabilized course of IPF and good clinical condition. While waiting, 32 patients (33%) received a *high urgency* (HU) status. Of total 29 patients (30%) died on the waiting list, including 7 patients with HU status. Finally, 52 patients received a lung transplant, 21 were SLT and 31 BLT. At the end of the study period 8 patients were still waiting for LTX (figure 1).

IPF patients listed for LTx	98

Removed of waiting list	9

Waiting list	89
Elective	57
High Urgency	32

Waiting list mortality	29
Elective	22
High Urgency	7

Transplanted	52
Single LTx	21
Bilateral LTx	31

Active waiting for LTx	8

Figure 1: Flowchart of 98 IPF patients listed for lung transplantation.

While waiting for LTX 29 patients (30%) died. Median survival on the waiting list for IPF patients was 22 months. Mean time listed to death was 8.7 months (mean 0 – 53 months, SD 12.6) and mean time listed to transplant was 9.0 months (0 – 3 months, SD 8.2). The waiting time in patients awaiting SLT vs. BLT did not differ (p= 0.978). The mean pulmonary artery pressure (mPAP) was higher in the patients who died on the waiting list (p= 0.039) (table 2). There were no other differences at time of screening between patients who died on the waiting list and patients who survived the waiting period.

Univariate Cox regression analysis revealed that patients with an elevated mPAP had an increased risk of dying while awaiting LTX. Furthermore, the use of supplemental oxygen and the six-minute walking distance (6MWD) were significantly related to waiting list mortality (table 4).

These significant variables from the univariate analysis were incorporated in a multivariate Cox regression analysis and only the use of supplemental oxygen remained significant (p= 0.042, HR 1.33, 95% CI 1.01 – 1.76).

There was no significant difference in mortality on the waiting list between patients received SLT versus BLT. Furthermore, univariate analysis showed that receiving a high urgency status was a strong predictor for surviving the period till LTX (p< 0.001, HR 3.12, 95% CI 1.77 – 5.52), but not a predictor for waiting list mortality. Statistical evaluation of the influence of delays in the transplant trajectory was done, but no significant delays related to waiting list mortality could be detected.

Fifty-two IPF patients received LTX with a median overall survival after LTX of 10 years (figure 2). Thirty-one patients underwent BLT and 21 SLT. The mean age at transplantation was 53 years, and patients who received SLT were significantly older than the BLT group (55 and 51 years, respectively; see table 3). There were no other significant clinical differences between the patients who received an SLT and BLT at time of screening (table 3).

	Data available (n)	Single LTX (n = 21)	Data available (n)	Bilateral LTX (n = 31)	*p*-value
Age, yr. at screening (+/- SD)	21	54 (7)	31	50 (9)	0.052
Age, yr. at transplantation (+/- SD)	21	55 (7)	31	51 (9)	0.046*
Male (%)	21	14 (67)	31	24 (77)	0.391
Body mass index, kg/m^2 (+/- SD)	21	26 (3)	31	26 (3)	0.605
Smoking, never (%)	21	6 (29)	31	9 (29)	0.971
Smoking, former (%)	21	15 (71)	31	22 (71)	0.971
Cigarette packyears[a] (range)	21	17 (1 – 40)	31	18 (1 – 45)	0.718
Use of oxygen (%)	21	15 (71)	30	22 (73)	0.881
Steroid treatment (%)	21	18 (86)	31	27 (87)	1.000
Pulmonary hypertension (%)	21	4 (19)	26	8 (31)	0.505
Mean PAP, mmHg (+/- SD)	21	20 (7)	26	22 (7)	0.317
6-minute walking test, m (+/- SD)	19	330 (126)	27	291 (169)	0.408
FVC, % predicted (+/- SD)	19	56 (20)	29	47 (14)	0.070
VC, % predicted (+/- SD)	21	55 (19)	30	48 (14)	0.143
TLC, % predicted (+/- SD)	19	52 (15)	22	54 (15)	0.731
DL_{CO}, % predicted (+/- SD)	18	29 (9)	27	27 (8)	0.525

Continued	**Data available (n)**	**Single LTX (n = 21)**	**Data available (n)**	**Bilateral LTX (n = 31)**	***p*-value**
Donor age, yr. (+/- SD)	18	41 (14)	28	48 (14)	0.108
Gender mismatch (%)	18	6 (33)	28	10 (36)	0.869
CMV mismatch (%)	18	9 (50)	28	12 (43)	0.635

Table 3: Patients characteristics of 52 IPF patients at time of screening – single versus bilateral lung transplantation
Abbreviations: FVC = forced vital capacity, VC = vital capacity, TLC = total lung capacity, DLCO = diffusing capacity for carbon monoxide, PAP = pulmonary artery pressure.
[a] *Pack-year data are shown for former smokers only.*

Factor	**Death on the waiting list HR and 95% CI**	***p*-value**
Waiting for single versus bilateral LTX	0.810 (0.355 – 1.849)	0.616
High urgency status	0.965 (0.405 – 2.229)	0.935
Age at listing	0.995 (0.953 – 1.038)	0.804
Body Mass Index	0.936 (0.843 – 1.040)	0.219
Oxygen requirement (litres/min)	1.322 (1.036 – 1.688)	0.025*
Pulmonary hypertension	2.421 (1.067 – 5.491)	0.034*
Mean PAP (mmHg)	1.031 (1.001 – 1.062)	0.043*
Six-minute walking distance	0.996 (0.994 – 0.999)	0.012*

Continued **Factor**	**Death on the waiting list HR and 95% CI**	***p*-value**
FVC (% of predicted)	0.977 (0.951 – 1.003)	0.082
DL_{CO} (% of predicted)	0.963 (0.915 – 1.013)	0.143

Table 4: Factors associated with mortality on the waiting list
Abbreviations: CI = confidence interval, LTX = lung transplantation, PAP = pulmonary artery pressure, FVC = forced vital capacity, DLco = diffusing capacity for carbon monoxide.

Survival analysis with Log Rank test between patients with BLT and SLT revealed a significant survival advantage for BLT (p= 0.023, HR 3.02; 95% CI 1.11 – 8.22) (figure 3). SLT had a median survival of 5.5 years and BLT had a median survival of longer than 10 years. Univariate Cox regression analysis showed that age, gender, lung function and donor characteristics (donor age, gender mismatch and CMV mismatch) were not confounding factors in the survival analysis. There was no significant differences in 1 and 3 months survival outcomes between SLT and BLT and survival analysis without including the first 3-months period after LTX also demonstrated a significant survival benefit in BLT (p= 0.035). Furthermore, there was no significant difference in survival before and after the introduction of high urgent (HU) status on the waiting list (p= 0.836). The year of transplantation did not correlate with survival (p= 0.242).
Survival after lung transplantation did not differ between sporadic IPF patients and familial IPF patients (p= 0.763). Delays in waiting time before transplantation were not significantly related to survival after LTX. The most common cause of death after transplantation was bronchiolitis obliterans syndrome (BOS) (in 44% of the patients), but the prevalence of BOS did not differ between the groups.

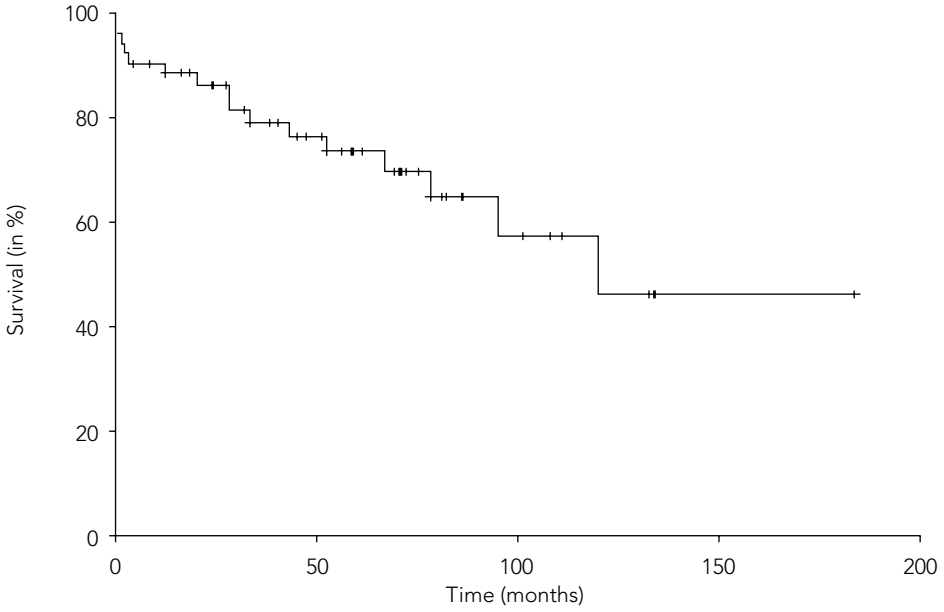


Figure 2: Survival of IPF patients after lung transplantation. This graph demonstrates a median survival is 120 months (10 years).

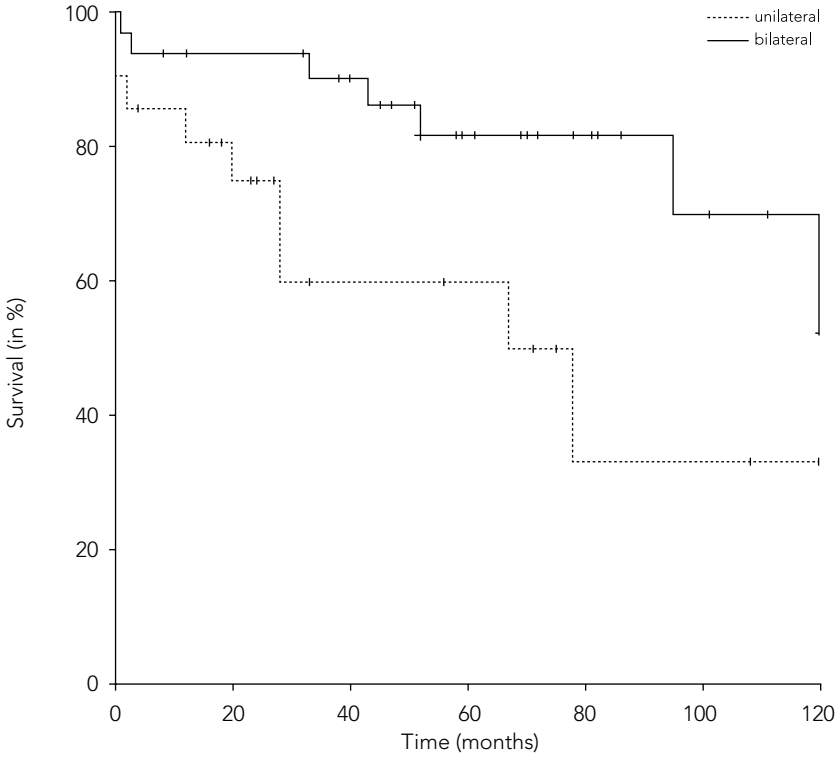


Figure 3: Survival of IPF patients after bilateral (solid line) or single lung transplantation (dotted line).
This graph shows a significant better survival of bilateral lung transplantation versus single lung transplantation (p = 0.023, Hazard ratio 3.02, 95% CI 1.11 – 8.22).

Discussion

The results of this multi-centre retrospective study demonstrated a mean survival of 10 years after LTX in end-stage IPF patients. To our knowledge, this is the longest median survival after LTX reported in IPF worldwide. Despite substantial progress in understanding the pathogenesis of IPF and trials with several potential agents, no progress has been made in improving survival for IPF patients (1, 11-13). Our study confirmed that presently LTX is still the most potent therapeutic option to improve survival in IPF.

Moreover, we demonstrated a survival benefit of BLT compared to SLT, with a median survival of BLT longer than 10 years. This is noteworthy, because our study demonstrated survival advantage of BLT in a well-characterized group of IPF patients for both short-term and long-term survival.

However, this benefit should be approached cautiously. Patients who received SLT were older, although univariate Cox regression analysis showed that age did not explain this difference in survival.

These findings contribute to the ongoing debate about the preferred transplant procedure in IPF. Independent of the underlying disease, both procedures have advantages. SLT is thought to be less invasive with shorter total ischemic time and therefore a lower likelihood of transplant complications. Another important advantage of SLT is that 2 patients can be accommodated by 1 donor and therefore provides a more efficient use of the limited donor pool (6, 14). Because of acceptable outcomes for SLT in IPF, together with the donor shortage and progressive increase of patients on the waiting list, it is generally supported that SLT had been the preferred option in IPF (15, 16).

On the other hand, BLT offers some important advantages over SLT, especially a larger pulmonary reserve in the setting of potential future allograft dysfunction. Moreover, better exercise performance and less long-term complications after LTX have been reported in BLT (6, 14).

In line with our results, some studies reported possible survival benefit of BLT and cautiously recommend BLT for patients in IPF (17, 18). Despite the absence of differences in overall survival benefit between BLT and SLT in general, *Algar and colleagues* reported a better long-term survival in BLT in IPF (19).

Although survival advantage could not be confirmed by *Weiss and colleagues,* they suggested BLT in high-risk patients, whereas high-risk patients were defined with a LAS ≥ 52 (20). Furthermore, *Force and colleagues* recommended that BLT should be considered in IPF patients younger than 57 years (21). Of note, recently reported data from the ISHLT also showed a probable long-term survival advantage for BLT in IPF (7). However, the majority of other studies that investigated survival outcomes of SLT versus BLT in IPF, did not find survival advantage for one of the procedures (19, 20, 22-24).

Survival after LTX in IPF in our study is longer than previously published survival data (4, 5, 7, 21-24). *Neurohr and colleagues* reported similar good survival after LTX in IPF patients, but only in the patients who received BLT (17). The overall differences in survival outcomes might be explained by large multi-institutional studies including centres with varied experience. Furthermore, the criteria used for selection of IPF patients are not always precisely described and contamination of study cohorts with other types of pulmonary fibrosis can be of importance because of varied prognoses and different outcomes after LTX (25, 26).

Survival after LTX is determined by improvements in timing of listing, surgical techniques, choice of procedure, lung preservation, patient characteristics, immunosuppressive regimes, therapy of infections, and episodes of acute rejections. Appropriately prioritized listing of patients for transplantation is a complex matter and therefore disease-specific guidelines for time of referral and listing were formulated (27). The ideal transplant candidate is free of medical comorbidities and has a relative good clinical condition to undergo this procedure (27, 28). Due to donor scarcity in the Netherlands, it is possible that some patient selection criteria are applied more strictly and led to the high survival after LTX. To the contrary, due to this donor scarcity and based on compliance with the donor criteria in the Netherlands it is not to be expected that donor selection was a factor contributing in the long-term survival after LTX in the Netherlands.
However, due to the same donor scarcity, waiting list mortality appeared to be high compared to other countries. Thirty percent of the IPF patients listed for LTX died while waiting for transplantation, of whom 76% died in the first 12 months after listing. A substantial part of all patients listed (31%) received a *high urgency* status, indicating that the patient's condition was indeed poor at time of LTX. Even so, regression analysis showed that none of the clinical parameters at time of screening were correlated with survival after transplantation (data not shown), and therefore the patient's poor condition did not seem to influence survival after LTX.
It is known that many centres have a bias towards BLT in IPF patients with pulmonary hypertension (PH), but remarkably we found no difference in prevalence of PH between SLT-group and BLT-group. And although PH was not related with survival after LTX, PH was associated with a significant risk of dying on the waiting list.
Furthermore, univariate regression analysis showed that also the use of supplemental oxygen and the 6MWD were predictors of mortality on the waiting list. These parameters are already known to be important independent predictors of mortality in IPF (29). Another study suggested that IPF patients listed for BLT might have an increased risk of pretransplant mortality, but our results could not support this finding (14).
Successful survival outcome might be explained by accurate follow up after LTX. Follow up is essential for detection of lung function decline due to chronic lung allograft dysfunction (e.g. BOS) or other medical problems, including infection or CMV-reactivation.

Our transplanted patients visited the transplant centers on a regular basis, unhampered by long travel distances in the Netherlands.
There are several limitations to our study. At first, retrospective analysis may be affected by multiple potential inherent biases, such as selection bias. The best study design to investigate potential differences between two procedures would be a randomized clinical trial. Secondly, our study population remains relatively small, despite the national character of the study and therefore some effects may have remained undetectable in the statistical analysis. At last, due to the national character of this study some results, concerning waiting list mortality and donor scarcity may not be able to be extrapolated to other countries.
In conclusion, we demonstrated a 10-year survival time after LTX in IPF. Therefore, LTX still remains the only treatment option to prolong life in IPF and should be considered at time of diagnosis for every IPF patient. Furthermore, our study demonstrated a significantly better survival after BLT in IPF as compared to SLT although BLT patients were significantly younger.

References

(1) Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE,Jr, Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schunemann HJ, ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788-824.

(2) King TE,Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet 2011;378:1949-1961.

(3) Chan AL, Rafii R, Louie S, Albertson TE. Therapeutic update in idiopathic pulmonary fibrosis. Clin Rev Allergy Immunol 2013;44:65-74.

(4) Thabut G, Christie JD, Ravaud P, Castier Y, Dauriat G, Jebrak G, Fournier M, Leseche G, Porcher R, Mal H. Survival after bilateral versus single-lung transplantation for idiopathic pulmonary fibrosis. Ann Intern Med 2009;151:767-774.

(5) Hosenpud JD, Bennett LE, Keck BM, Edwards EB, Novick RJ. Effect of diagnosis on survival benefit of lung transplantation for end-stage lung disease. Lancet 1998;351:24-27.

(6) Kotloff RM, Thabut G. Lung transplantation. Am J Respir Crit Care Med 2011;184:159-171.

(7) Stehlik J, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, Dobbels F, Kirk R, Rahmel AO, Hertz MI, International Society of Heart and Lung Transplantation. The registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report--2012. J Heart Lung Transplant 2012;31:1052-1064.

(8) Nathan SD, Shlobin OA, Weir N, Ahmad S, Kaldjob JM, Battle E, Sheridan MJ, du Bois RM. Long-term course and prognosis of idiopathic pulmonary fibrosis in the new millennium. Chest 2011;140:221-229.

(9) Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J 2011;37:356-363.

(10) Whelan TP, Dunitz JM, Kelly RF, Edwards LB, Herrington CS, Hertz MI, Dahlberg PS. Effect of preoperative pulmonary artery pressure on early survival after lung transplantation for idiopathic pulmonary fibrosis. J Heart Lung Transplant 2005;24:1269-1274.

(11) Richeldi L, Costabel U, Selman M, Kim DS, Hansell DM, Nicholson AG, Brown KK, Flaherty KR, Noble PW, Raghu G, Brun M, Gupta A, Juhel N, Kluglich M, du Bois RM. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med 2011;365:1079-1087.

(12) Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, King TE,Jr, Lancaster L, Sahn SA, Szwarcberg J, Valeyre D, du Bois RM, CAPACITY Study Group. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet 2011;377:1760-1769.

(13) Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, Suga M, Taguchi Y, Takahashi H, Nakata K, Sato A, Takeuchi M, Raghu G, Kudoh S, Nukiwa T, Pirfenidone Clinical Study Group in Japan. Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J 2010;35:821-829.

(14) Nathan SD, Shlobin OA, Ahmad S, Burton NA, Barnett SD, Edwards E. Comparison of wait times and mortality for idiopathic pulmonary fibrosis patients listed for single or bilateral lung transplantation. J Heart Lung Transplant 2010;29:1165-1171.

(15) Rinaldi M, Sansone F, Boffini M, El Qarra S, Solidoro P, Cavallo N, Ruffini E, Baldi S. Single versus double lung transplantation in pulmonary fibrosis: a debated topic. Transplant Proc 2008;40:2010-2012.

(16) Meyer DM, Edwards LB, Torres F, Jessen ME, Novick RJ. Impact of recipient age and procedure type on survival after lung transplantation for pulmonary fibrosis. Ann Thorac Surg 2005;79:950-7; discussion 957-8.

(17) Neurohr C, Huppmann P, Thum D, Leuschner W, von Wulffen W, Meis T, Leuchte H, Baumgartner R, Zimmermann G, Hatz R, Czerner S, Frey L, Ueberfuhr P, Bittmann I, Behr J, Munich Lung Transplant Group. Potential functional and survival benefit of double over single lung transplantation for selected patients with idiopathic pulmonary fibrosis. Transpl Int 2010;23:887-896.

(18) Mason DP, Brizzio ME, Alster JM, McNeill AM, Murthy SC, Budev MM, Mehta AC, Minai OA, Pettersson GB, Blackstone EH. Lung transplantation for idiopathic pulmonary fibrosis. Ann Thorac Surg 2007;84:1121-1128.

(19) Algar FJ, Espinosa D, Moreno P, Illana J, Cerezo F, Alvarez A, Baamonde C, Redel J, Vaquero JM, Santos F, Salvatierra A. Results of lung transplantation in idiopathic pulmonary fibrosis patients. Transplant Proc 2010; 42:3211-3213.

(20) Weiss ES, Allen JG, Merlo CA, Conte JV, Shah AS. Survival after single versus bilateral lung transplantation for high-risk patients with pulmonary fibrosis. Ann Thorac Surg 2009;88:1616-25; discussion 1625-6.

(21) Force SD, Kilgo P, Neujahr DC, Pelaez A, Pickens A, Fernandez FG, Miller DL, Lawrence C. Bilateral lung transplantation offers better long-term survival, compared with single-lung transplantation, for younger patients with idiopathic pulmonary fibrosis. Ann Thorac Surg 2011;91:244-249.

(22) Meyers BF, Lynch JP, Trulock EP, Guthrie T, Cooper JD, Patterson GA. Single versus bilateral lung transplantation for idiopathic pulmonary fibrosis: a ten-year institutional experience. J Thorac Cardiovasc Surg 2000;120:99-107.

(23) Thabut G, Mal H, Castier Y, Groussard O, Brugiere O, Marrash-Chahla R, Leseche G, Fournier M. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. J Thorac Cardiovasc Surg 2003;126:469-475.

(24) Keating D, Levvey B, Kotsimbos T, Whitford H, Westall G, Williams T, Snell G. Lung transplantation in pulmonary fibrosis: challenging early outcomes counterbalanced by surprisingly good outcomes beyond 15 years. Transplant Proc 2009;41:289-291.

(25) Navaratnam V, Ali N, Smith CJ, McKeever T, Fogarty A, Hubbard RB. Does the presence of connective tissue disease modify survival in patients with pulmonary fibrosis? Respir Med 2011;105:1925-1930.

(26) Monaghan H, Wells AU, Colby TV, du Bois RM, Hansell DM, Nicholson AG. Prognostic implications of histologic patterns in multiple surgical lung biopsies from patients with idiopathic interstitial pneumonias. Chest 2004;125:522-526.

(27) Orens JB, Estenne M, Arcasoy S, Conte JV, Corris P, Egan JJ, Egan T, Keshavjee S, Knoop C, Kotloff R, Martinez FJ, Nathan S, Palmer S, Patterson A, Singer L, Snell G, Studer S, Vachiery JL, Glanville AR, Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. International guidelines for the selection of lung transplant candidates: 2006 update--a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2006;25:745-755.

(28) Kreider M, Hadjiliadis D, Kotloff RM. Candidate selection, timing of listing, and choice of procedure for lung transplantation. Clin Chest Med 2011;32:199-211.

(29) du Bois RM, Weycker D, Albera C, Bradford WZ, Costabel U, Kartashov A, Lancaster L, Noble PW, Raghu G, Sahn SA, Szwarcberg J, Thomeer M, Valeyre D, King TE,Jr. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2011;184:459-466.

CHAPTER FOUR

IgA in serum: an old acquaintance as a new prognostic biomarker in idiopathic pulmonary fibrosis

Liesbeth ten Klooster, Coline H M van Moorsel, Johanna M Kwakkel-van Erp, Heleen van Velzen-Blad, Jan C. Grutters

Clin Exp Immunol. 2015 Aug; 181(2): 357-61

SUMMARY

IgA is an important immunoglobulin in mucosal immunity and protects the lungs against invading pathogens. The production of IgA is regulated by transforming growth factor-ß (TGF-ß), a versatile cytokine and key player in the pathogenesis of pulmonary fibrosis. TGF-ß is upregulated in patients with idiopathic pulmonary fibrosis (IPF), but difficult to use as biomarker. The aim of this study was to evaluate the prognostic value of IgA in serum in patients with IPF. We examined IgA levels at time of diagnosis in 86 patients diagnosed with IPF. Mean serum IgA level in IPF is 3.22 g/L and regression analyses showed a significant association with mortality (HR 1.445, p= 0.002). A significant worse survival was found in patients with IgA serum levels > 2.85 g/L compared to patients with lower IgA serum levels (p= 0.003). These findings were confirmed in a duplication cohort. In conclusion, the level of IgA in blood is a promising prognostic marker in IPF and can be easily implemented in hospital setting. Future studies are warranted to investigate if repeated measurements of serum IgA can further improve the performance of serum IgA as a prognostic marker.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive parenchymal lung disease with a poor prognosis of 3 – 4 years after diagnosis. The pathogenesis of IPF remains unclear, although several environmental factors may contribute, for example cigarette smoking and chronic viral infections (1). To protect the lungs against pathogens invading the mucosa, IgA is present in the mucous secretions of the lungs (2). Human IgA can be found in various mucous secretions and produced locally by plasma cells. IgA also circulates in blood and although the overall function in the circulation is not well understood, it is thought that its main function is to clear the circulation of immune complexes by phagocytosis (3). The majority of IgA is produced locally by plasma cells. In vivo studies in transforming growth factor-ß (TGF-ß) knock-out mice demonstrated that TGF-ß plays an important role in IgA production (4-6). TGF-ß is a multifunctional cytokine that is involved in multiple signalling processes and has profound regulatory effects on many developmental and physiological processes, such as embryogenesis, cell growth, immune functions, and wound healing (7,8). In the lungs, secretion and activation of latent TGF-ß is caused by abnormally activated alveolar epithelial cells (AECs) in IPF. Activated TGF-ß is responsible for the differentiation of fibroblasts to myofibroblasts and the formation of the typical fibroblast foci in IPF[1]. In the different mechanisms proposed to be involved in the pathogenesis of IPF, all identified TGF-ß as a key player in the development of IPF (1,9-11). Activated TGF-ß is upregulated by AECs in IPF (12-14) and we hypothesized that increase in activated TGF-ß is reflected by an increase in IgA in blood. Therefore IgA serum could be a practical and well-needed biomarker for the prognosis of IPF, since IgA serum measurements are easy accessible. To study the potential role of serum IgA as a prognostic biomarker in IPF we examined a retrospective cohort of IPF patients and reproduced our findings in a prospective duplication cohort.

Materials and Methods

Study patients

The initial study cohort consisted of 86 patients with IPF diagnosed before 2007. Diagnoses were revised and patients were included when the criteria set by the ATS/ERS/JRS/ALAT were met (10). We recruited a prospective duplication cohort of 83 IPF patients all diagnosed after 2007 till July 2013.

Time of diagnosis was defined as the date of first multidisciplinary meeting between pulmonologist, radiologist and pathologist.
Patient demographics, smoking status and lung function were determined at diagnosis. One patient was excluded because of an IgA deficiency and hypogammaglobulinemia due to a common variable immune deficiency (CVID). The research was conducted using appropriate ethical guidelines and approved by the Medical-ethical Committee VCMO (*Verenigde Commissies Mensgebonden Onderzoek*) of St. Antonius Hospital in Nieuwegein. All participants gave written informed consent.

Evaluation of Serum immunoglobulin IgA, IgG and IgM

Serum samples were collected at time of diagnosis. The majority of the serum samples were collected routinely for monitoring purposes and in a few cases immunoglobulin measurements were performed on stored serum samples. Immunoglobulin measurements were performed by the Immage ® 800 Beckman Turbid meter (Woerden, The Netherlands).

Statistical analysis

Differences in demographic and clinical characteristics between both cohorts were compared with the use of the independent samples T-test for continuous variables and the χ^2 test or Fisher exact test for categorical variables, as appropriate. There are no adult age-specific and gender-specific values for serum IgA concentrations. Exploratory analysis demonstrated a linear relationship between increasing IgA level and risk of mortality. The Cox proportional hazard regression model was used for the primary analysis to evaluate the associations of serum immunoglobulins with mortality. Survival estimates were calculated with the Kaplan-Meier method. For survival calculation the patients were censored at time of transplantation or when patients were still alive at the end of the study. The optimal cut-off point of IgA between the two survival groups was calculated with a ROC analysis. Comparison of survival in different groups was done with the log-rank test. Cox regression analysis was used to test the effect on the variables on overall survival. All tests were evaluated at the 0.05 alpha level and *p*-values were 2-sided. Analyses were performed using Statistical Package for the Social sciences version 21.0 (SPSS Inc., New York, VS).

Results

The initial cohort included 86 IPF patients and during a follow up of 48 months 69 patients died and 13 patients underwent lung transplantation. The duplication cohort consisted of 83 patients and during a follow up of 48 months 43 patients died and 2 patients underwent lung transplantation. Demographic and clinical characteristics of both cohorts are shown in table 1.

Subgroup analyses demonstrated no significant differences in IgA levels between patients who were current or former smokers and the non-smokers.

The mean serum IgA level was 3.22 g/L (range, 0.9 – 6.8 g/L). No statistically significant associations were found between serum IgA levels and baseline demographic and clinical variables, except for serum IgA levels and DLCO% of predicted, which showed a moderate Spearman's correlation (r_s= -0.5, $p< 0.001$). The immunoglobulin levels in the duplication cohort are not significantly different compared to the initial cohort, see figure 1 and table 2.

	Initial cohort (n = 86)	Duplication cohort (n = 83)	Significance
Gender (%)			
male	67 (78)	73 (88)	NS
female	19 (22)	10 (12)	NS
Age (SD)	59.4 (12.9)	62.9 (8.7)	NS
Smoking status (%)			
yes	9 (10)	3 (4)	NS
never	24 (28)	20 (24)	NS
former	42 (49)	58 (70)	NS
unknown	11 (13)	2 (2)	NS
Pulmonary function tests (SD)			
DLco% of predicted	45.1 (16.3)	46.6 (15.1)	NS
FVC% of predicted	72.6 (23.5)	82.8 (24.3)	NS
TLC% of predicted	66.1 (18.9)	68.4 (14.4)	NS

Table 1: Baseline characteristics of the initial and duplication cohort
DLco = diffusing capacity for carbon monoxide, FVC = forced vital capacity, TLC = total vital capacity, SD = standard deviation, NS = not significant.
Statistical significant difference between both groups at the 0.05 alpha level

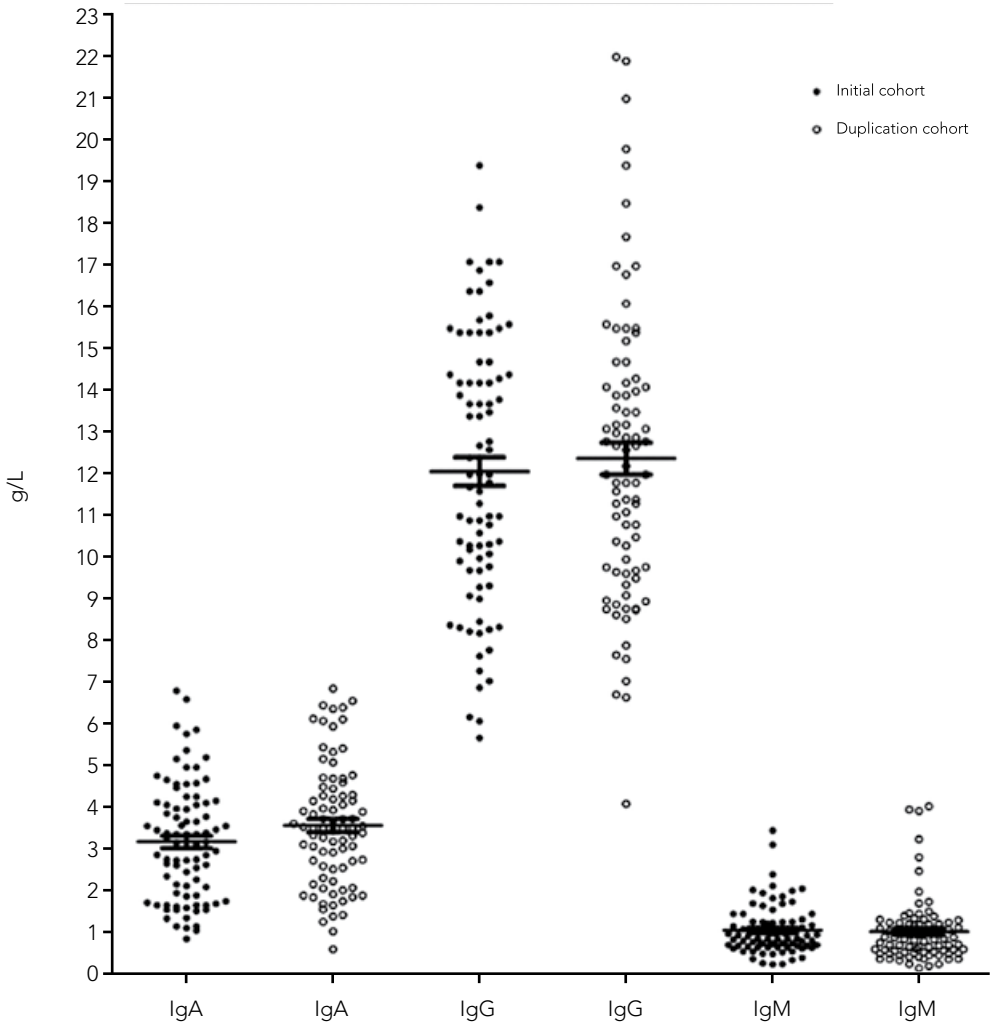


Figure 1 shows a comparison of immunoglobulin levels of both cohorts. There are no significant differences between the cohorts (IgA: p = 0.08, IgG: p = 0.5, IgM: p = 0.8).

	N	Mean g/L (SD)	Range
Cohort 1			
Ig A	86	3.22 (1.38)	0.9 – 6.8
Ig G	85	12.07 (3.16)	5.7 – 19.4
Ig M	85	1.10 (0.58)	0.3 – 3.5
Cohort 2			
Ig A	83	3.61(1.45)	0.7 – 6.9
Ig G	83	12.42 (3.54)	4.1 – 22.0
Ig M	82	1.07 (0.79)	0.2 – 4.1

Table 2: Mean serum immunoglobulin levels of both IPF cohorts
SD = standard deviation; Ig = immunoglobulin

Serum IgA levels associated with mortality

Univariate regression analyses revealed that serum IgA, but not IgG and IgM serum levels, was associated with early mortality (Hazard ratio 1.445, CI 95% 1.14 – 1.83, p= 0.002; table 3). Regression analysis of both cohorts together showed a significant correlation between IgA in serum and long term survival (HR 1.424, CI 95% 1.216 – 1.667, p< 0.001). Multivariate regression analysis correcting for pulmonary function tests showed that serum IgA levels remain predictive of survival (HR 1.576, CI 95% 1.224 – 2.029, p< 0.001).

The median overall survival of the initial cohort is 4.4 years and the median survival of the duplication cohort is 3.4 years. None of the patients were lost to follow up. After dividing the patients in groups according to the cause of death mean IgA serum levels were calculated: progressive lung fibrosis mean IgA 2.71 g/L; infectious complications mean IgA 3.89 g/L; acute exacerbation (AE) of IPF 4.05 g/L. Highest levels were found in patients who died after AE or infections and the lowest levels in progressive lung fibrosis (infectious complications vs progressive lung fibrosis p= 0.045; AE vs progressive lung fibrosis p= 0.038; infectious complication vs AE = NS). The cut-off value defining whether a patient is at risk of dying within the first years after diagnosis with highest specificity as well as sensitivity was found at approximately 2.85 g/L (ROC AUC= 0.71, sensitivity 0.79 and specificity 0.43). This cut-off value was used for a Kaplan Meier analyses, which were shown in figure 2a – c.

Factor	Survival after diagnosis (HR and 95% CI)	*p*-value
Initial cohort		
Ig A	1.445 (1.142 – 1.829)	0.002*
Ig G	1.087 (0.976 – 1.210)	0.129
Ig M	1.161 (0.659 – 2.043)	0.606
Duplication cohort		
Ig A	1.360 (1.099 – 1.683)	0.005*
Ig G	1.066 (0.988 – 1.149)	0.098
Ig M	0.891 (0.601 – 1.320)	0.564

Table 3: Correlation between immunoglobulin in blood serum and survival after diagnosis in IPF
HR = hazard ratio; CI = confidence interval; Ig = immunoglobulin

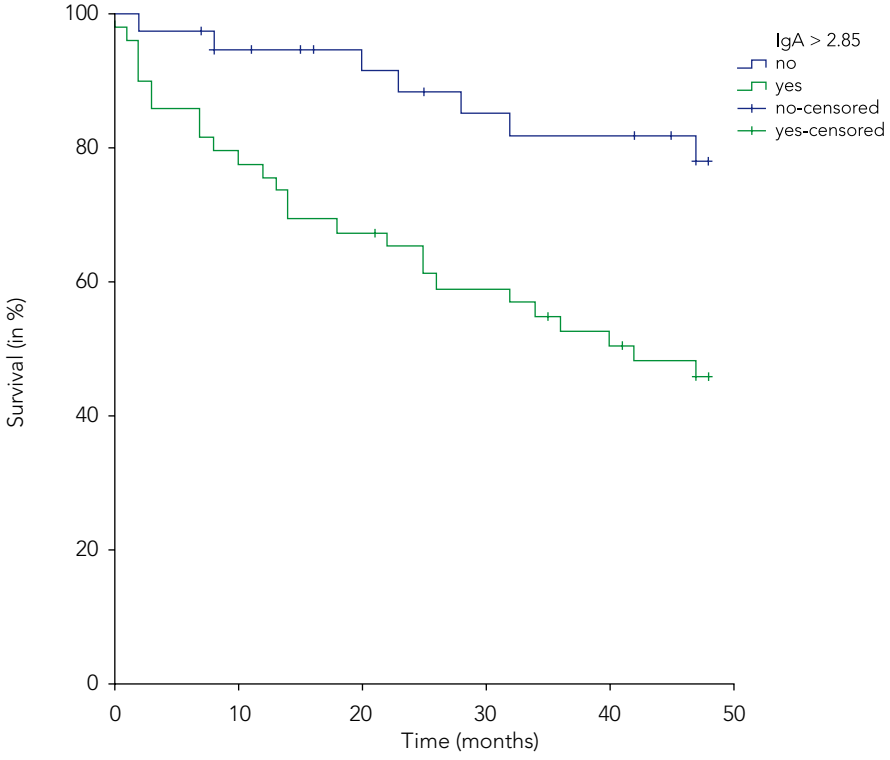


Figure 2a demonstrates survival of 86 IPF patients (initial cohort). Significant survival difference between patients with IgA > 2.85 g/L compared to patients with IgA < 2.85 g/L at time of diagnosis (p= 0.003).

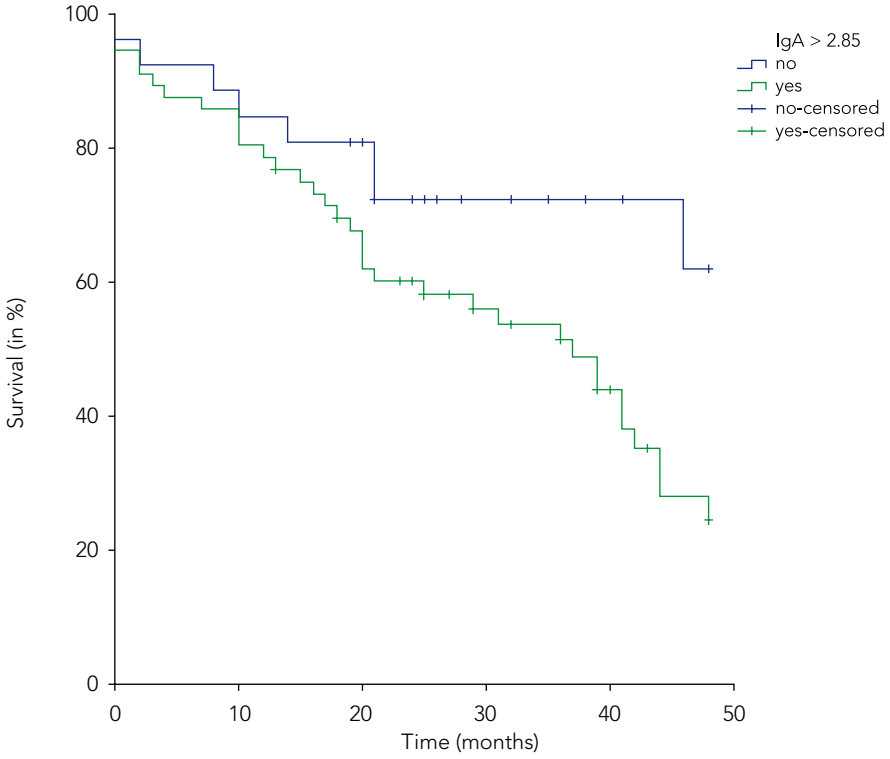


Figure 2b shows the survival of 83 IPF patients (duplication cohort) and also demonstrates a significant difference in survival between patients with IgA > 2.85 g/L compared to patients with IgA < 2.85 g/L at time of diagnosis (p= 0.03).

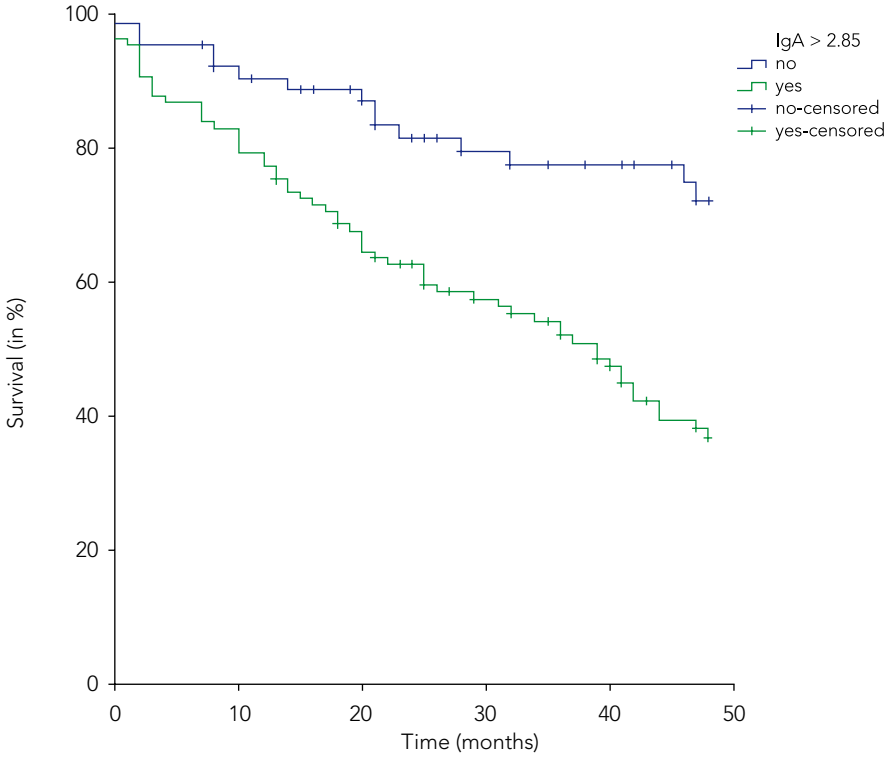


Figure 2c shows difference in survival of both cohorts together (n = 168; p <0.001).

Discussion

The results of this study indicate that the IgA level in serum is a promising prognostic biomarker for IPF, in which high IgA levels indicate a worse prognosis. The search for a biomarker to diagnose and follow up fibrotic diseases continues, and although many possible prognostic biomarkers have been studied in IPF, to our knowledge, this is the first study that investigated serum IgA as a potential biomarker for IPF (15).

Normal IgA levels in serum range from 0.7 – 4 g/L. Most of the serum IgA levels in both of our cohorts varied between normal and slightly elevated levels. Moreover, the established cut off value of 2.85 g/L lies within the normal range.

Former studies demonstrated that both bacterial and viral infections cause acute exacerbations in IPF and a higher risk of mortality (16,17). Furthermore, increased numbers of immunoglobulin-secreting cells in bronchiolar lavage fluid and blood of patients with IPF have been reported (18).

However, it is not clear if the increase of immunoglobulin-secreting cells is caused by the pathogenic process or may be secondary to subclinical triggers such as infection, smoking or air pollution. Recent study demonstrated an increased bacterial load in IPF patients at the time of diagnosis and that this was associated with rapidly progressive IPF and increased risk of mortality (19). Analysis of IgA serum levels in different causes of death in our cohort demonstrated higher IgA serum levels in patients who died after an acute exacerbation of IPF or infectious complications, which suggest that levels of IgA are associated with an external cause of death. Further investigation on this topic is warranted.

Production of IgA is regulated by multiple molecules, including TGF-ß (20,21). TGF-ß is required for the development of plasma cells secreting all secondary isotypes. One of the best characterized effects of TGF-ß is its ability to stimulate isotype switching to IgA (22).The role of TGF-ß1 in mucosal and systemic IgA production is further studied in TGF-ß1 knock-out mice and it has been demonstrated that these mice had significantly lower levels of IgA in both blood and mucosal secretions (23). TGF-ß has been characterized as a key profibrotic molecule and involved in tissue repair processes. TGF-ß is upregulated during wound healing and it is thought that persistent activation of TGF-ß signalling causes fibrotic diseases, like lung, liver and kidney fibrosis. In line with this observation, TGF-ß levels were found to be increased in the lungs of IPF patients (13,24). Due to the relation between TGF-ß and IgA, this would - in theory - mean that IgA could be a marker for fibrosis in general. Studies in other organ fibrosis are required to investigate this.

TGF-ß has a dual role in immune homeostasis, it inhibits Th1 cells, Th2 cells and cytotoxic lymphocytes (CTL), whereas it induces differentiation of regulatory T cells and Th17 cells. Furthermore, together with IL-10 and IL-21, TGF-ß induces CD40-activated B cells to switch into IgA^{+}B cells (20,25).

At first glance, determination of TGF-ß in blood would be a logical step in diagnosing and following disease progression in fibrotic diseases like IPF. However, there is controversy about measuring functional TGF-ß and the determination of TGF-ß-concentrations in blood. Different ranges for the concentrations of TGF-ß in blood have been described and several different recommendations for preanalytical sample handling can be found (26). It has been suggested that serum measurements are not useful, due to the abundance of TGF-ß in platelets and therefore should be corrected by simultaneous measurement of markers of platelet degranulation (27).

However, others stated that direct measurements could provide a reliable estimate of active and total TGF-ß in plasma (28). In contrast to many other promising biomarkers, IgA assays are part of everyday routine in most clinical laboratories worldwide, with well-known standard values and the analysis is run on a daily basis.

Prognostic biomarkers for IPF are well needed to identify the individual clinical course and to predict prognosis. IPF patients have a poor prognosis and different patterns of survival are identified (1). Identification of these different survival patterns is important, because the only medical option to prolong life in these patients is lung transplantation and early referral of suitable patients can be potential life-saving (29). Assessment for lung transplantation is advisable when patients have a 2 to 3-year predicted survival or less (30). Lung function and lung function decline seems to be a good marker for early mortality, however biomarker like IgA might better capture activity of disease (31). Future studies might show if repeated measurements of serum IgA level provide a more specific marker of mortality risk and disease severity for long-term follow-up than pulmonary function tests alone.

The strengths of the current study are: its well-characterized patient population; classification of patients according to the most recent consensus criteria; the length and completeness of the follow-up; and confirmation of our findings in a duplication cohort.

However, a limitation should also be considered when interpreting our results. Despite of no major significant differences in baseline clinical characteristics, the duplication cohort was derived from a different time span and in the last few years awareness of IPF has increased.

In conclusion, we demonstrated that serum IgA level at the time of diagnosis is a predictor of survival in IPF. Increased serum levels of IgA may identify more 'fibrotic active' disease that increases the risk of death in the following year and was not predicted by other baseline, non-invasive clinical predictors, such as pulmonary function tests. These results and along with the broad availability to measure serum IgA levels in many hospitals, makes it easy to use IgA levels for prognostic purposes in IPF. Moreover, IgA level in serum might be an interesting parameter for determination timing of referral for lung transplantation.

References

1. King TE,Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet 2011; 378:1949-61.

2. Macpherson AJ, Geuking MB, McCoy KD. Immune responses that adapt the intestinal mucosa to commensal intestinal bacteria. Immunology 2005; 115:153-62.

3. Woof JM, Russell MW. Structure and function relationships in IgA. Mucosal Immunol 2011; 4:590-7.

4. McIntyre TM, Klinman DR, Rothman P, Lugo M, Dasch JR, Mond JJ, Snapper CM. Transforming growth factor beta 1 selectivity stimulates immunoglobulin G2b secretion by lipopolysaccharide-activated murine B cells. J Exp Med 1993; 177:1031-7.

5. Kim PH, Kagnoff MF. Transforming growth factor-beta 1 is a costimulator for IgA production. J Immunol 1990; 144:3411-6.

6. Brandtzaeg P, Johansen FE. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. Immunol Rev 2005; 206:32-63.

7. Yaswen L, Kulkarni AB, Fredrickson T et al. . Autoimmune manifestations in the transforming growth factor-beta 1 knockout mouse. Blood 1996; 87:1439-45.

8. Massague J, Blain SW, Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. Cell 2000; 103:295-309.

9. Wolters PJ, Collard HR, Jones KD. Pathogenesis of Idiopathic Pulmonary Fibrosis. Annu Rev Pathol 2013.

10. Raghu G, Collard HR, Egan JJ et al. . An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011; 183:788-824.

11. Sime PJ, O'Reilly KM. Fibrosis of the lung and other tissues: new concepts in pathogenesis and treatment. Clin Immunol 2001; 99:308-19.

12. Sakai N, Tager AM. Fibrosis of two: Epithelial cell-fibroblast interactions in pulmonary fibrosis. Biochim Biophys Acta 2013; 1832:911-21.

13. Khalil N, O'Connor RN, Unruh HW et al. . Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol 1991; 5:155-62.

14. Lepparanta O, Sens C, Salmenkivi K, Kinnula VL, Keski-Oja J, Myllarniemi M, Koli K. Regulation of TGF-beta storage and activation in the human idiopathic pulmonary fibrosis lung. Cell Tissue Res 2012; 348:491-503.

15. Prasse A, Muller-Quernheim J. Non-invasive biomarkers in pulmonary fibrosis. Respirology 2009; 14:788-95.

16. Wootton SC, Kim DS, Kondoh Y et al. . Viral infection in acute exacerbation of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2011; 183:1698-702.

17. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J 2011; 37:356-63.

18. Izumi T, Fujimura N, Oshima S. T-lymphocyte alveolitis and B-lymphocyte alveolitis: a new classification of interstitial pneumonitis based on broncho-alveolar lavage findings. Asian Pac J Allergy Immunol 1983; 1:131-4.

19. Molyneaux PL, Cox MJ, Willis-Owen SA, Mallia P, Russell KE, Russell AM, Murphy E, Johnston SL, Schwartz DA, Wells AU, Cookson WO, Maher TM, Moffatt MF. The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2014;190:906-13

20. Banchereau J, Pascual V, O'Garra A. From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. Nat Immunol 2012; 13:925-31.

21. Cerutti A, Cols M, Gentile M et al. . Regulation of mucosal IgA responses: lessons from primary immunodeficiencies. Ann N Y Acad Sci 2011; 1238:132-44.

22 Stavnezer J, Kang J. The surprising discovery that TGF beta specifically induces the IgA class switch. J Immunol 2009; 1:5-7

23. van Ginkel FW, Wahl SM, Kearney JF et al. . Partial IgA-deficiency with increased Th2-type cytokines in TGF-beta 1 knockout mice. J Immunol 1999; 163:1951-7.

24. Khalil N, O'Connor RN, Flanders KC, Unruh H. TGF-beta 1, but not TGF-beta 2 or TGF-beta 3, is differentially present in epithelial cells of advanced pulmonary fibrosis: an immunohistochemical study. Am J Respir Cell Mol Biol 1996; 14:131-8.

25. Sonoda E, Matsumoto R, Hitoshi Y et al. . Transforming growth factor beta induces IgA production and acts additively with interleukin 5 for IgA production. J. Exp. Med. 1989. 170: 1415-1420. J Immunol 2009; 182:14-9.

26. Kropf J, Schurek JO, Wollner A, Gressner AM. Immunological measurement of transforming growth factor-beta 1 (TGF-beta1) in blood; assay development and comparison. Clin Chem 1997; 43:1965-74.

27. Wakefield LM, Letterio JJ, Chen T et al. . Transforming growth factor-beta1 circulates in normal human plasma and is unchanged in advanced metastatic breast cancer. Clin Cancer Res 1995; 1:129-36.

28. Grainger DJ, Mosedale DE, Metcalfe JC, Weissberg PL, Kemp PR. Active and acid-activatable TGF-beta in human sera, platelets and plasma. Clin Chim Acta 1995; 235:11-31.

29. Thabut G, Mal H, Castier Y et al. . Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. J Thorac Cardiovasc Surg 2003; 126:469-75.

30. Orens JB, Estenne M, Arcasoy S et al. . International guidelines for the selection of lung transplant candidates: 2006 update--a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2006; 25:745-55.

31. Schmidt SL, Tayob N, Han MK, Zappala C, Kervitsky D, Murray S, Wells AU, Brown KK, Martinez FJ, Flaherty KR. Predicting pulmonary fibrosis disease course from past trends in pulmonary function. Chest 2014; 145: 579-85

-

CHAPTER FIVE

Poor survival in TERT mutation carriers with pulmonary fibrosis

L. ten Klooster, J.J. van der Vis, R. Snetselaar, J.H. van Kwakkel-van Erp, J.C. Grutters, C.H.M. van Moorsel

ABSTRACT

Introduction

Mutations in telomere maintenance genes are a well-known cause of familial and sporadic forms of idiopathic pulmonary fibrosis (IPF). However, little is known about the clinical differences between *TERT* mutations carriers and sporadic IPF patients. We assessed clinical characteristics, survival, prognostic biomarkers and cell-profile in bronchoalveolar lavage (BAL).

Methods

The characteristics of 26 unrelated probands with a *TERT* mutation were compared to a cohort of 113 sporadic IPF patients. Patient's demographics, smoking status, and pulmonary function tests were collected at baseline. Blood tests and bronchoalveolar lavage were determined at diagnosis.

Results

Median survival of *TERT* probands was poor with 31 months (2.6 years). Compared to sporadic IPF patients, significantly worse survival in the first 1.5 year after the diagnosis is set (Hazard ratio 2.43, 95% CI 0.208 – 0.877, p= 0.016). Macrocytosis was present in 10 *TERT* probands and mean corpuscular value (MCV) was significantly higher compared to sporadic IPF patients. Furthermore, serum IgA levels in *TERT* probands were significantly elevated compared to sporadic IPF patients (3.31 g/L versus 4.84 g/L, p< 0.001) and BAL analysis demonstrated a significant lower percentage of lymphocytes in the BAL of *TERT* probands compared to sporadic IPF patients.

Conclusion

This study demonstrated a significant difference in survival between *TERT* probands and sporadic IPF patients. Furthermore, we hypothesized that high serum levels of IgA and low amount of CTL in BAL fluid in *TERT* mutation carriers is caused by activated TGF-β and that poor survival in *TERT* mutation carriers is probably due to severe fibrotic active lung disease.

Introduction

Idiopathic pulmonary fibrosis (IPF) is the most common and severe form of all idiopathic interstitial pneumonias (IIP) with a poor survival of approximately 3 years after diagnosis. Patients characteristically present with coughing and progressive dyspnea with a typical radiological pattern of usual interstitial pneumonia (UIP) seen on high resolution CT scan (1, 2). Several clinical risk factors are known to be linked to IPF such as older age, male sex, cigarette smoking and a positive family history for pulmonary fibrosis (3).

IPF is familial in up to 20% of cases (3, 4), and major progress has been made in the discovery of genetic factors that contribute to the disease. Nowadays, rare mutations in ten different genes have been identified, including four genes involved in surfactant homeostasis and six genes involved in telomere maintenance (5, 6). Telomeres shorten with each cell division and finally induce a DNA-damage response that leads to apoptosis of the cell or cell-cycle arrest. In other words, the length of telomeres limits the replicative capacity of tissues and therefore has been implicated in age-related diseases like IPF (7). Former studies demonstrated that not only patients with familial fibrosis, but also sporadic IPF patients have significantly shorter telomeres compared to age-related healthy controls (7-9). Telomerase, encoded by the telomerase reverse transcriptase (*TERT*) gene, is a specialized enzyme that adds telomere repeats *(TTAGGG)* to the end of chromosomes.

Mutations in *TERT* underlie the inheritance in 8-15% of familial interstitial pneumonia (FIP) (7, 8). In most populations, TERT mutations are the most frequent cause of FIP, although Dutch studies showed that mutations in surfactant genes are found in up to 25% of end-stage FIP patients (5, 10). FIP is inherited as an autosomal dominant trait with reduced penetrance. Heterozygous *TERT* mutations lead to haploinsufficiency that results in an overall minor decrease in telomerase activity. More importantly, this decrease in telomerase activity seems to cause a significant reduction of telomere length over generations, a phenomenon called genetic anticipation. It is thought that not only the presence of the mutation confers the risk of disease, but that genetic anticipation mainly causes critically short telomere length and finally leads to disease (7).

Premature aging syndromes associated with mutated telomere maintenance genes can develop disease in different types of tissue, for example bone marrow, liver and lungs. *TERT* mutation carriers have been characterised with significantly

lower red blood cells (RBC) and platelet counts (without overt thrombocytopenia) and significantly higher mean corpuscular volume (MCV) than noncarrying family members (11). Clinical reviews to differentiate between the pulmonary fibrosis in *TERT* mutation carriers and sporadic IPF patients showed minor clinical differences, however only limited data on survival and prognostic disease biomarkers in mutation carriers is available (9, 12, 13). A potential prognostic disease biomarker is IgA in serum. Recently, our study group showed that high levels of IgA correlated with worse survival in both an original and a duplication cohort of sporadic IPF patients, but data of IgA levels in *TERT* mutation carriers is lacking (14).
In this study we described the characteristics of 26 unrelated probands with a *TERT* mutation and their survival in comparison with sporadic IPF patients. Furthermore, we examined the biomarker status of IgA in serum and cell-profile in bronchoalveolar lavage (BAL) in *TERT* mutation carriers.

Methods

Characteristics of 26 unrelated probands with a *TERT* mutation were compared to a cohort of sporadic IPF patients. Patients in the IPF cohort were included when the criteria for IPF set by the ATS/ERS/JRS/ALAT were met (2). Patients with IIP report having one or more first degree family members with the disease (10, 15) were referred to as familial interstitial pneumonia (FIP). Patient's demographics, smoking status, pulmonary functions tests, blood tests and bronchoalveolar lavage were determined at diagnosis. The Ethical Committee of St. Antonius Hospital approved the study and all subjects gave written informed consent.

TERT mutation analysis

Genomic DNA was extracted from peripheral white blood cells using a magnetic beads-based method (chemagic DNA blood 10k kit; Perkin Elmer Inc.). Mutations in *TERT* were detected by targeted sequencing of coding regions. Furthermore, the sporadic IPF cohort and 100 self-reported healthy hospital employees of Dutch origin were screened for the revealed mutations using high resolution melting analysis (ABI Fast 7500RT; Applied Biosystems, Foster City, CA). Sequence primers are listed in table 1 and HRM primers are listed in table 2 of the supplementary data.

To predict the effect of amino acid substitution on protein function and structure Sorting Intolerant From T (SIFT) and PolyPhen-2 were used with TERT protein identifier NP_937983.2. SIFT score was calculated with the Blink algorithm (http://sift.jcvi.org/). SIFT predicts deleterious, not tolerated effect if p<=0.05. PolyPhen-2 predictions were calculated from http://genetics.bwh.harvard.edu/pph2/ and choosing the humvar prediction in the Results section. PolyPhen predicts a probably damaging effect if the probabilistic score is greater than 0.909, possibly damaging effect if 0.447<pp2_hdiv<=0.909 and benign if pp2_hdiv<=0.446.

Quantitative Polymerase Chain Reaction Telomere Length

Telomere length was measured using the monochrome multiplex, quantitative polymerase chain reaction (PCR) method previously described (16). All quantitative PCR telomere length measurements were performed in triplicate. Along each PCR run, four independent samples were measured for quality control and an average interassay coefficient of variation of < 10% was found and an average intra-assay variation of < 5% was found. To correct for age the difference between observed T/S ratio and the expected T/S ratio was calculated. Expected T/S ratios were based on linear regression of healthy controls T/S in Snetselaar et al, 2015 (17).

Evaluation of Bronchoalveolar Lavage and Immunophenotyping

Bronchoalveolar lavage was performed according to previously described methods (18) in patients who required lavage for diagnostic purposes. All samples were stored at -80 °C.

Evaluation of Serum immunoglobulin IgA, IgG and IgM

Serum samples were collected at time of diagnosis. The majority of the serum samples were collected routinely for monitoring purposes and in a few cases immunoglobulin measurements were performed on stored serum samples. Immunoglobulin measurements were performed by the Immage ® 800 Beckman Turbid meter (Woerden, The Netherlands).

Statistical analysis

Univariate group comparisons of continuous and categorical variables were performed using two-sample *t* test and χ^2 tests or Fisher Exact test when appropriate. Survival estimates were calculated with the Kaplan-Meier method. For survival calculation the patients were censored when they were still alive at the end of the study or underwent lung transplantation (LTX). Comparison of survival in different groups was done with the log-rank test. Cox regression analysis was used to test the effect of the variables on overall survival in *TERT* mutation carriers and sporadic IPF patients. Analyses were performed using Statistical Package for the Social Sciences version 22.0 (SPSS Inc., New York, VS). All tests were evaluated at the 0.05 alpha level and *p*-values were 2-sided.

Results

TERT mutations

The cohort of 26 unrelated probands with a *TERT* mutation consisted of 21 patients with familial disease and five patients with sporadic disease. In case of familial disease, 10 probands were the first generation with a clinical phenotype consistent with telomere syndrome, while 11 were part of the second affected generation (table 1). There was no significant difference in age at diagnosis and T/S ratio between the first and second affected generation. Three *TERT* mutations were detected in more than one proband (table 2). The R901W mutation in FPF4 was also identified in spIIP3, *TERT* haplotype analysis of family members revealed that the R901W mutation was of independent origin in both patients (data not shown). *TERT* mutation C528W and mutation R669W were present in multiple probands. The independent origin of both mutations could not be confirmed. Of the 22 different *TERT* mutations, one mutation caused an inframe deletion, one gained a stop codon and 20 mutations caused a nonsynonymous amino acid substitutions. All amino acid substitutions were predicted to be possibly deleterious by SIFT or polyphen-2, of which 15 were supported by both prediction programs. Mutation V96L, R671W, A716T, A716V and R901W mutations were reported previously in patients with telomere related disease (9, 19-22).

None of the mutations were present in the Dutch healthy control subjects. All mutations except R669W were absent from whole genome databases comprising over 30,000 individuals. R669W is registered in the Exac database with an extremely low frequency of 6*10-5. An overview of mutations and predictions is given in table 2.

Sample ID	Generation	Diagnosis	Age diagnosis	Sex	Survival time of death (mo)	T/S observed	T/S observed-expected
FPF53	II	UIP/IPF	45	female	transplanted 12 mo after dx	0.75	-0.23
FPF64	II	FIP/NCIP	60	female	alive 9 mo after dx	0.68	-0.27
spIIP7	NA	UIP/IPF	62	male	7	0.69	-0.26
FPF41	II	UIP/IPF	57	female	alive 43 mo after dx	0.84	-0.12
FPF56	II	UIP/IPF	58	male	39	0.65	-0.31
FPF27	I	FIP/NCIP	56	male	46	0.69	-0.27
FPF40	I	UIP/IPF	75	male	10	0.75	-0.18
FPF34	II	UIP/IPF	50	male	21	0.62	-0.35
FPF8	I	UIP/IPF	74	male	2	0.77	-0.17
FPF36	II	UIP/IPF	66	male	alive 60 mo after dx	0.68	-0.26
FPF24	II	UIP/IPF	64	male	0	0.65	-0.30
FPF63	II	UIP/IPF	79	male	3	0.79	-0.13
FPF17	II	UIP/IPF	58	male	31	0.68	-0.28
FPF11	I	UIP/IPF	63	male	3	0.86	-0.09
FPF26	I	UIP/IPF	70	female	alive 63 mo after dx	0.79	-0.15
FPF70	I	UIP/IPF	68	male	alive 2 mo after dx	0.83	-0.11
FPF23	II	FIP/NCIP	63	male	82	0.86	-0.09
spIIP5	NA	UIP/IPF	59	male	4	0.91	-0.42
spIIP2	NA	UIP/IPF	41	male	alive 68 mo after dx	0.80	-0.18
spIIP1	NA	UIP/IPF	46	male	transplanted 27 mo after dx	0.76	-0.21
FPF6	I	UIP/IPF	58	male	12	0.68	-0.28
FPF31	II	UIP/EAA	62	male	7	0.62	-0.33
FPF4	I	UIP/IPF	56	male	86	0.56	-0.39
spIIP3	NA	UIP/IPF	57	female	1	0.78	-0.18
FPF51	II	NSIP	72	male	alive 28 mo after dx	0.67	-0.27
FPF28	I	UIP/IPF	58	male	14	0.76	-0.20

Table 1: Identified coding variants in TERT mutation carriers

Sample ID	Site of variant	cDNA position	Consequence	Variant name	SIFT*	PolyPhen-2	Dutch controls	Reference nr	Exac	1000G	HGMD public	Publication
		NM_198253					N=100					
FPF53	Exon 2	c.232A>T	stop gain	K78X	NA	NA	0	-	no	no		
FPF64	Exon 2	c.250G>A	nonsynonymous	V84M	D	P	0	-	no	no		
spIIP7	Exon 2	c.286G>T	nonsynonymous	V96L	D	P	0	-	no	no	DKC	Aspesi (2010) Pediatr Blood Cancer 55, 550
FPF41	Exon 2	c.299G>A	nonsynonymous	G100D	D	D	0	-	no	no		
FPF56	Exon 2	c.375C>G	nonsynonymous	N125K	D	D	0	-	no	no		
FPF27	Exon 2	c.395G>A	nonsynonymous	R132Q	D	B	0	-	no	no		
FPF40	Exon 2	c.455T>A	nonsynonymous	L152Q	D	D	0	-	no	no		
FPF34	Exon 2	c.515G>A	nonsynonymous	G172E	D	D	0	-	no	no		
FPF8	Exon 3	c.1584T>G	nonsynonymous	C528W	T	P	0	-	no	no		
FPF36	Exon 3	c.1584T>G	nonsynonymous	C528W	T	P	0	-	no	no		
FPF24	Exon 3	c.1698_1700delCAC	inframe deletion	T567del	NA	NA	0	-	no	no		
FPF63	Exon 3	c.1726T>C	nonsynonymous	Y576H	D	D	0	-	no	no		
FPF17	Exon 3	c.1729C>T	nonsynonymous	R577W	D	D	0	-	no	no		
FPF11	Exon 5	c.2005C>T	nonsynonymous	R669W	D	B	0	rs372140951	0.00006376	no		
FPF26	Exon 5	c.2005C>T	nonsynonymous	R669W	D	B	0	rs372140951	0.00006376	no		
FPF70	Exon 5	c.2005C>T	nonsynonymous	R669W	D	B	0	rs372140951	0.00006376	no		
FPF23	Exon 5	c.2011C>T	nonsynonymous	R671W	D	P	0	-	0.00006342	no	PF	Diaz de Leon (2010) PLOS one 5, 5
spIIP5	Exon 5	c.2032G>A	nonsynonymous	A678T	D	P	0	-	no	no	AA	Parry (2011) Blood 117, 5607

spIIP2	Exon 6	c.2146G>A	nonsynonymous	A716T	D	D	0	rs387907249	no	no	AA and PF	Du (2009) Blood 113, 309
spIIP1	Exon 6	c.2147C>T	nonsynonymous	A716V	D	D	0	rs199422298	no	no		
FPF6	Exon 7	c.2303A>T	nonsynonymous	D768V	D	D	0	-	no	no		
FPF31	Exon 8	c.2406C>G	nonsynonymous	S802R	D	D	0	-	no	no		
FPF4	Exon 11	c.2701C>T	nonsynonymous	R901W	T	P	0	rs199422304	no	no	HHS	Marrone (2007) Blood 110, 4198
spIIP3	Exon 11	c.2701C>T	nonsynonymous	R901W	T	P	0	rs199422304	no	no		
FPF51	Exon 15	c.3208G>A	nonsynonymous	V1070M	D	P	0	-	no			
FPF28	Exon 15	c.3256C>T	nonsynonymous	R1086C	T	P	0	-	no	no		

Table 2: Overview of mutations and predictions in TERT mutation carriers
SIFT prediction: Damaging (D)<=0,05; Tolerated (T)>0,05; PolyPhen-2: probably damaging (D) >=0,909; possibly damaging (P) 0,447<hum_div<=0,909; benign (B) <=0,446; DKC = dyskeratosis congenita; AA = aplastic anaemia; PF = pulmonary fibrosis; HHS = Hoyeraal-Hreidarsson syndrome

Clinical characteristics of 26 proband TERT mutation carriers

Twenty-one patients were classified as IPF based on HRCT findings and/or lung biopsy. One patient was diagnosed with non-specific interstitial pneumonia (NSIP) based on HRCT, in one patient features of UIP/ extrinsic allergic alveolitis (EAA) were present on HRCT and biopsy. Three patients were classified as inconsistent with UIP based on HRCT and/or biopsy and were diagnosed as FIP with non-classifiable interstitial pneumonia (NCIP) in a consensus meeting.

The mean age at diagnosis was 61 years (SD 9, range 41 – 79). Twenty-one probands (81%) were male, which is significantly different ($p< 0.02$) from the expected 50% in case of plain autosomal inheritance. Sixty-four percent was a current or former smoker with a mean of 12 packyears. Pulmonary function tests demonstrated a mean diffusing capacity of monoxide (DL_{CO}) of predicted of only 48% at diagnosis, comparable to that found in spIPF. However, a relative preserved lung volume was found in *TERT* probands compared with spIPF; both VC and TLC were significantly higher in *TERT* probands (table 3).

Blood test analyses at time of diagnosis demonstrated macrocytosis in 10 *TERT* probands and the mean corpuscular value (MCV) was significantly higher compared to sporadic IPF patients. Four *TERT* mutation carriers had a thrombocytopenia at time of diagnosis, but the mean platelets count was in the normal range. The measured T/S ratio was significantly shorter in *TERT* mutation carriers compared to sporadic IPF patients (0.822 vs 0.734, $p= 0.001$). All baseline characteristics are shown in table 3.

Patients characteristics	Sporadic IPF (N= 113)	TERT probands (N= 26)	*p*-value
Age at diagnosis, yr. (mean ± SD)	63 (11)	61 (9)	NS
Male sex, no. (%)	99 (88)	21 (81)	NS
Cigarette smoking	(available data= 97)	(available data= 25)	
Current smoker no. (%)	7 (7)	0 (0)	NS
Former smoker no. (%)	67 (69)	16 (64)	NS
Never smoker no. (%)	23 (24)	9 (36)	NS
Packyears, mean (SD)	18 (22)	12 (15)	NS
Pulmonary function tests			
Forced vital capacity % pred: mean ± SD	75 ± 23	86 ± 25	NS
Vital capacity % pred: mean ± SD	73 ± 23	85 ± 23	0.025
Total lung capacity % pred: mean ± SD	65 ± 15	72 ± 14	0.036
DLco % pred: mean ± SD	46 ± 16	48 ± 11	NS
Blood test results			
Hemoglobulin (g/L), mean ± SD	8.9 ± 0.96	8.9 ± 0.66	NS
Mean corpular volume (fL), mean ± SD	92 ± 6.1	98 ± 6.2	0.002
Platelets (x109/L), mean ± SD	252 ± 73	221 ± 68	NS
Leukocytes (x109/L), mean ± SD	10.1 ± 3.4	8.9 ± 3.2	NS
T/S ratio (mean ± SD)			
Objected T/S ratio	0.822 (0.119)	0.734 (0.087)	0.001
T/S ratio difference (observed – expected)	-0.122 (0.119)	-0.217 (0.900)	<0.001

Table 3**:** Characteristics of *TERT* probands and sporadic IPF patients at time of diagnosis

Survival and Lung Transplantation in TERT probands

Seventeen *TERT* probands died during the follow up. The median survival was 31 months (2.6 years, figure 1). The most common cause of death was respiratory insufficiency evoked by infections or progressive fibrosing disease (N= 10, 60%). One patient died because of an abdominal sepsis and in 6 patients (35%) the cause of death was unknown (patients died at home or in another hospital). Importantly, *TERT* probands had a significant worse survival compared to sporadic IPF patients in the first 1.5 year after the diagnosis is set (Hazard ratio 2.43, 95% CI 0.208 – 0.877, p= 0.016; figure 2).

Eight *TERT* probands were screened for lung transplantation (LTX). Two patients were rejected for LTX because of coronary artery disease and 1 patient died before listing on the waiting list.

Eventually 5 patients were listed for LTX. Two out of 26 *TERT* probands (8%) underwent LTX. The other 3 patients died awaiting LTX. The first patient received a single LTX 27 months after diagnosis at the age of 48 years. Twenty-nine months after transplantation the patient died because of multi organ failure with a pancytopenia suggesting bone marrow failure based on a refractory septic shock. The second patient received a bilateral LTX 15 months after diagnosis at the age of 47 years. At the end of the study (12 months after LTX) this patient was still alive.

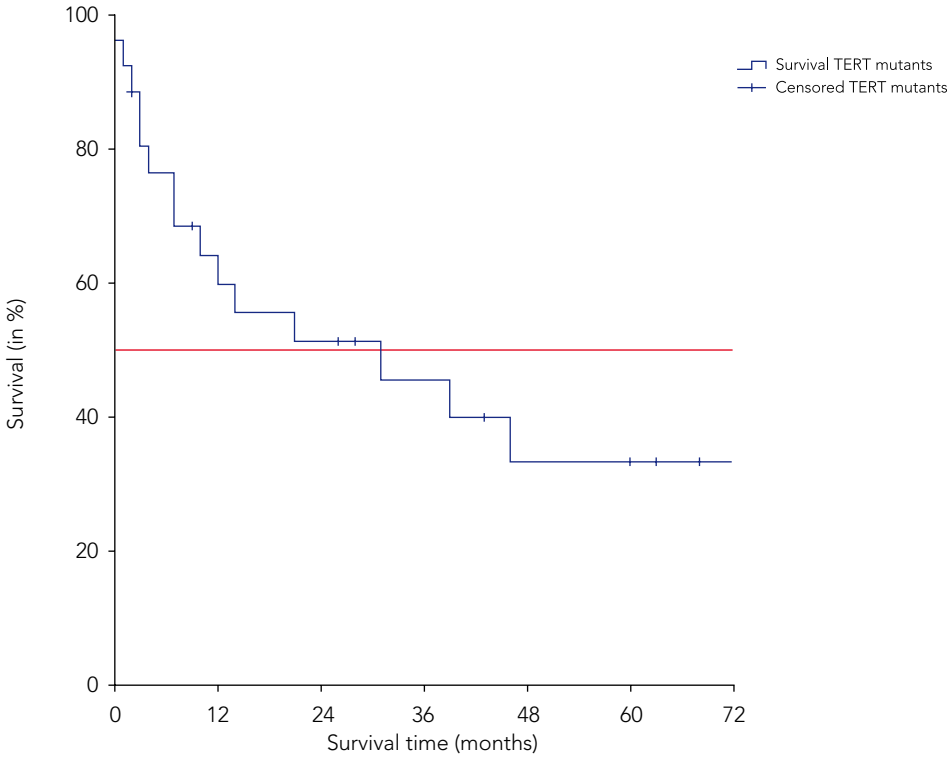


Figure 1: Survival of 26 *TERT* probands after diagnosis. This graph shows a median survival of 31 months (2.6 years).

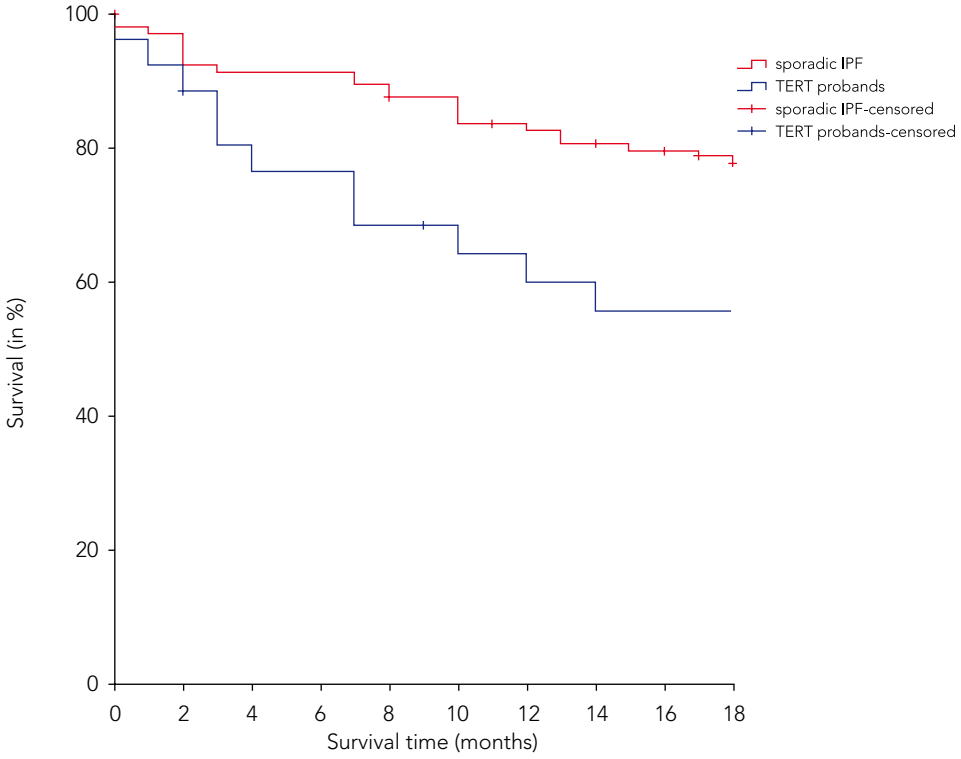


Figure 2: Survival of *TERT* probands compared to sporadic IPF patients the first 18 months after diagnosis. This graph shows a significant worse survival of *TERT* probands (p = 0.016, Hazard ratio 2.43, 95% CI 0.208 – 0.877).

Serum Immunoglobulin Levels in TERT probands

Analysis of immunoglobulins in serum at time of diagnosis showed significant high levels of immunoglobulin A (IgA) in *TERT* mutation carriers compared to sporadic IPF (4.85 g/L versus 3.31 g/L, p <0.001, figure 3). Cox regression analysis showed a significant univariate correlation of IgA with survival in sporadic IPF and *TERT* mutation carriers together (p= 0.002, Hazard ratio 1.292 (95% CI 1.097 – 1.522). However, probably due to the small numbers of events, significant correlation with survival in *TERT* mutation carriers alone could not be demonstrated (p= 0.80, Hazard ratio 0.951, 95% CI 0.646 – 1.398).

Immunoglobulins test results	IPF (N= 86)	*TERT* probands (N= 24)	P- value
Immunoglobulin A (g/L)	3.31 ± 1.5	4.84 ± 1.4	< 0.001
Immunoglobulin G (g/L)	12.0 ± 3.5	14.3 ± 5.0	0.01
Immunoglobulin M (g/L)	1.05 ± 0.68	0.82 ± 0.54	NS

Table 4: Immunoglobulin serum levels at time of diagnosis in *TERT* probands and sporadic IPF patients.

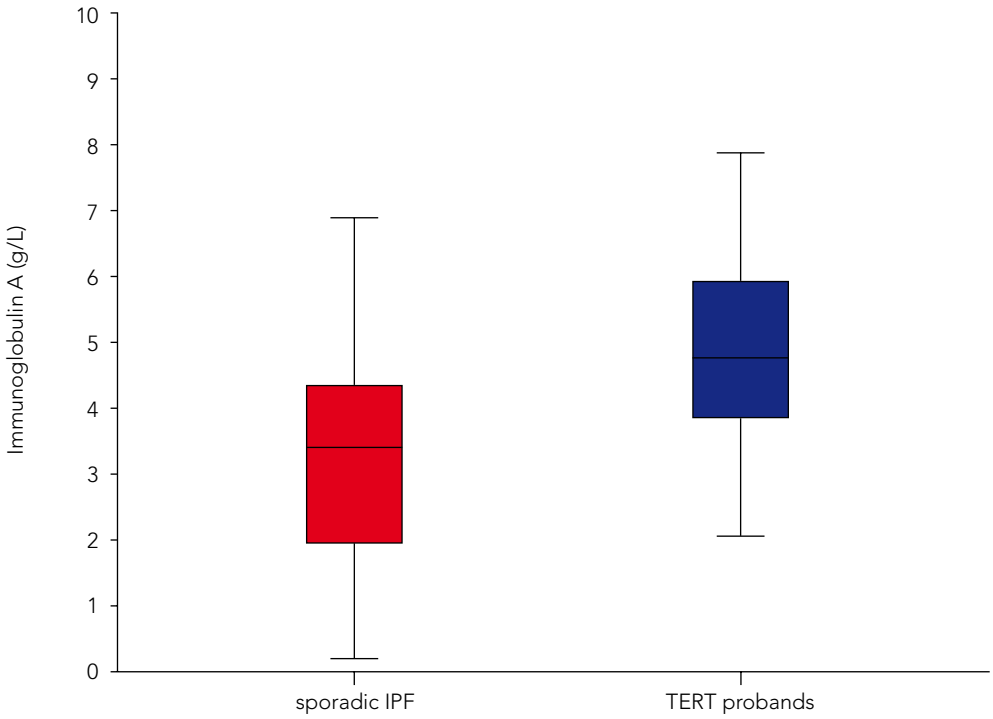


Figure 3: IgA serum levels at time of diagnosis in TERT probands and sporadic IPF patients. Serum IgA levels in TERT probands are significantly elevated compared to sporadic IPF patients (3.31 g/L versus 4.84 g/L, p <0.001).

Bronchoalveolar Lavage Results in TERT probands

Eleven of the 26 *TERT* probands underwent a BAL for diagnostic purposes. We found a significant lower percentage of lymphocytes in the BAL of *TERT* mutation carriers compared to sporadic IPF patients. Furthermore, immunophenotyping of the lymphocytes demonstrated low numbers of different types of lymphocytes compared to sporadic IPF patients and especially a significant lower amount of CD3+CD8+ cells, also named cytotoxic T-cells. The results of the BAL are demonstrated in table 5.

	IPF (N= 70)	***TERT* probands (N= 11)**	***p*-value**
Total cell count (x10^4) (SD)	27.4 (18)	27.3 (13.6)	NS
Macrophages (10^4/ml) (SD) Macrophages %	19.9 (11.3) 74	22.4 (10.8) 84	NS NS
Lymphocytes (10^4/ml) (SD) Lymphocytes %	2.2 (2.4) 8	1.3 (1.0) 5	NS 0.01
Neutrophils (10^4/ml) (SD) Neutrophils %	3.4 (8.6) 10	1.4 (1.8) 5	NS NS
Eosinophils (10^4/ml) (SD) Eosinophils %	2.1 (4.9) 8	2.0 (3.9) 7	NS NS
Basophils (10^4/ml) (SD) Basophils %	0.1 (0.1) 0.4	0.1 (0.1) 0.4	NS NS
Immunophenotyping	**(N= 48)**	**(N= 4)**	
CD3+ (10^4/ml) CD3+ %	2.5 87	1.7 93	NS NS
CD3+CD4+ (10^4/ml) CD3+CD4+ %	1.5 49	1.2 58	NS NS
CD3+CD8+ (10^4/ml) CD3+CD8+ %	0.7 36	0.1 31	0.000 NS
CD19+ (10^4/ml) CD19+ %	0.02 1	0.01 0.9	NS NS
CD3+CD16+CD56+ (10^4/ml) CD3+CD16+CD56+ %	0.09 4	0.04 4	NS NS
CD103+ (10^4/ml) CD103+ %	1.3 45	0.7 35	NS NS
CD4+/CD8+ ratio (10^4/ml)	2.0	2.4	NS

Table 5: Bronchoalveolar lavage (BAL) fluid analysis in *TERT* probands (N= 11) and patients with sporadic IPF (N= 70).

Discussion

This study demonstrated a significant worse survival in *TERT* mutation carriers compared to sporadic IPF patients in the first 18 months after diagnosis (p= 0.016). To our knowledge, this is the first study that directly compared survival between *TERT* mutation carriers and sporadic IPF patients. Furthermore, the median survival in *TERT* mutations carriers after a follow up period of 4 years was only 2.6 years. Median survival in our Dutch IPF cohort has been consistent at around 4 years (14, 23).

The difference between sporadic IPF and *TERT* mutation carriers is striking, one year from diagnosis only 60% survival in *TERT* mutation carriers is observed compared with 84% in IPF patients. One previous study showed a median survival of 3 years after diagnosis of 47 different *TERT* mutation carriers with pulmonary fibrosis from 21 unrelated families (9). Although a comparison with sporadic IPF was not provided in their study, it was concluded that the clinical outcome of *TERT* mutation carriers mirrors that of IPF.

It is very well possible that patients in that study were diagnosed earlier, because they identified *TERT* mutation carriers through family members. Increased awareness for disease-related symptoms within the families can lead to early diagnosis, which is a common phenomenon in investigations of familial disease. Nevertheless, median survival was very poor in these patients.

The finding that patients with *TERT* mutations have worse survival corresponds well with reports on an association between short telomere length and survival. A significant proportion of patients with IIPs have reduced telomere lengths compared to healthy control subjects (8, 9, 17, 24). Furthermore, telomere lengths differ significantly among different IIPs and the shortest telomeres were seen in patients with FIP carrying a mutation in *TERT* (17). Short telomere length have been shown to be independently of age, sex and lung function, associated with worse survival in IPF patients (25). However, a correlation between age-adjusted telomere length and survival was not observed in our cohort of IPF patients and TERT probands.

Up till now, LTX is the only medical treatment with a very marked survival advantage in IPF patients (1, 26). However, little is known about LTX in *TERT* mutation carriers with pulmonary fibrosis. Of the 26 *TERT* probands 8 patients were referred for LTX of which 4 patients (50%) died during the process.

Finally, only 2 patients underwent LTX of which one patient is still alive after 12 months, while the other died from a septic shock with multi organ failure after 29 months. These complications were very likely to be related to consequences of his inherited TERT mutations and short telomeres. A recent article reported an international series of 8 *TERT* mutation carriers who underwent LTX with reasonable survival (27). Nevertheless, the authors suggest that *TERT* mutation carriers require attention to specific complications, including haematological toxicities with medications, and requirement for transfusion support for significant cytopenias (27).

In our cohort of *TERT* probands we found no signs of increased awareness or early onset diagnosis. At time of diagnosis we found no differences in age, smoking history, sex and diffusion capacity measurements between *TERT* probands and IPF patients. Up till now, it was thought that pulmonary function tests in mutation carriers was identical to those with sporadic IPF, however, we found that the lung volumes were significantly less affected in *TERT* mutation carriers. Interestingly, observations in families with telomerase mutations indicate that emphysema may be a first manifestation of telomere-related lung disease in some telomerase mutation carriers (28). A previous study examining radiographs of telomerase mutation carriers with IPF, there was evidence of superimposed emphysema in 20% of cases (9). It is possible that in *TERT* mutation carriers expected reductions in lung volumes because of interstitial abnormalities are masked by concomitant emphysema (29).

Analysis of blood cells in *TERT* probands revealed a subclinical effect of TERT mutations on bone marrow function. We found a significantly higher MCV in *TERT* mutation carriers compared to sporadic IPF patients. Previous studies described blood dyscrasias in *TERT* mutation carriers before, but a significant difference in macrocytosis with IPF patients has never been demonstrated (11, 12, 30).

Analyses of immunoglobulin levels of *TERT* mutation carriers showed significant higher levels of IgA in serum at time of diagnosis compared to sporadic IPF patients. It is thought that repeated injury of alveolar epithelial cells (AECs) in IPF patients cause upregulation of profibrotic factors, such as transforming growth factor β (TGF-β) (1). Activated TGF-β induce the differentiation of fibroblasts to myofibroblasts and the formation of typical fibroblast foci in IPF (1). TGF-β1 is besides a potent inducer of pulmonary fibrosis (31), involved in multiple signalling processes (32, 33).

It has a prominent role in the immune system by inhibiting Thelper (Th1) 1, Th2 and cytotoxic lymphocytes (CTL) and B cell isotype switching (34). One of the characterised effects of TGF-β1 is its ability to stimulate immunoglobulin (Ig) isotype switching to IgA (35). We hypothesized that an increase in activated TGF-β is reflected by an increase in IgA in blood and therefore high levels of IgA in blood reflects the degree of fibrosis in the lungs (see figure 4). In a previous study of our group we demonstrated that higher serum IgA levels at time of diagnosis was a predictor of worse survival in IPF (14). However, probably due to small numbers, we did not found an association between survival and IgA serum levels in *TERT* mutation carriers alone. We suggest that high serum IgA levels in *TERT* mutation carriers may identify severe 'fibrotic active' disease and might explain the poor survival in *TERT* mutation carriers.

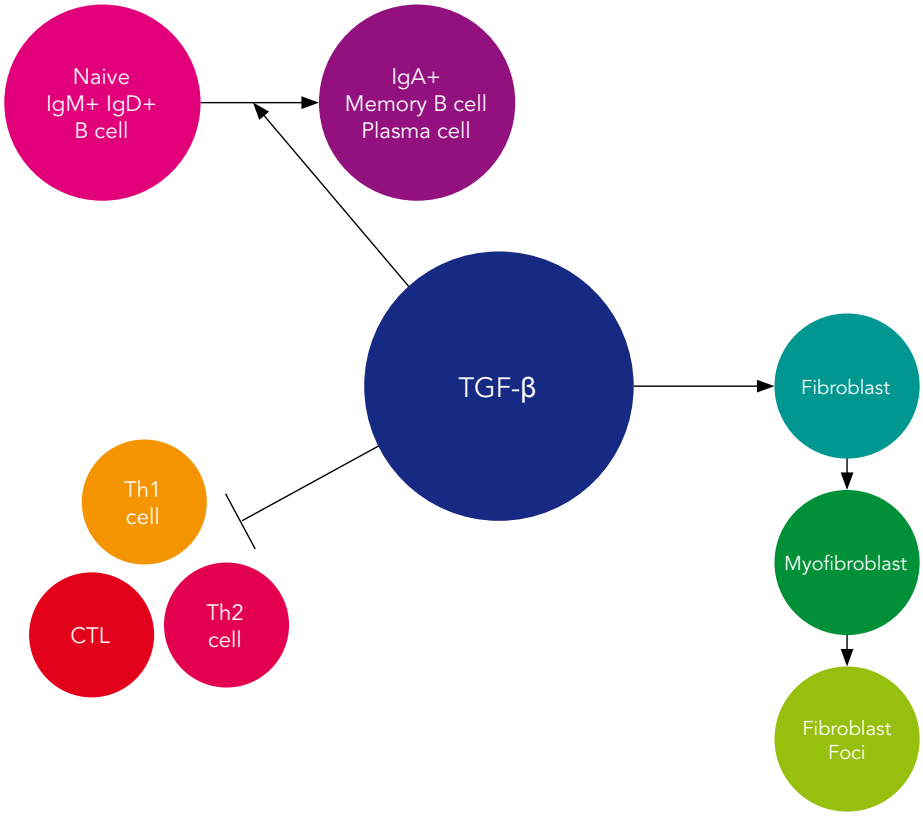


Figure 4: The different roles of transforming growth factor-β (TGF-β) in fibrosis and immunity. TGF-β induces the immunoglobulin (Ig) isotype switching to IgA and the formation of fibroblasts into myofibroblasts and finally the formation of fibroblast foci. Activated TGF-β inhibits Thelper (Th) 1 and Th 2 cells and cytotoxic lymphocytes (CTL).

To our knowledge this study is the first to demonstrate BAL results of *TERT* mutation carriers. The cellular pattern of BAL in IPF patients is characterised by a neutrophilic cellular pattern with a lack of prominent lymphocytosis or eosinophilia (36). BAL analysis showed an equal amount of cells in BAL of TERT mutation carriers compared to sporadic IPF patients, however, we found that *TERT* mutation carriers had a significant lower percentage of lymphocytes.

Furthermore, immunophenotyping demonstrated a significant low amount of cytotoxic lymphocytes (CTL) in the BAL of *TERT* mutations carriers compared to sporadic IPF patients. The low percentage of lymphocytes and low amount of CTL in the BAL supports the hypothesis of severe fibrotic active disease in *TERT* mutation carriers because of the low amount of CTL in lavage fluid caused by inhibition by activated TGF-β (figure 4).

The strength of the current study is its well-characterized population and the length and completeness of the follow-up. However, some limitations should be considered when interpreting our results. Due to the rarity of *TERT* mutation in pulmonary fibrosis and infrequent use of BAL for diagnostic purposes the number of lavaged patients are small.

Although care was taken to present data from independent unrelated subject by checking names and dates for up to two generations backwards, three out of 22 mutations were found in multiple probands. In one case we could rule out kinship, but for two mutations mutual relatedness remains possible. Especially when we take into account the small size of the Netherlands, national adherence of our center and genetic anticipation due to a *TERT* mutation. It may exist that several generations pass before haplo-insufficiency of telomerase results in critically short telomeres.

None of the mutations was present in the 1000Genome project. Only the R669W mutation occurred in the database of the exome aggregation consortium (Exac).The allele frequency of this mutation was very low, 0.00006, only 1 of 15684 alleles was affected. Furthermore, in our cohort R669W mutation segregated with disease.

Strikingly, 5 out of 22 mutations have been found before in patients with telomere syndromes, including R901W. We cannot rule out the possibility that these patients and our probands share common ancestry. However, for the duplicate finding of R901W in our cohort we discovered that the mutations resides on a different haplotype in each family.

Future whole genome SNP analyses could provide an answer about medium deep ancestry of patients sharing the same mutation.
In conclusion, we compared clinical characteristics of 26 *TERT* mutation carriers with pulmonary fibrosis with sporadic IPF patients. Most importantly, we demonstrated a significant poor survival in *TERT* mutation carriers compared to sporadic IPF patients in the first 18 months after the diagnosis is set. Furthermore, we hypothesized that high serum levels of IgA and low amount of CTL in BAL fluid in *TERT* mutation carriers is caused by activated TGF-β and that poor survival in *TERT* mutation carriers is probably due to severe fibrotic active lung disease.

References

(1) King TE,Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet* 2011;378:1949-1961.

(2) Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE,Jr, Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schunemann HJ, ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788-824.

(3) Loyd JE. Pulmonary fibrosis in families. *Am J Respir Cell Mol Biol* 2003;29:S47-50.

(4) Garcia-Sancho C, Buendia-Roldan I, Fernandez-Plata MR, Navarro C, Perez-Padilla R, Vargas MH, Loyd JE, Selman M. Familial pulmonary fibrosis is the strongest risk factor for idiopathic pulmonary fibrosis. *Respir Med* 2011;105:1902-1907.

(5) van Moorsel CH, Hoffman TW, van Batenburg AA, Klay D, van der Vis JJ, Grutters JC. Understanding Idiopathic Interstitial Pneumonia: A Gene-Based Review of Stressed Lungs. *Biomed Res Int* 2015;2015:304186.

(6) Nathan N, Giraud V, Picard C, Nunes H, Dastot-Le Moal F, Copin B, Galeron L, De Ligniville A, Kuziner N, Reynaud-Gaubert M, Valeyre D, Couderc LJ, Chinet T, Borie R, Crestani B, Simansour M, Nau V, Tissier S, Duquesnoy P, Mansour-Hendili L, Legendre M, Kannengiesser C, Coulomb-L'Hermine A, Gouya L, Amselem S, Clement A. Germline SFTPA1 mutation in familial idiopathic interstitial pneumonia and lung cancer. *Hum Mol Genet* 2016.

(7) Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA,3rd, Lansdorp PM, Greider CW, Loyd JE. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 2007;356:1317-1326.

(8) Cronkhite JT, Xing C, Raghu G, Chin KM, Torres F, Rosenblatt RL, Garcia CK. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008;178:729-737.

(9) Diaz de Leon A, Cronkhite JT, Katzenstein AL, Godwin JD, Raghu G, Glazer CS, Rosenblatt RL, Girod CE, Garrity ER, Xing C, Garcia CK. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. *PLoS One* 2010;5:e10680.

(10) van Moorsel CH, van Oosterhout MF, Barlo NP, de Jong PA, van der Vis JJ, Ruven HJ, van Es HW, van den Bosch JM, Grutters JC. Surfactant protein C mutations are the basis of a significant portion of adult familial pulmonary fibrosis in a dutch cohort. *Am J Respir Crit Care Med* 2010;182:1419-1425.

(11) Diaz de Leon A, Cronkhite JT, Yilmaz C, Brewington C, Wang R, Xing C, Hsia CC, Garcia CK. Subclinical lung disease, macrocytosis, and premature graying in kindreds with telomerase (TERT) mutations. *Chest* 2011;140:753-763.

(12) Fernandez BA, Fox G, Bhatia R, Sala E, Noble B, Denic N, Fernandez D, Duguid N, Dohey A, Kamel F, Edwards L, Mahoney K, Stuckless S, Parfrey PS, Woods MO. A Newfoundland cohort of familial and sporadic idiopathic pulmonary fibrosis patients: clinical and genetic features. *Respir Res* 2012;13:64-9921-13-64.

(13) Dai J, Cai H, Zhuang Y, Wu Y, Min H, Li J, Shi Y, Gao Q, Yi L. Telomerase gene mutations and telomere length shortening in patients with idiopathic pulmonary fibrosis in a Chinese population. *Respirology* 2015;20:122-128.

(14) Ten Klooster L, van Moorsel CH, Kwakkel-van Erp JM, van Velzen-Blad H, Grutters JC. Immunoglobulin A in serum: an old acquaintance as a new prognostic biomarker in idiopathic pulmonary fibrosis. *Clin Exp Immunol* 2015;181:357-361.

(15) Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, DiMaio JM, Kinch LN, Grishin NV, Garcia CK. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am J Hum Genet* 2009;84:52-59.

(16) Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res* 2009;37:e21.

(17) Snetselaar R, van Moorsel CH, Kazemier KM, van der Vis JJ, Zanen P, van Oosterhout MF, Grutters JC. Telomere Length in Interstitial Lung Diseases. *Chest* 2015;148:1011-1018.

(18) Heron M, Grutters JC, ten Dam-Molenkamp KM, Hijdra D, van Heugten-Roeling A, Claessen AM, Ruven HJ, van den Bosch JM, van Velzen-Blad H. Bronchoalveolar lavage cell pattern from healthy human lung. *Clin Exp Immunol* 2012;167:523-531.

(19) Aspesi A, Vallero S, Rocci A, Pavesi E, Lanciotti M, Ramenghi U, Dianzani I. Compound heterozygosity for two new TERT mutations in a patient with aplastic anemia. *Pediatr Blood Cancer* 2010;55:550-553.

(20) Parry EM, Alder JK, Qi X, Chen JJ, Armanios M. Syndrome complex of bone marrow failure and pulmonary fibrosis predicts germline defects in telomerase. *Blood* 2011;117:5607-5611.

(21) Du HY, Pumbo E, Ivanovich J, An P, Maziarz RT, Reiss UM, Chirnomas D, Shimamura A, Vlachos A, Lipton JM, Goyal RK, Goldman F, Wilson DB, Mason PJ, Bessler M. TERC and TERT gene mutations in patients with bone marrow failure and the significance of telomere length measurements. *Blood* 2009;113:309-316.

(22) Marrone A, Walne A, Tamary H, Masunari Y, Kirwan M, Beswick R, Vulliamy T, Dokal I. Telomerase reverse-transcriptase homozygous mutations in autosomal recessive dyskeratosis congenita and Hoyeraal-Hreidarsson syndrome. *Blood* 2007;110:4198-4205.

(23) Barlo NP, van Moorsel CH, van den Bosch JM, van de Graaf EA, Kwakkel-van Erp JM, Grutters JC. Idiopathic pulmonary fibrosis; description of a Dutch cohort. *Ned Tijdschr Geneeskd* 2009;153:B425.

(24) Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, Vulto I, Xie M, Qi X, Tuder RM, Phillips JA,3rd, Lansdorp PM, Loyd JE, Armanios MY. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proc Natl Acad Sci U S A* 2008;105:13051-13056.

(25) Dai J, Cai H, Li H, Zhuang Y, Min H, Wen Y, Yang J, Gao Q, Shi Y, Yi L. Association between telomere length and survival in patients with idiopathic pulmonary fibrosis. *Respirology* 2015;20:947-952.

(26) Thabut G, Mal H, Castier Y, Groussard O, Brugiere O, Marrash-Chahla R, Leseche G, Fournier M. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. *J Thorac Cardiovasc Surg* 2003;126:469-475.

(27) Silhan LL, Shah PD, Chambers DC, Snyder LD, Riise GC, Wagner CL, Hellstrom-Lindberg E, Orens JB, Mewton JF, Danoff SK, Arcasoy MO, Armanios M. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. *Eur Respir J* 2014;44:178-187.

(28) Alder JK, Guo N, Kembou F, Parry EM, Anderson CJ, Gorgy AI, Walsh MF, Sussan T, Biswal S, Mitzner W, Tuder RM, Armanios M. Telomere length is a determinant of emphysema susceptibility. *Am J Respir Crit Care Med* 2011;184:904-912.

(29) Washko GR, Hunninghake GM, Fernandez IE, Nishino M, Okajima Y, Yamashiro T, Ross JC, Estepar RS, Lynch DA, Brehm JM, Andriole KP, Diaz AA, Khorasani R, D'Aco K, Sciurba FC, Silverman EK, Hatabu H, Rosas IO, COPDGene Investigators. Lung volumes and emphysema in smokers with interstitial lung abnormalities. *N Engl J Med* 2011;364:897-906.

(30) Armanios M. Telomerase mutations and the pulmonary fibrosis-bone marrow failure syndrome complex. *N Engl J Med* 2012;367:384; author reply 384.

(31) Gauldie J, Bonniaud P, Sime P, Ask K, Kolb M. TGF-beta, Smad3 and the process of progressive fibrosis. *Biochem Soc Trans* 2007;35:661-664.

(32) Yaswen L, Kulkarni AB, Fredrickson T, Mittleman B, Schiffman R, Payne S, Longenecker G, Mozes E, Karlsson S. Autoimmune manifestations in the transforming growth factor-beta 1 knockout mouse. *Blood* 1996;87:1439-1445.

(33) Massague J, Blain SW, Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell* 2000;103:295-309.

(34) Banchereau J, Pascual V, O'Garra A. From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. *Nat Immunol* 2012;13:925-931.

(35) Stavnezer J, Kang J. The surprising discovery that TGF beta specifically induces the IgA class switch. *J Immunol* 2009;182:5-7.

(36) Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, Drent M, Haslam PL, Kim DS, Nagai S, Rottoli P, Saltini C, Selman M, Strange C, Wood B, American Thoracic Society Committee on BAL in Interstitial Lung Disease. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. *Am J Respir Crit Care Med* 2012;185:1004-1014.

Supplementary data

Exon	Forward primer (5′ – 3′)	Reverse primer (5′ – 3′)	Annealing temperature	Internal sequence primer
1	GTCCTGCCCCTTCACCTT	GACACCTGCGGGGGAAG	64	CTC TCC GCA TGT CGC
2a	CAGTGCCTGGTGTGCGTG	ACTCGGTCCACGCGTCCT	55	
2b	AACGGGCCTGGAACCATA	GAGCAGAGGCAGAGATC	59	TTC CTC TAC TCC TCA
3	CTTGGTGAGCTGGATGTG	GGCAGTCAGAGCCTTGCA	72	
4	CTGCAAGTAGAGGGGCTC	CCACGCTGCTTTTCTGGA	59	
5	GCATGAGGATCCCGTGTG	TCAACAGTGACAGGGTCA	59	
6	GGCAGAGGTGATGTCTGA	AGATACATGCACCACGAC	59	
7	GTAGCTACTTTGCGTCCTC	CCAAGGCACACAGCTCAT	59	
8	ACGATGGCCCTGCATTCC	ACACCCCGCTTGCCATTT	62	
9	CCGGCTGAATGGTAGACG	AGCAGTCATGGTCTCCAG	59	
10	CTTCAGCTGGCACAGAAT	CTGCTCTTGCGGATCCAG	59	
11	GGAAAGCACCCCGAAGTC	AGCAGAGGTGGAGCGAA	62	
12	TGGAGTTTGGTCATGCAG	CTGAACTCTGTGCTGACC	59	
13	TGACACAGAGTCTTGACT	CGCGAACAGAACTGTGCA	59	
14	ACCTGTGCCCATGAGGAA	GCCTGACAGTGGTTGGGT	59	
15	TTGTGGAAATTTCACCTGG	GGGACACCAGCGTTTAAT	59	
16	TTCACTGAGGTTCCCACCA	TGGCTCACAGCATCAGAA	59	TGG AAG CCG GGC TCC

Table 1: Primers for TERT sequencing

HRM assay	Forward primer (5′ – 3′)	Reverse primer (5′ – 3′)	Annealing temperature (°C)
Exon 2 G100D	ACATGCGGAGAGCAGCGCAG	GTCACCGTGTTGGGCAGGTAGC	62
Exon 2 N125K and R132Q	GAGGCCTTCACCACCAGCGT	CAGCGTGCCAGCAGGTGAAC	65
Exon 2 L152Q and G172E	GGCGACGACGTGCTGGTTCA	CCGGGCCTGAGTGGCAGC	60
Exon 3 C528W	CTTGGTGAGCTGGATGTGCGG	GAGGAGATCCTGGCCAAGTTCC	72
Exon 3 T567del and R577W	TGTTCCGGCCGCAGAGCAC	AGAGGCCTGGCGTGGGGATA	65
Exon 5 R669W; R671W and A678T	GGATCCACTTTCCTGACTGTCTCC	AAGGTCCAGCAGGGCTGCTCA	60
Exon 6 A716T and A716V	GGCAGAGGTGATGTCTGATTT	CACACATCCTGGACACGACT	65
Exon 7 D768V	ACATTTGTGGCTCATGCCCTC	AGCCACGAACTGTCGCATGT	60
Exon 8 S802R	CATGTGTCTCCCGTCTGCTT	AGAGAGGTGAGCAGAAGCCC	62
Exon 11 R901W	ATCCTGGGGCTGACATTGCC	TGGGCCGGCATCTGAACAA	60
Exon 15 V1070M and R1086C	GGGCTGGGCCTGTGACTCCTC	GTGTGGCTGGAGGCCAGTGC	72

Table 2: Primers for TERT HRM assays

CHAPTER SIX

Case Report: Telomerase mutation in patient with idiopathic pulmonary fibrosis and a complicated course after lung transplantation

L. ten Klooster, C. van Moorsel, J. Kuball, E. van de Graaf and J. Grutters

ABSTRACT

Telomerase dysfunction can cause age-related pathologies most commonly in the bone marrow and liver. We report a detrimental outcome of lung transplantation (LTX) due to bone marrow failure in a patient with IPF. Post-mortem genetic analysis detected a TERT-mutation.

The patient presented with IPF at the relatively early age of 47 and very mild pathology of the bone marrow, and after LTX he developed recurrent infections and pancytopenia.

We recommend a careful clinical evaluation and, if indicated, genetic screening of patients with IPF before LTX. Furthermore, we suggest the possibility of combined hematopoietic stem cell and lung transplantation in patients with TERT mutations, to improve general outcome.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive life-threatening parenchymal lung disease with mean survival after diagnosis of approximately 3 years (1, 2). There is no sufficient evidence to support the use of medicinal treatment for patients with IPF and therefore the only proven treatment to prolong life is lung transplantation (3).

The etiology of IPF is still unknown and the pathogenic mechanisms involved in its cause and disease progression are poorly understood. As potential risk factors smoking, chronic aspiration, infections and environmental factors, like long-term exposition to dust and farming have been suggested (4).

A significant percentage of IPF patients report to have familial disease (5) and mutations in the genes surfactant protein C (*SFTPC*) (6, 7), surfactant protein A2 (*SFTPA*) (8), telomerase reverse transcriptase (*TERT*) , and telomerase RNA component (*TERC*) (9, 10) have been linked to disease development in these families. In 18% of familial and 3% of sporadic patients, heterozygous loss-of-function mutations in TERT have been identified (11, 12). Telomeres are repetitive, non-coding DNA elements (*TTAGGG*) at the ends of chromosomes. In most human somatic cells, telomeres shorten with each cell division due to incomplete lagging DNA strand synthesis and oxidative damage. When a critical length is reached, cellular senescence occurs. The enzyme telomerase solves the end-replication problem by adding multiple copies of the repetitive DNA sequence to the ends of chromosomes, thereby producing a template that allows replications of the lagging strand to be completed. The TERT gene encodes for the enzyme telomerase and loss of function mutations are known to result in shortened telomeres in mutation carriers.

It has been reported that telomeres are shorter in older individuals compared to younger individuals and telomerase dysfunction causes early age-related pathology. Syndromes of telomere shortening have clinical manifestations in multiple organs, but the bone marrow, lungs and liver are most commonly affected. The intensity of infestation of the affected organ ranges from asymptomatic laboratory abnormalities to decompensated organ failure (13).

Here we describe a case of detrimental outcome of lung transplantation in a IPF-patient with underlying *TERT*-mutation as an incentive for careful clinical evaluation and, if indicated, genetic screening of patients with IPF before LTX.

Case presentation

In March 2005, a 47-year-old man presented with progressive dyspnea and chronic cough without hemoptysis. He had never smoked and his family history was negative for pulmonary diseases. Auscultation of the lungs revealed bibasilar crackles. Laboratory tests were normal besides an isolated thrombocytopenia of 94 x 10^9/L. Serum precipitins against birds could not be demonstrated. Autoimmune laboratory tests were positive for antibodies to extractable nuclear antigens, negative for both antineutrophil cytoplasmic antibodies and anti-double-stranded DNA. However, the rheumatologist could not find any indications for underlying autoimmune disease. High resolution computed tomography (HRCT) scan showed fibrotic interstitial deviations located at the periphery. Pulmonary function tests showed total lung capacity (TLC) of 4.1L (55% of predicted), forced expiratory volume in one second (FEV1) of 2.29L/s (57% of predicted) and diffusion capacity of 50% of predicted. His histological diagnosis was usual interstitial pneumonia (UIP) on tissue obtained by open lung biopsy. Based on these findings the diagnosis IPF was established.

His thrombocyte count was monitored and remained low around 80 x 10^9/L. In October 2005, bone marrow analysis was performed and showed poor to normal cell count, small increase of myelopoiesis and increased granulation. The results were explained to be a reactive, or possibly toxic process. No diagnosis was established and he did not receive any treatment. The thrombocytopenia seemed to be largely self-limiting with a thrombocyte count of 140 x 10^9/L in November 2005 .

The patient was treated with high doses of oral and intravenous steroids. Because of his thrombocytopenia it was decided not to add azathioprine or cyclophosphamide. Despite his medicinal treatment and in congruence with the natural evolution of IPF, his lung function deteriorated progressively and he required oxygen. Therefore, he was listed for lung transplantation in December 2006.

Posttransplant period

Because of clinical deterioration due to further disease progression during the waiting list period, the patient was classified as a highly urgent patient in Eurotransplant.

After a waiting list period of 9 months, the patient successfully received unilateral left lung transplant in September 2009. Laboratory testing the day after transplantation revealed a thrombocytopenia of 78 x 10^9/L and hemoglobin count of 7.8 mmol/L. Virology showed less than 50 CMV copies per mL. Immunosuppression consisted of mycophenolate mofetil, tacrolimus and prednisolone. Valganciclovir was added because of the patient's negative CMV serostatus and the donors positive CMV serostatus. Furthermore, he received prophylactic treatment with co-trimoxazole. In the following period our patient was admitted to the hospital several times for infectious complications.
After discontinuing CMV prophylaxis the patient suffered from relapsing CMV reactivation with tissue invasive disease, based on a mild colitis seen by colonoscopy. In April 2008 his CMV copies count was 3,830,000 per mL. He was treated with intravenous valganciclovir and received a maintenance dose of 900 mg valganciclovir twice a day. A few months later he again developed a CMV-reactivation with an incomplete response to intravenous valganciclovir. After addition of megalotect complete remission was achieved and the combination of valganciclovir and megalotect dose of 4500 units per 2 weeks has been continued as maintenance therapy.
During the posttransplant period of 27 months his thrombocyte count remained low. At the first hospitalization period his thrombocyte count was 25x10^9/L and he had a leucopenia of 4.1x10^9/L, which was thought to be caused by the use of mycophenolate mofetil. However, after discontinuing this medication, both thrombocyte and leucocyte count remained low. The decrease was now seen as a side effect of co-trimoxazole and therefore the patient received pentamidine inhalations instead of co-trimoxazole. However, the changes in medication did not have the desired effect on the thrombocytopenia and tendency to have leucopenia in the patient (figure 1).
During the post-transplant period the patient's lung function remained stable and showed no signs of BOS. He was staged BOS stage 0-p (figure 2).

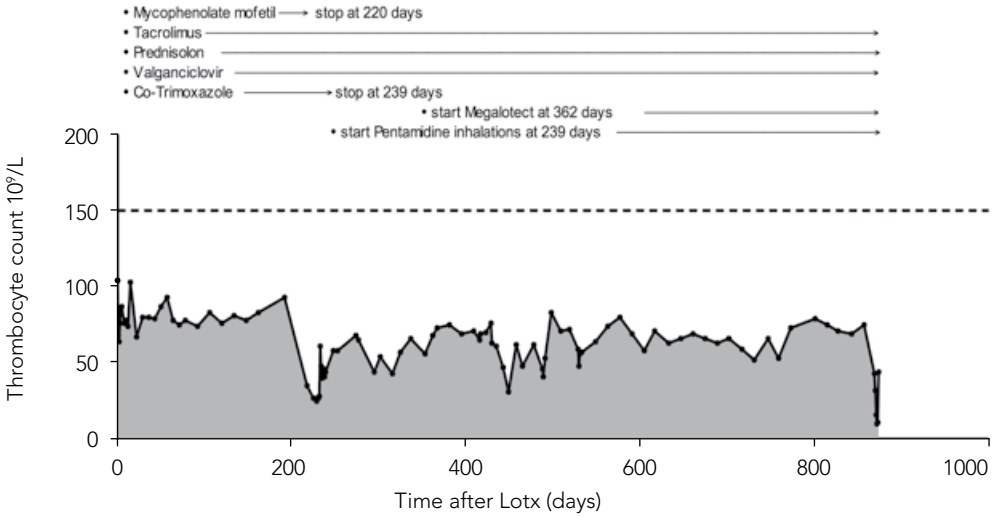


Figure 1: Thrombocyte count and medication after lung transplantation. Normal thrombocyte count ranges from 150 to 450x10⁹L.

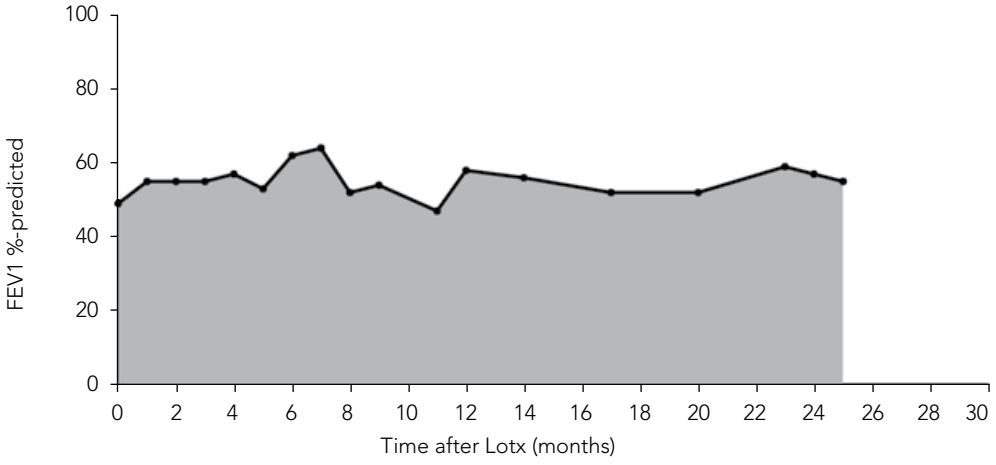


Figure 2: Forced Expiratory Volume in 1 second (FEV1) percentage of predicted after lung transplantation. Bronchiolitis Obliterans Syndrome (BOS) stage 0-p.

Twenty-seven months after transplantation he was admitted to the Intensive Care Unit suspected of a community acquired pneumonia (CAP). He had severe hypoxemia and required invasive ventilation.
Laboratory testing revealed an elevated C-reactive protein (CRP) of 381 and a neutropenia along with a leucopenia of $2.3x10^9$/L and thrombocytopenia of $43x10^9$/L. He received antibiotic treatment with ceftriaxone, co-trimoxazole, and ciproxin. Our patient developed refractory multiple organ failure based on a septic shock. Complete blood count now revealed a pancytopenia suggesting bone marrow failure (BMF). Because of limited therapeutic options and lack of improvement in disease it was decided to end medical treatment and to start palliative sedation. The same day the patient died.
During post-mortem evaluation of this case, the suspicion arose of a TERT-mutation because of the early age at onset of IPF in combination with the idiopathic thrombocytopenia, although the patient had no family history which could have suggested TERT-mutation. After post-mortem genetic evaluation of his DNA a TERT-mutation was found on exon 6; c.2147 C>T; A716V, SIFT 0.01 (intolerant) that caused a substitution of Alanine to Valine at codon position 716. In silico analysis with the computer programme SIFT (Sorting Intolerant From Tolerant) predicted the amino acid substitution to be intolerant (0.01) with deleterious consequence for enzymatical functionability (14).

Discussion

Our patient suffered from IPF, persistent thrombocytopenia and a trend towards leucopenia after LTX. After a relative short posttransplant period, characterized by recurrent infections and CMV reactivations, he died of CAP complicated by refractory multi-organ failure including BMF. Postmortem DNA-analysis revealed an A716V mutation in *TERT*. An identical mutation, A716V, has previously been described in a child with severe pancytopenia and a family history of aplastic anemia and lung disease (15).

Mutations in *TERT* are associated with a group of disorders which are referred to as syndromes of telomere shortening. Not only telomerase mutations are involved in telomere shortening, also other factors are involved in telomere shortening, like the physiological process of aging and environmental factors, for example cigarette smoking, alcohol and psychological stress, and iatrogenically by intensive chemotherapy (16, 17). Dyskeratosis Congenita (DC), based on a triad of oral leukoplakia, skin hyperpigmentation, and nail dystrophy/ridging, is a disease of short telomeres and causes premature mortality due to bone marrow failure in aplastic anemia. Besides bone marrow failure, other presentations of telomere shortening syndromes are pulmonary fibrosis, liver cirrhosis, and limitation of the replicative capacity of hematopoietic stem cells (13).

TERT-mutations cause accelerated telomere shortening and erosion, which is proven to be prognostic and directly correlated with bone marrow failure (17). Excessive loss of telomeres permeates the pathogenesis of bone marrow failure, because maintenance of an adequate telomere length is essential for hematopoiesis. It is reported that granulocytes were mainly affected by telomere erosion and that patients with short telomeres were less likely to respond to immunosuppression (17). When critical telomere shortening takes place, the cell no longer proliferates and becomes either senescent or undergoes apoptosis (16). Presumably, this and the 'stressed' hematopoiesis after transplantation, resulted in bone marrow failure in our patient although we cannot exclude that subsequent medication such as valganciclovir and megalotect was partially responsible for the observed pancytopenia.

Three recent observational studies evaluated in total 31 patients with a mutation in the telomerase complex (*TERT* or *TR*) who underwent LTX (18-20). The most common complications after LTX were haematological, including thrombocytopenia, anaemia, leukopenia and a few patients developed myelodysplastic syndrome and/or bone marrow failure. Significant adjustment of the immunosuppressive regimen was required in almost all cases and most patients were able to tolerate only a two-drug immunosuppressive regimen. Other frequent observed complications were renal failure and respiratory infections or even sepsis, while three patients developed a malignancy.

Hematopoietic stem cell transplantation (HSCT) is used as very powerful treatment for many hematologic malignancies, but also for inherited and acquired bone marrow and immune system disorders (21, 22). Considering that TERT mutation associates with a weakened hematopoiesis, HSCT may be a reasonable option in order to correct the defective bone marrow prior to or shortly after LTX. A prospective study in patients with DC showed positive outcomes and demonstrated that these patients need specifically designed nonmyeloablative conditioning regimens (23). Furthermore, carrier detection in family members is important for both genetic counseling and donor selection. *Giri et al.* recently described LTX after HSCT in a young boy with DC with good clinical outcome (24). However, to our knowledge, HSCT before or combined with LTX has not been applied to an IPF-patient.

This case report is an incentive to improve knowledge of underlying genetic disorders in lung diseases and possible consequences for lung transplantation. It is needed to create more awareness among physicians of telomere mutations as underlying mechanism for pulmonary fibrosis. Although our patient had subtle hematologic deviations, genetic evaluation in this case had indicated that the patient had limited marrow reserves which probably required vigilance during treatment with myelosuppressive drugs in the setting of lung transplantation. For the future, we recommend genetic screening for *TERT*-mutation in IPF-patients registered for lung transplantation with indistinct hematological deviations. Thereby, we suggest exploring the option of HSCT before LTX or combined HSCT and LTX in this defined group of patients to improve bone marrow capacity and achieve better outcomes after lung transplantation.

References

(1) Caminati A, Harari S. IPF: New insight in diagnosis and prognosis. *Respir Med* 2010;104 Suppl 1:S2-10.

(2) King TE,Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet* 2011;378:1949-1961.

(3) Thabut G, Mal H, Castier Y, Groussard O, Brugiere O, Marrash-Chahla R, Leseche G, Fournier M. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. *J Thorac Cardiovasc Surg* 2003;126:469-475.
(4) Harari S, Caminati A. IPF: new insight on pathogenesis and treatment. *Allergy* 2010;65:537-553.

(5) van Moorsel CH, van Oosterhout MF, Barlo NP, de Jong PA, van der Vis JJ, Ruven HJ, van Es HW, van den Bosch JM, Grutters JC. Surfactant protein C mutations are the basis of a significant portion of adult familial pulmonary fibrosis in a dutch cohort. *Am J Respir Crit Care Med* 2010;182:1419-1425.

(6) Nogee LM, Dunbar AE,3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med* 2001;344:573-579.

(7) Thomas AQ, Lane K, Phillips J,3rd, Prince M, Markin C, Speer M, Schwartz DA, Gaddipati R, Marney A, Johnson J, Roberts R, Haines J, Stahlman M, Loyd JE. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. *Am J Respir Crit Care Med* 2002;165:1322-1328.

(8) Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, DiMaio JM, Kinch LN, Grishin NV, Garcia CK. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am J Hum Genet* 2009;84:52-59.

(9) Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA,3rd, Lansdorp PM, Greider CW, Loyd JE. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 2007;356:1317-1326.

(10) Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, Rosenblatt RL, Shay JW, Garcia CK. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci U S A* 2007;104:7552-7557.

(11) Diaz de Leon A, Cronkhite JT, Katzenstein AL, Godwin JD, Raghu G, Glazer CS, Rosenblatt RL, Girod CE, Garrity ER, Xing C, Garcia CK. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. *PLoS One* 2010;5:e10680.

(12) Cronkhite JT, Xing C, Raghu G, Chin KM, Torres F, Rosenblatt RL, Garcia CK. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008;178:729-737.

(13) Armanios M. Syndromes of telomere shortening. *Annu Rev Genomics Hum Genet* 2009;10:45-61.

(14) Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009;4:1073-1081.

(15) Du HY, Pumbo E, Ivanovich J, An P, Maziarz RT, Reiss UM, Chirnomas D, Shimamura A, Vlachos A, Lipton JM, Goyal RK, Goldman F, Wilson DB, Mason PJ, Bessler M. TERC and TERT gene mutations in patients with bone marrow failure and the significance of telomere length measurements. *Blood* 2009;113:309-316.

(16) Young NS. Telomere biology and telomere diseases: implications for practice and research. *Hematology Am Soc Hematol Educ Program* 2010;2010:30-35.

(17) Calado RT, Young NS. Telomere maintenance and human bone marrow failure. *Blood* 2008;111:4446-4455.

(18) Borie R, Kannengiesser C, Hirschi S, Le Pavec J, Mal H, Bergot E, Jouneau S, Naccache JM, Revy P, Boutboul D, Peffault de la Tour R, Wemeau-Stervinou L, Philit F, Cordier JF, Thabut G, Crestani B, Cottin V, Groupe d'Etudes et de Recherche sur les Maladies "Orphelines" Pulmonaires (GERM"O"P). Severe hematologic complications after lung transplantation in patients with telomerase complex mutations. *J Heart Lung Transplant* 2015;34:538-546.

(19) Silhan LL, Shah PD, Chambers DC, Snyder LD, Riise GC, Wagner CL, Hellstrom-Lindberg E, Orens JB, Mewton JF, Danoff SK, Arcasoy MO, Armanios M. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. *Eur Respir J* 2014;44:178-187.

(20) Tokman S, Singer JP, Devine MS, Westall GP, Aubert JD, Tamm M, Snell GI, Lee JS, Goldberg HJ, Kukreja J, Golden JA, Leard LE, Garcia CK, Hays SR. Clinical outcomes of lung transplant recipients with telomerase mutations. *J Heart Lung Transplant* 2015;34:1318-1324.

(21) Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med* 2006;354:1813-1826.

(22) Gadalla SM, Savage SA. Telomere biology in hematopoiesis and stem cell transplantation. *Blood Rev* 2011;25:261-269.

(23) Dietz AC, Orchard PJ, Baker KS, Giller RH, Savage SA, Alter BP, Tolar J. Disease-specific hematopoietic cell transplantation: nonmyeloablative conditioning regimen for dyskeratosis congenita. *Bone Marrow Transplant* 2011;46:98-104.

(24) Giri N, Lee R, Faro A, Huddleston CB, White FV, Alter BP, Savage SA. Lung transplantation for pulmonary fibrosis in dyskeratosis congenita: Case Report and systematic literature review. *BMC Blood Disord* 2011;11:3-2326-11-3.

CHAPTER SEVEN

SFTPA2 mutations in familial and sporadic idiopathic interstitial pneumonia

van Moorsel CH, ten Klooster L, van Oosterhout MF, de Jong PA, Adams H, Wouter van Es H, Ruven HJ, van der Vis JJ, Grutters JC

This chapter was published as a letter in Am J Respir Crit Care Med. 2015 Nov 15; 192(10): 1249-52

Introduction

Idiopathic interstitial pneumonias (IIPs) are diffuse lung diseases of unknown cause and high mortality. Up to 20% of patients with IIP report having one or more family members with the disease (1, 2), referred to as familial interstitial pneumonia (FIP). Familial occurrence of a rare disease strongly suggests that genetic factors play an important role, especially when multiple generations are involved. Up to 20% of patients report to have at least one first-degree family member with IIP (3-4, 5-8). So far, genetic analysis of affected families have revealed deleterious mutations in the genes encoding surfactant protein C (*SFTPC*), surfactant protein A2 (*SFTPA2*), telomerase reverse transcriptase (*TERT*) and telomerase RNA component (*TERC*). These mutations were rarely found in sporadic cases and never in healthy controls. Genetic analysis has previously revealed deleterious mutations in surfactant protein (*SFTPA2*) in two families (2). Here, we report the genetic analysis of *SFTPA2* in a cohort of familial and sporadic patients with IIP.

Methods

All coding regions in *SFTPA2* were sequenced in a cohort of 39 unrelated patients with FIP and 118 sporadic patients with IIP, using previously published primers (2). FIP was defined as two or more first-degree family members with IIP. Diagnoses were established using current classification guidelines (3, 4). Patients with mutations in *SFTPC*, *TERT*, *TERC*, or *TINF2* were not included. The control cohort comprised 100 healthy white Dutch subjects. The Ethical Committee of St. Antonius Hospital approved the study, and all subjects gave written informed consent.

Results

In the FIP cohort, we identified three new mutations in exon 6 of *SFTPA2*: N210T, G231R, and N171Y. Mutation carriers were heterozygous for the mutation. In the sporadic IIP cohort, one heterozygous carrier of the mutation, N210T, was discovered; none of the mutations was detected in Dutch controls or in any of the public databases representing more than 60,000 individuals worldwide (table 1).

All three mutations caused nonsynonymous amino acid substitutions that were predicted to have deleterious consequences *in silico* by the program Sorting Intolerant from Tolerant (SIFT) (5) and PolyPhen-2 (6) (table 1). We additionally evaluated *in silico* all seven common and unique nonsynonymous *SFTPA2* variants that were identified by Wang and co-workers (2). In contrast to common polymorphisms, only mutations F198S and G231V were predicted to have deleterious consequences in both SIFT and PolyPhen-2 (table 1).

Patient FPF5 carried mutation *SFTPA2* N210T (figure 1A). He was a 40-year-old man with a pattern of nonspecific interstitial pneumonia (NSIP; Figure 1B) on high-resolution computed tomography (HRCT) and usual interstitial pneumonia (UIP) with features of desquamative interstitial pneumonia (DIP) on biopsy. Sixty-four months after diagnosis, he underwent unilateral lung transplantation of the left lung. Posttransplantation, he soon developed severe primary graft dysfunction and respiratory failure. Therefore, a rescue bilateral retransplantation was performed. Pathological examination of the explanted native right lung revealed a poorly differentiated adenocarcinoma situated in the right lower lobe with invasive growth into the pleural wall. Furthermore, five intrapulmonary lymph nodes and three hilar lymph nodes were tumour positive. No malignancy was found in the native left lung. The patient died 19 months after lung transplantation as a result of metastasized lung cancer. In the sporadic IIP cohort, an identical *SFTPA*-N210-T mutation was present in a 67-year-old male patient. His father had died of lung cancer, and his mother had suffered from chronic obstructive pulmonary disease. Patients demographics of this patient and FPF5 differed up to two generations back, but genetic analysis could not exclude the possibility of a common ancestor.

Our second *SFTPA2* mutation, G231R, was found in patient FPF15 (figure 1A), a 43-year-old man. HRCT showed a pattern of NSIP (figure 1C) and UIP on biopsy (figure 1F). Fourteen months after diagnosis, he developed an area of consolidation (figure 1E), and he died 18 months after the diagnosis of respiratory insufficiency. Autopsy demonstrated an adenocarcinoma with a poorly differentiated invasive component and a component of lepidic growth (formerly known as bronchioloalveolar cell carcinoma) in the left lower lobe (figure 1G).

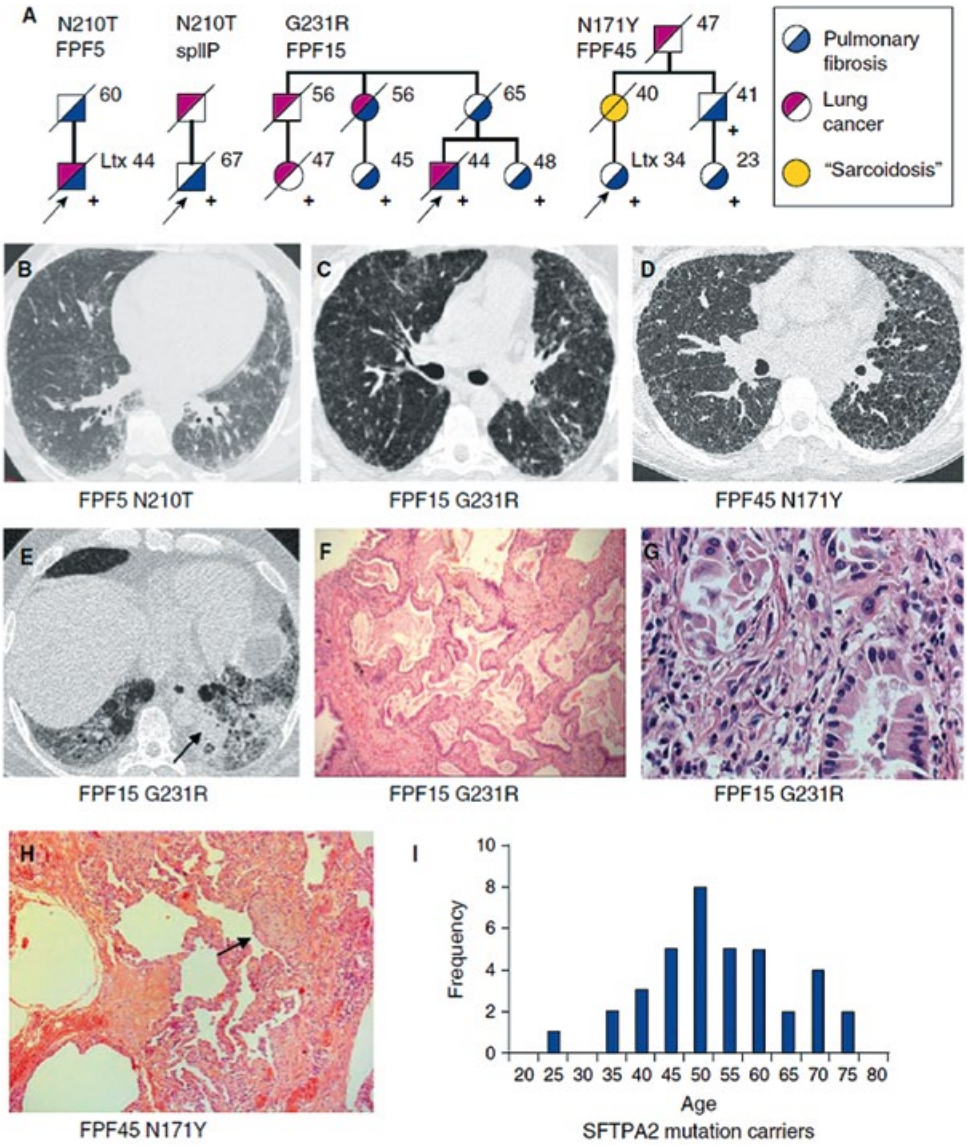
The location coincided with increased uptake on a nuclear [^{18}F] fluoro-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET)/computed tomography (CT) scan in the left lower lobe without focal lesions (*see* figure 2). His sister and niece recently developed pulmonary fibrosis and presented with an HRCT pattern of NSIP.

The third family, FPF45, carried the mutation N171Y (figure 1A). The proband developed pulmonary fibrosis at 33 years of age and received bilateral lung transplantation 11 months after diagnosis. HRCT showed a pattern of diffuse nonclassifiable interstitial lung disease with many thin-walled cysts and honeycombing without peripheral or bibasilar predominance. Furthermore, no traction bronchiectasis were present (figure 1D). The biopsy (figure 1H) showed varying degrees of predominantly subpleural fibrosis with few fibroblast foci, a DIP-like reaction, and thickening of alveolar septa. The mother of the patient died from sarcoidosis at age 40 years, but no medical reports were available to confirm the diagnosis.

Site of Variant	cDNA Position	Variant Name	SIFT	PolyPhen-2	FIP proband	Family Name	spIIP	Controls
New variants					N= 39 (1)		N= 118	N= 100
Exon 6	c.629A>C	N210T	0.03*	1.00*	1 proband	FPF5	1 patient	0†
Exon 6	c.691G>A	G231R	0.00*	1.00*	1 proband	FPF15	0	0†
Exon 6	c.511A>T	N171Y	0.00*	1.00*	1 proband	FPF45	0	0†
Previously reported variants					N= 59 (2)			N > 27,000 ExAC
Exon 3	c.26C>A	T9N	0.49	0.00	0.53 (A)			0.41 (A)
Exon 3	c.35T>G	L12W	0.09	0.91*	1 proband	Family: no segregation with disease		0
Exon 3	c.148G>C	V50L	0.42	0.12	0.08 (C)			0.05 (C)
Exon 4	c.271G>C	A91P	1.00	0.00	0.17 (C)			0.15 (C)
Exon 6	c.593T>C	F198S	0.00*	0.99*	1 proband	CKG810		0
Exon 6	c.667C>A	Q223K	0.36	0.00	0.15 (A)			0.20 (A)
Exon 6	c.692G>T	G231V	0.00*	1.00*	1 proband	F27		0

Table 1: Nonsynonymous coding variants in SFTPA2 in familial and sporadic IIP.
Definition of abbreviations: cDNA = complementary DNA; ExAC = Exome Aggregation Consortium; FIP = familial interstitial pneumonia; IIP = idiopathic interstitial pneumonia; n = number of individuals; SFTPA2 = surfactant protein A2; SIFT = Sorting Intolerant from Tolerant; spIIP = sporadic IIP.

Prediction of effect of substitution on protein structure and function. cDNA positions are based on sequence NM_0010998668.2, and the variant names are based on protein NP001092138.1. SIFT score was calculated with the Blink algorithm (sift.jcvi.org) with SFTPA2 gene identifier GI:14886696, SIFT predict deleterious, not tolerated effect if $P < 0.05$. PolyPhen-2 predictions were calculated from genetics.bwh.harvard.edu/pph2 with protein identifier NP_001092138.1 and choosing the humvar prediction in the Results section. PolyPhen predicts a damaging effect if the probabilistic score is greater than 0.85. FIP probands numbers correspond to Van Moorsel and colleagues (1) and Wang and colleagues (2). For common polymorphism T9N, V50L, A91P, and Q223K, the frequency of the minor allele is given. Population frequencies of common variants were derived from the ExAC European (Non-Finnish) population and comprised at least 27,000 successful genotypes. *Significant result. †The mutation was not found July 7th 2015, in the following databases: National Center for Biotechnology Information Single Nucleotide Polymorphism database (www.ncbi.nlm.nih.gov/projects/SNP and www.ncbi.nlm.nih.gov/clinvar), 1,000 Genomes Project (www.1000genomes.org), public version of the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac), and ExAC (exac.broadinstitute.org).



(A) Condensed pedigrees: +, mutation present; current age, age at death, or age at lung transplantation (LTX) is indicated at the upper right of each symbol, *arrows* point at investigated probands. (B) FPF5, high-resolution computed tomography (HRCT) at diagnosis with nonspecific interstitial pneumonia pattern. (C) FPF15, HRCT at diagnosis with nonspecific interstitial pneumonia pattern. (D) FPF45, HRCT with diffuse nonspecific interstitial lung disease pattern, presence of many scattered cysts in both lungs, and absence of classic subpleural honeycombing and traction bronchiectasis. (E) FPF15, HRCT scan 14 months after diagnosis showing consolidation in the lower left lobe. (F and G) FPF15, tissue from autopsy 18 months after diagnosis. (F) Low-power (25x) hematoxylin and eosin (H&E) image of area of honeycomb changes in fibrotic lung. Also, fibroblast foci and more normal lung were present (not shown), fitting with usual pneumonia pattern. (G) High-power (200x) H&E image of adenocarcinoma, partly with lepidic growth, partly with a poorly differentiated invasive component. (H) Microhoneycomb changes and fibrosis; *arrow* points to fibroblast foci. Also, a desquamative interstitial pneumonia component was present (not shown). (I) Frequency distribution of age at death, lung transplantation, or current age of affected carriers of a SFTPA2 mutation; combined data from present study and Wang and coworkers (2). spIIP = sporadic idiopathic interstitial pneumonia.

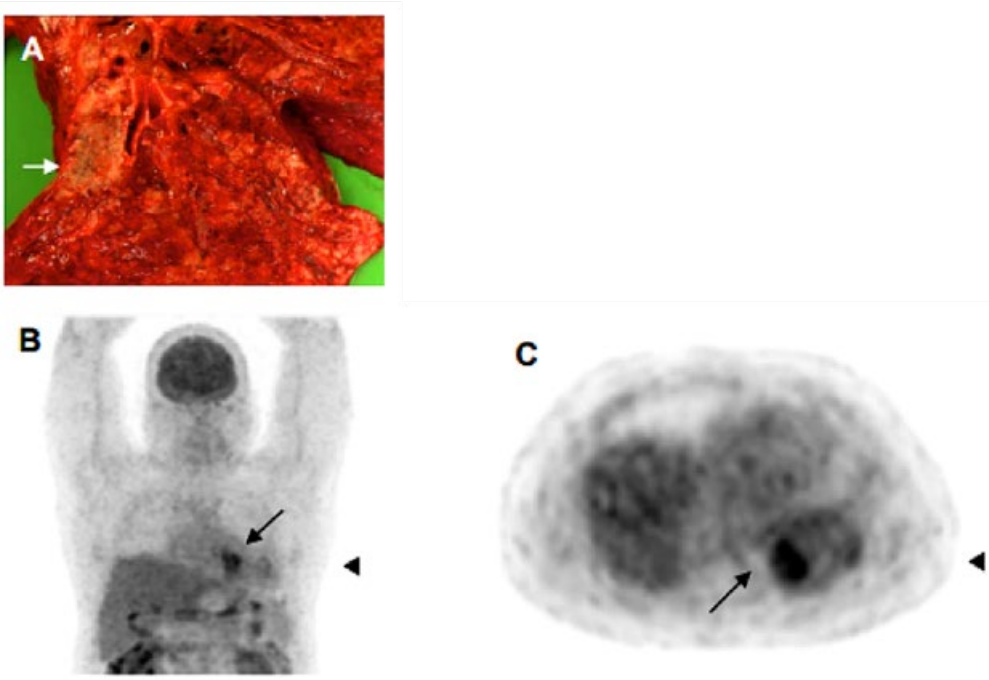


Figure 2: A: macro (0.5x) image of carcinoma (white arrow)in fibrotic lower left lobe at autopsy. B and C: 18F-FDG PET scan of malignancy, black arrows points to an area of intense FDG accumulation of 47 x 41 x 24 mm and a SUV max of 4.85. Comparison with serial HRCT scans demonstrated consolidation in the corresponding area.

Discussion

This report illustrates several important points in the detection and management of *SFTPA2* mutation carriers. Both previous reported mutations, F198S and G231V (2), and current mutations were found in *SFTPA2* exon 6. One other study described an exon 6 *SFTPA2* mutation, V178M, in a patient with FIP but presented no clinical data (7). Moreover, G231R abrogates the same wild-type amino acid as the previously reported G231V mutation (2). Exon 6 encodes the carbohydrate-recognition domain of surfactant protein A2 and seems to be a hot spot for mutations in FIP. Surfactant protein A2 is a C-type lectin important in the defence against pathogens. In contrast to SFTPC, SFPTA2 is expressed not only by alveolar epithelial type II cells but also by Clara cells and submucosal glands of the respiratory airways (8). The protein can be found in alveolar macrophages, but these cells do not express SFTPA2 mRNA (8). A limitation of the current research is that the new mutations have not been experimentally tested.

Experiments are particularly interesting in light of discovering cellular processes that might become targets for therapy. Previously, it was shown that mutant surfactant protein A2 expression induced endoplasmic reticulum (ER) stress (9). The role of ER stress in tumorigenesis is unclear, but recently a positive feed-forward loop was discovered whereby a transient increase of ER stress caused reduced senescence and promotion of tumorigenesis (10). ER stress promoting tumorigenesis and the involvement of Clara cells might explain the co-occurrence of lung cancer and pulmonary fibrosis in families with a *SFTPA2* mutation. The sporadic patient with a mutation had a father who died from lung cancer (Figure 1A), demonstrating that not only IIP, but also lung cancer, can point towards familial disease and can be the starting point for genetic analysis.
In FPF5, lung cancer developed in the native lung after unilateral lung transplantation. Because of the increased risk for lung cancer, transplantation should be bilateral in patients with a *SFTPA2* mutation. At screening for lung transplantation, distinction between dense fibrosis and tumour might be aided by ^{18}F-FDG-PET/CT evaluation. ^{18}F-FDG-PET/CT has a higher sensitivity than HRCT alone (11), but its potential role in detecting adenocarcinoma in IIP requires further investigation.

In the family of FPF45, the youngest patient with a *SFTPA2* mutation ever reported was present. At presentation, she was 20 years old, with a FVC of 57% of predicted and a diffusing capacity of the lung for carbon monoxide (DL_{CO}) of 49% of predicted. Age distribution of the six families so far with a *SFTPA2* mutation shows that most patients die around the fifth decade (Figure 1I). In contrast to families carrying a *SFTPC* mutation (12), paediatric disease was not reported.
In conclusion, *SFTPA2* mutations occurred in 3 of 39 FIP families and 1 in 118 sporadic patients. Both nonclassifiable and nonspecific interstitial pneumonia patterns on HRCT were observed, whereas biopsies show a combination of UIP and DIP. Lung cancer is present in all *SFTPA2* families and warrants special attention in case of lung transplantation.

References

(1) van Moorsel CH, van Oosterhout MF, Barlo NP, de Jong PA, van der Vis JJ, Ruven HJ, van Es HW, van den Bosch JM, Grutters JC. Surfactant protein C mutations are the basis of a significant portion of adult familial pulmonary fibrosis in a dutch cohort. *Am J Respir Crit Care Med* 2010;182:1419-1425.

(2) Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, DiMaio JM, Kinch LN, Grishin NV, Garcia CK. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am J Hum Genet* 2009;84:52-59.

(3) Travis WD, Costabel U, Hansell DM, King TE,Jr, Lynch DA, Nicholson AG, Ryerson CJ, Ryu JH, Selman M, Wells AU, Behr J, Bouros D, Brown KK, Colby TV, Collard HR, Cordeiro CR, Cottin V, Crestani B, Drent M, Dudden RF, Egan J, Flaherty K, Hogaboam C, Inoue Y, Johkoh T, Kim DS, Kitaichi M, Loyd J, Martinez FJ, Myers J, Protzko S, Raghu G, Richeldi L, Sverzellati N, Swigris J, Valeyre D, ATS/ERS Committee on Idiopathic Interstitial Pneumonias. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013;188:733-748.

(4) Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE,Jr, Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schunemann HJ, ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788-824.

(5) Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009;4:1073-1081.

(6) Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248-249.

(7) Coghlan MA, Shifren A, Huang HJ, Russell TD, Mitra RD, Zhang Q, Wegner DJ, Cole FS, Hamvas A. Sequencing of idiopathic pulmonary fibrosis-related genes reveals independent single gene associations. *BMJ Open Respir Res* 2014;1:e000057-2014-000057. eCollection 2014.

(8) Madsen J, Tornoe I, Nielsen O, Koch C, Steinhilber W, Holmskov U. Expression and localization of lung surfactant protein A in human tissues. *Am J Respir Cell Mol Biol* 2003;29:591-597.

(9) Maitra M, Wang Y, Gerard RD, Mendelson CR, Garcia CK. Surfactant protein A2 mutations associated with pulmonary fibrosis lead to protein instability and endoplasmic reticulum stress. *J Biol Chem* 2010;285:22103-22113.

(10) Zhu B, Ferry CH, Markell LK, Blazanin N, Glick AB, Gonzalez FJ, Peters JM. The nuclear receptor peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) promotes oncogene-induced cellular senescence through repression of endoplasmic reticulum stress. *J Biol Chem* 2014;289:20102-20119.

(11) Silvestri GA, Gould MK, Margolis ML, Tanoue LT, McCrory D, Toloza E, Detterbeck F, American College of Chest Physicians. Noninvasive staging of non-small cell lung cancer: ACCP evidenced-based clinical practice guidelines (2nd edition). *Chest* 2007;132:178S-201S.

(12) Ono S, Tanaka T, Ishida M, Kinoshita A, Fukuoka J, Takaki M, Sakamoto N, Ishimatsu Y, Kohno S, Hayashi T, Senba M, Yasunami M, Kubo Y, Yoshida LM, Kubo H, Ariyoshi K, Yoshiura K, Morimoto K. Surfactant protein C G100S mutation causes familial pulmonary fibrosis in Japanese kindred. *Eur Respir J* 2011;38:861-869.

CHAPTER EIGHT

The occurrence of Hermansky Pudlak Syndrome in patients with idiopathic pulmonary fibrosis – a cohort study

L. ten Klooster*, J.J. van der Vis*, M.F.M. van Oosterhout , J.C. Grutters, C.H.M. van Moorsel

*These authors contributed equally to this work

Genet Syndr Gene Ther. vol.4: article 414, 2013

ABSTRACT

Background

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive multisystem disease characterized by bleeding disorders and oculocutaneous albinism. In some patients the syndrome is accompanied by granulomatous colitis, immunodeficiency or pulmonary fibrosis. To date nine genes are associated with the syndrome, resulting in subtypes HPS-1 to HPS-9. Pulmonary fibrosis is most commonly associated with HPS-1. The clinical course of patients with HPS and pulmonary fibrosis resembles that of idiopathic pulmonary fibrosis (IPF). Our aim was to establish the prevalence of HPS in a cohort of IPF patients.

Methods

127 patients diagnosed with IPF based on the criteria set by the ATS/ERS/JRS/ALAT were retrospectively screened for clinical features of HPS. Genetic analysis was performed in patients with pulmonary fibrosis and 2 or more clinical features of HPS. Genomic DNA was extracted from peripheral blood of each individual using standard methods. The coding exons were amplified and sequenced and their exon-intron boundaries of *HPS1* gene.

Results

Out of 127 IPF patients, 22 patients had 1 feature and 4 patients had ≥ 2 features of HPS. These 4 patients were genetically analyzed. Genetic analysis identified one compound heterozygous patient with 2 mutations in exon 13, Q397S**fs**X1 and R439X. Both mutations were not present in our IPF cohort and healthy controls. In the other 3 IPF patients no mutations were found.

Conclusion

Out of 127 IPF patients we identified one compound heterozygous patient with 2 mutations in the *HPS1* gene. HPS is a very rare syndrome, but recognizing is important because of the clinical consequences, particularly when the patient is referred for lung transplantation. Therefore, pulmonologists should be aware of HPS as an underlying cause of pulmonary fibrosis.

Introduction

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive and genetically heterogeneous disorder. It is characterized by reduced pigmentation of skin, hair and eyes and bleeding diathesis due to absent platelet delta granules (1, 2). Pathogenesis in HPS is caused by disrupted biogenesis or function of lysosome-related organelles due to mutations in organelle related genes (3). Nowadays, HPS is reported in more than 800 patients. The estimated prevalence of HPS worldwide is 1:500,000 – 1:1.000,000 and therefore almost certainly seems to be underestimated because of mis-diagnosis or un-diagnosis (3). At our center for interstitial lung diseases, specialized in diagnosing and treating of pulmonary fibrosis, we were visited by a patient with clinical features of HPS. Because of the assumption of underestimation of HPS and the clinical consequences of HPS for further medical treatment, we aimed to establish the prevalence of HPS in a well-described cohort of IPF patients.

To date, mutations in nine different genes (*HPS1, AP3B1, and HPS3 to HPS9*) have been identified in human, resulting in subphenotypes HPS-1 to HPS-9 (4). HPS-1 is the most frequent subphenotype of HPS. HPS-1, together with HPS-4, is a subunit of the lysosomal complex BLOC-3. Loss of either subunit results in destabilization of the remaining subunits, consequently resulting in clinical features of HPS (3). One of the clinical features of HPS is pulmonary fibrosis. It is most commonly associated with HPS-1, however pulmonary fibrosis is also reported in cases of HPS-4 and HPS-2 (5). Pulmonary fibrosis in HPS is characterized by extensive proliferation of type II epithelial cells with characteristic foamy swelling, patchy fibrosis with lymphocytic and histiocytic infiltration, and honeycomb changes (6). However, clinical features of HPS are not always distinct, in fact the severity of the clinical features and the degree of albinism can vary significantly in patients. Therefore, pulmonary fibrosis in unidentified HPS patients with mild extrapulmonary involvement might be easily mistaken for idiopathic pulmonary fibrosis (IPF).

IPF is associated with a histopathological or radiological pattern of *usual interstitial pneumonia* (UIP) (7, 8). However, lung histopathology of patients with HPS also typically demonstrates the UIP pattern found in IPF, whereas the radiographic CT findings are not always consistent with UIP (9,10). The course of pulmonary fibrosis in both IPF and HPS is progressive and lethal.

Currently, there is no medical treatment to prolong life in these patients and without lung transplantation they usually die due to rapid clinical deterioration (5, 6, 11).

Methods

Patient selection

We performed a cohort study of patients diagnosed with IPF enrolled in the Interstitial Lung Disease database of our hospital until March 2013. Patients were included after revision of the diagnosis based on the IPF criteria set by the ATS/ERS/JRS/ALAT (8). Medical records were screened for clinical features of HPS by two independent investigators (table 1) (3,4). In patients who had in addition to pulmonary fibrosis two or more clinical features of HPS DNA sequence analysis of the *HPS1* gene was performed. To determine the frequency of sequence variation, DNA of our Dutch control group of 100 self-reported healthy hospital employees was used. All subjects gave formal written informed consent.

DNA analysis

Genomic DNA was extracted from peripheral blood of each individual using standard methods. We amplified and sequenced the coding exons and their exon-intron boundaries of *HPS1* gene. (For further information see table 1 of the supplementary data.)

The frequency of sequence variations were determined in the IPF cohort and in 100 controls using high-resolution melting (HRM) analysis (ABI Fast 7500RT; Applied Biosystems, Foster City, CA). The primers used were HPS1 13fw HRM 5′-CCTCTCGGCCCTTACCCTCA-3′ and HPS1 12-13 rv primer 5′-CTGCTGTGGACCGGATGTA-3′.

To determine allelic phase, two allele specific PCR's were performed. The forward primer was either specific for the wildtype sequence (primer 5′-GGCCCTGGTTCTGTCCCA-3′) or for the mutant sequence (primer 5′-GCCCTGGTTCTGTCCA-3′). While the reverse primer was similar in both reactions (primer 5′- CTGCTGTGGACCGGATGTA-3′). Subsequently, both PCR products were sequenced using only the reverse primer in each sequence reaction.Prediction of deleterious amino acid change was performed online at http://sift.jcvi.org/ using default settings in Sorting Intolerant From Tolerant (SIFT) (12).

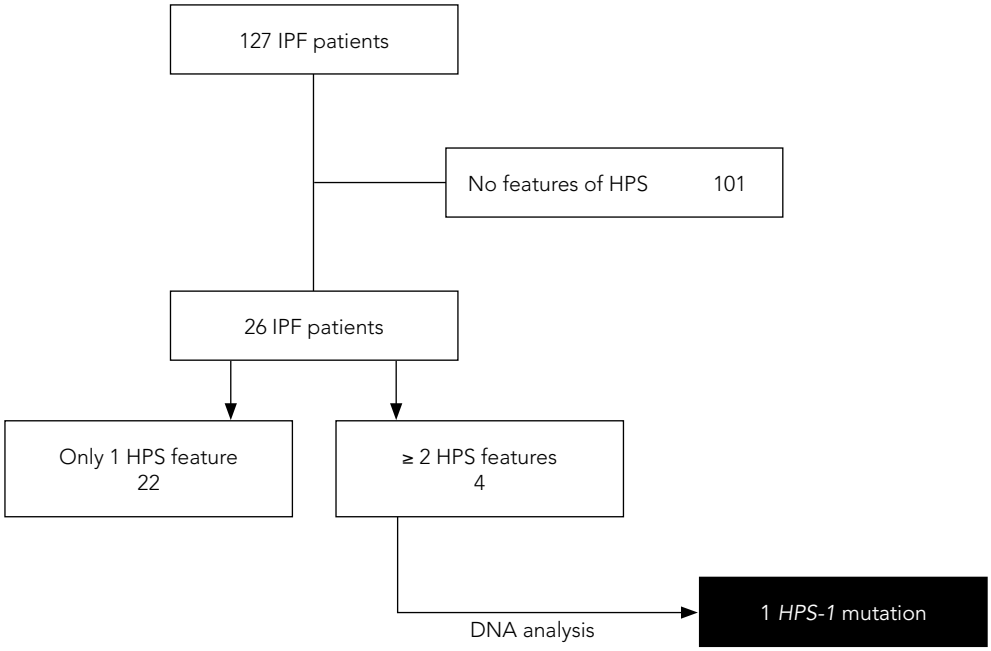
Results

IPF cohort

The cohort consisted of 127 patients including 108 male and 19 female. Mean age at time of diagnosis was 64,0 years (SD 10.6 years). Out of 127 patients, 26 patients had besides pulmonary fibrosis, at least one clinical feature consistent with HPS. Most frequently patients had decreased visual acuity, hypercholesterolemia or bleeding diathesis (table 1). Within this group of 26 patients, 4 patients (PF1, PF2, PF3 and PF4) had 2 or more clinical characteristics of HPS (flowchart 1).

Clinical feature	Associated with HPS subtype	No. of patients with clinical feature
Restrictive lung disease	1, 2, 4	127
Variable pigment dilution	1, 2, 4, 7-9	3
Decreased visual acuity	1-4, 9	11
Nystagmus	1-9	1
Haemorrhage, prolonged bleeding, easy bruising	1-8	7
Granulomatous colitis	1, 4	1
Conductive hearing loss	2	1
Neutropenia, immunodeficiency	2	1
Dysplastic acetubulae	2	0
Poor balance	2	0
Hypercholesterolemia	5	8

Table 1: Overview of clinical features of HPS with associated subtype in our IPF cohort



Flowchart 1: Selection of IPF cohort by HPS clinical features

DNA sequence analysis of the *HPS1* gene was performed in these 4 patients and identified two mutations in exon 13, Q397S**fs**X1 and R439X mutation in patient PF1. Allele specific PCR revealed that the mutations were located on separate alleles (figure 1).

The Q397S**fs**X1 mutation found was a deletion of a cytosine, predicted to change a glutamine to a serine at codon 397 and causing a frameshift resulting in a stop codon at position 398. The R439X mutation was a thymine to cytosine substitution resulting in a stop codon at position 439 of the HPS1 protein. Allelic phase analysis determined that each allele carried one of the mutations, meaning that this patient is compound heterozygote.

Genotyping showed that both mutations were not present in the IPF patients or in the healthy control subjects. The summary of sequence variations is given in table 2. In patient PF4 two non-synonymous variants Pro491Arg (rs2296434) and Gln603Arg (rs2296436) were found. SIFT analysis predicted no consequences on protein function for these variants.

cDNA position*	**Site of variant**	**Variant name^**	**Consequence**	**Patient**	**rs number**
c.297C>T	exon 5	p. Thr99=	synonymous	PF4	rs11539873
c.399-35G>A	intron 5			PF2	rs11591594
c.507+61C>G	intron 6			PF1, PF4	rs1886728
c.636C>T	exon 7	p.Leu212=	synonymous	PF1, PF3	rs1801287
c.937+78C>T	intron 10			PF4	rs1061134
c.937+88C>T	intron 10			PF4	rs34533614
c.1187delC	exon 13	p.Q397SfsX1	Frameshift	PF1	
c.1315C>T	exon 13	p.R439X	Stop codon	PF1	
c.1335+48G>A	intron 13			PF4	rs41317034
c.1397+7G>C	intron 14			PF3	rs2296432
c.1397+8G>C	intron 14			PF3	rs2296433
c.1472C>G	exon 15	p.Pro491Arg	nonsynonymous	PF4	rs2296434
c.1808A>G	exon 18	p.Gln603Arg	nonsynonymous	PF4	rs2296436

Table 2: Identified variants in *HPS1*gene

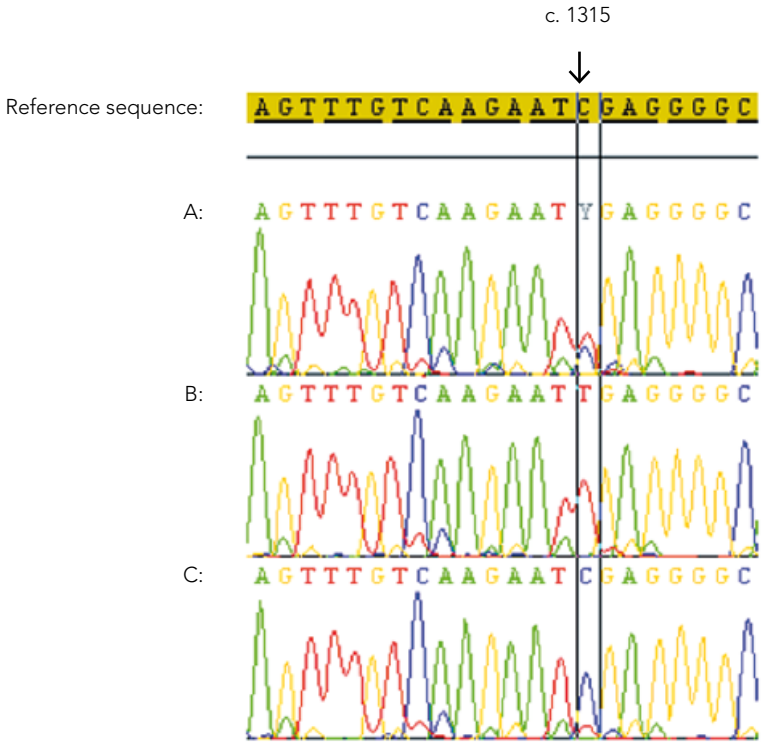


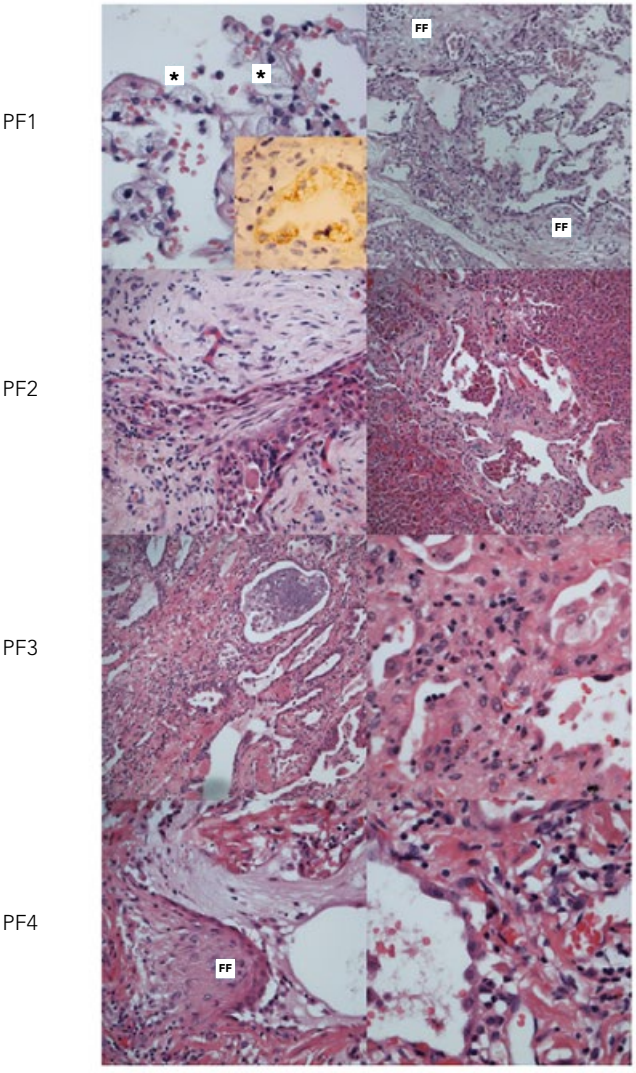
Figure 1: Allelic phase analysis for Q397SfsX1 (at cDNA position c. 1187) and R439X (at cDNA position c.1315); A: sequence of generic PCR product made of both alleles, demonstrating a heterozygous C →T substitution at position c.1315; B: sequence of allele specific PCR product made of c.1187C wildtype allele, demonstrating a C → T substitution at c.1315; C: sequence of allele specific PCR product made of c. 1187C deletion allele, demonstrating a wildtype C nucleotide at position c.1315.

Patient characteristics

Patient PF1 is a 62-year-old Caucasian female diagnosed in 2006 with PA-proven idiopathic pulmonary fibrosis. She is a non-smoker and without significant family history for pulmonary diseases. She had a medical history of oculocutaneous albinism, hysterectomy for excessive bleeding, easy bruising, normocytic anemia and actinic keratosis.

She consulted our outpatient clinic in 2008 for expert opinion. She complained about dyspnea and dry cough. Saturation of peripheral oxygen (SpO2) was 94% and pulmonary function testing demonstrated a vital capacity of predicted of 35% (1.04 L) and a Tiffeneau index of 85%. High-resolution computed tomography showed bronchiectasis, diffuse ground-glass opacities and interstitial fibrosis predominantly in the bases of the lungs, but no presence of honeycombing.

Revision of the lung biopsy confirmed a pattern consistent with UIP. Furthermore, pro-sp-C stained biopsy showed foamy type II cells which are characteristic for HPS (figure 2). After suspicion for HPS platelet function tests determined impaired secondary aggregation response and decreased concentration of ADP, ATP and serotonin, which are features of platelet storage pool disease.
Patient PF2 is a Caucasian female diagnosed with IPF in 2002. She has a distinct pale skin and light-colored hair. She is visually impaired and has severe conductive hearing loss. Furthermore, she had a hysterectomy due to menometrorrhagia.
Patient PF3 was a Caucasian male diagnosed in 1999 with IPF for which he received an unilateral lung transplantation in 2005. Seven years post-lung transplantation he died at the age of 70 years because of metastasized atypical fibroxanthoma. Besides the restricted lung function due to IPF, he had an impaired visual function and hypercholesterolaemia.
Patient PF4 was a Caucasian female with a medical history of hysterectomy and common variable immune deficiency (CVID). She was diagnosed with IPF in 1999 and listed for unilateral lung transplantation in 2005. She died 8 months after listing at the age of 58 years.



FF = fibroblastic foci
***** = foamy type 2 pneumocytes

Figure 2: Histopathology of patient PF1, PF2, PF3 and PF4
PF1: Left high power H&E stained lung tissue showing slender alveolar septae with foamy type II pneumocytes. This was immunohistochemically confirmed with the pro-surfactant C staining (inset). The cells where negative with the CD68 macrophage marker (not shown). Right: more fibrous lung tissue with fibroblast foci. To a lesser extent also in these areas foamy type II pneumocytes were found.
PF2: High power H&E stained fibrotic lung tissue with a large fibroblast focus. The alveolar spaces are filled with macrophages (also right panel), consistent with DIP pattern.
PF3: Left and right: medium power H&E stained fibrotic lung tissue. The pneumocytes do not show the foamy aspect as was seen in PF2.
PF4: Left and right: medium and high power fibrotic lung tissue. Foamy macrophages were not seen. In some alveoli debris was seen, possible associated with passed pneumonia.

Discussion

The prevalence of HPS seems to be underestimated because clinical features of HPS are not always very distinct which can result in mis-diagnosis or un-diagnosis. Pulmonary fibrosis is one of the clinical features of HPS and has several similarities to IPF. We conducted a cohort study and we identified one compound heterozygous patient with two mutations in the *HPS1* gene.

The two identified mutations were Q397S**fs**X1 and R439X. Both mutations cause an early stop codon resulting in a truncated protein, like most of the *HPS1* gene mutations reported to date. The Q397S**fs**X1 mutation was previously reported in patients with HPS-1 of Caucasian origin (13). Frequency analysis in 100 healthy controls of Dutch origin showed no carriership for this mutation, suggesting this mutation is not common in the Dutch population.

Furthermore, we found two non-synonymous variants in PF4, Pro491Arg (rs2296434) and Gln603Arg (rs2296436). These variants have previously been described in literature and are considered to be non-pathologic DNA polymorphisms (14). Indeed SIFT analysis predicted no consequences on protein function for these variants.

It should be noted that we screened the study population for all clinical features identified in the different subtypes of HPS-1 to HPS-9, however the analysis has concentrated on HPS-1. Although pulmonary fibrosis is most commonly associated with HPS-1, it is also reported in a few cases of HPS-2. HPS-2 is caused by mutations in the *AP3B1* gene. HPS-1 and HPS-2 have similar clinical features, however immune-related comorbidities are specifically associated with HPS-2 (13,15). Patient PF4 was known with common variable immunodeficiency (CVID). CVID is an autosomal dominant or recessive inherited disorder affecting the humeral immune system resulting in increased susceptibility to infections and diminished responses to protein and polysaccharide vaccines (16). To investigate the possibility that this patient had HPS-2 we also sequenced the coding regions of the *AP3B1* gene, but no mutation was found (data not shown).

This study shows that specific clinical features can identify patients suspected for HPS. Out of 127 IPF patients only 4 patients had 2 or more clinical features of HPS besides pulmonary fibrosis and 1 patient could be diagnosed with the syndrome. Genetic analysis should be considered in these patients to exclude HPS as an underlying cause of pulmonary fibrosis. However, in general these patients undergo extensive clinical tests, whereas targeted genetic analysis is fast and

simple and can easily establish the diagnosis. It illustrates, due to increasing knowledge of genetic disorders, that cooperation between the physician and geneticist is in favor of the patient. Notwithstanding its limitations of a retrospective study design, this study suggests that increased awareness of clinical features of HPS followed by gene specific analysis, can adequately diagnose these patients.

Although HPS is a rare syndrome, because of the clinical consequences physicians should be aware that it can be the underlying cause of pulmonary fibrosis.

This is particularly important when HPS patients have severe pulmonary fibrosis and are in need of lung transplantation. In the past lung transplantation in patients with HPS has been considered not possible due to bleeding diathesis, however successful lung transplantation is reported for the first time in 2005 (17). Therefore, lung transplantation is considered to be a treatment option in HPS, although careful assessment of bleeding risks and of pre-operative and intra-operative precautions should be taken.

To our knowledge this is the first study that investigates the prevalence of HPS1 in an IPF cohort. Further investigation is needed for better determination of the occurrence of HPS and to increase awareness for HPS among physicians worldwide. Moreover, we would like to emphasize that due to advancing knowledge, cooperation between chest physicians and clinical geneticists should be encouraged.

References

(1) HERMANSKY F, PUDLAK P. Albinism associated with hemorrhagic diathesis and unusual pigmented reticular cells in the bone marrow: report of two cases with histochemical studies. *Blood* 1959;14:162-169.

(2) Gahl WA, Brantly M, Kaiser-Kupfer MI, Iwata F, Hazelwood S, Shotelersuk V, Duffy LF, Kuehl EM, Troendle J, Bernardini I. Genetic defects and clinical characteristics of patients with a form of oculocutaneous albinism (Hermansky-Pudlak syndrome). *N Engl J Med* 1998;338:1258-1264.

(3) Wei AH, Li W. Hermansky-Pudlak syndrome: pigmentary and non-pigmentary defects and their pathogenesis. *Pigment Cell Melanoma Res* 2013;26:176-192.

(4) Cullinane AR, Curry JA, Carmona-Rivera C, Summers CG, Ciccone C, Cardillo ND, Dorward H, Hess RA, White JG, Adams D, Huizing M, Gahl WA. A BLOC-1 mutation screen reveals that PLDN is mutated in Hermansky-Pudlak Syndrome type 9. *Am J Hum Genet* 2011;88:778-787.

(5) Gochuico BR, Huizing M, Golas GA, Scher CD, Tsokos M, Denver SD, Frei-Jones MJ, Gahl WA. Interstitial lung disease and pulmonary fibrosis in Hermansky-Pudlak syndrome type 2, an adaptor protein-3 complex disease. *Mol Med* 2012;18:56-64.

(6) Grutters JC, du Bois RM. Genetics of fibrosing lung diseases. *Eur Respir J* 2005;25:915-927.

(7) King TE,Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet* 2011;378:1949-1961.

(8) Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE,Jr, Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schunemann HJ, ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788-824.

(9) Avila NA, Brantly M, Premkumar A, Huizing M, Dwyer A, Gahl WA. Hermansky-Pudlak syndrome: radiography and CT of the chest compared with pulmonary function tests and genetic studies. *AJR Am J Roentgenol* 2002;179:887-892.

(10) Pierson DM, Ionescu D, Qing G, Yonan AM, Parkinson K, Colby TC, Leslie K. Pulmonary fibrosis in hermansky-pudlak syndrome. a case report and review. *Respiration* 2006;73:382-395.

(11) Thabut G, Mal H, Castier Y, Groussard O, Brugiere O, Marrash-Chahla R, Leseche G, Fournier M. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. *J Thorac Cardiovasc Surg* 2003;126:469-475.

(12) Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009;4:1073-1081.

(13) Oh J, Ho L, Ala-Mello S, Amato D, Armstrong L, Bellucci S, Carakushansky G, Ellis JP, Fong CT, Green JS, Heon E, Legius E, Levin AV, Nieuwenhuis HK, Pinckers A, Tamura N, Whiteford ML, Yamasaki H, Spritz RA. Mutation analysis of patients with Hermansky-Pudlak syndrome: a frameshift hot spot in the HPS gene and apparent locus heterogeneity. *Am J Hum Genet* 1998;62:593-598.

(14) Bailin T, Oh J, Feng GH, Fukai K, Spritz RA. Organization and nucleotide sequence of the human Hermansky-Pudlak syndrome (HPS) gene. *J Invest Dermatol* 1997;108:923-927.

(15) Shotelersuk V, Hazelwood S, Larson D, Iwata F, Kaiser-Kupfer MI, Kuehl E, Bernardini I, Gahl WA. Three new mutations in a gene causing Hermansky-Pudlak syndrome: clinical correlations. *Mol Genet Metab* 1998;64:99-107.

(16) Salzer U, Warnatz K, Peter HH. Common variable immunodeficiency - an update. *Arthritis Res Ther* 2012;14:223.

(17) Lederer DJ, Kawut SM, Sonett JR, Vakiani E, Seward SL,Jr, White JG, Wilt JS, Marboe CC, Gahl WA, Arcasoy SM. Successful bilateral lung transplantation for pulmonary fibrosis associated with the Hermansky-Pudlak syndrome. *J Heart Lung Transplant* 2005;24:1697-1699.

Supplementary Information

exon	Forward primer	5′ – 3′ sequence	Reverse primer	5′ – 3′ sequence	Product size (bp)	Anneal temperature
3	3fw	GCCCCGTGGTGGGTCTGTTG	3rv	AGGATGCTGGACCCCACCCAG	587	68
4 and 5	4+5fw	GTGTCTGGCCCACTCTAAGC	4+5rv	GTGCTCGGCAAAGGACAG	589	60
6	6fw	GAGCTCGGCCGGAAACCTGG	6rv	AGGGGCTGCTTGTGCCTTCA	378	68
7 and 8	7+8fw	GTGGGGCAAGGTTGTTGG	7+8rv	CAGGTTTTCTGAACTACCTCCCTA	833	60
9 and 10	9+10fw	TGGAGGCTTCTCTGTGAAGC	9+10rv	CTTTAGGGATCAGTGGAGTGGT	630	62
11	11fw	GCCATTGCTTACATCTCATGG	11rv	TACTCACTGCGGCATCTCAG	164	60
12 and 13	12+13fw	CAATGGGTGGGTCCAACTTA	12+13rv	CTGTGGACCGGATGTA	580	62
14	14fw	TGTGTGCCTGTGCCCGTCAG	14rv	TCCCAGCTTCTCCACGCGGT	478	72
15 and 16	15+16fw	AACAAGGTGGTCCACACAGG	15+16rv	CCCCACCCCTCGTGGAA	670	62
17	17fw	ACAGCTAGAAGCCCAGGACA	17rv	ATCACACCAGGAAGGGCTAC	289	60
18	18fw	CTTTCCTTCCTTCGGAGGTG	18rv	TGGGCTTACCAGCAACAAGA	320	60
19 and 20	19+20fw	ACTCAGGCCCTTGCACTACC	19+20rv	GGGAACAGTGGCAAGCAA	814	68

All PCR amplifications were performed using HotStarTaq DNA Polymerase (Qiagen, Venlo, the Netherlands), 3 mM MgCl2. Reaction conditions: 10 min. 95 °C, 30 cycles of 30 sec. 94°C, 60 sec Ta (°C), 60 sec. 72°C, followed by a cycle of 3 min. 72°C. Sequencing was performed on purified PCR amplicons using Big Dye® Terminator v3.1 Cycle Sequencing Chemistry (Applied Biosystems, Foster City, USA).

CHAPTER NINE

GENERAL DISCUSSION

Aim

The broad aim of the thesis was to evaluate the results of lung transplantation (LTX) in patients with idiopathic pulmonary fibrosis (IPF) in the Netherlands in order to identify factors influencing the outcome of this highly complex treatment modality and find points of application to optimize LTX in the future. In addition, potentially relevant molecular and genetic characteristics were studied in the Antonius IPF cohort aiming at finding new targets for treatment optimisation of patients care.

Chapter 2 described all IPF patients referred for LTX in the Netherlands and revealed that one-third of the IPF patients died awaiting LTX.

Chapter 3 demonstrated a 10-year survival time after LTX in IPF patients in the Netherlands.

Chapter 4 demonstrated that IgA in serum is significantly correlated to survival outcomes in IPF patients and might be a potential prognostic biomarker.

Chapter 5 described clinical and molecular characteristics in *TERT* mutations carriers and demonstrated a significant worse survival in the first 18 months after diagnosis compared to sporadic IPF patients. Furthermore, data showed significant higher levels of IgA in serum in *TERT* mutations carriers.

Chapter 6 reported the clinical course after LTX in *TERT* mutation carrier and identified possible *TERT*-related problems in the post-LTX period .

Chapter 7 reported the genetic analysis of *SFTPA2* in a cohort of familial and sporadic patients with IIP and described the clinical course of these patients, which is characterised by the occurrence of malignancies.

Chapter 8 described the prevalence of Hermansky-Pudlak syndrome (HPS) in a cohort of IPF patients and discussed possible consequences of HPS for LTX.

Waiting list mortality

The key finding in chapter 2 was that the mortality of IPF patients on the waiting list for LTX in the Netherlands was high: 33% of the IPF patients died awaiting LTX. Evaluation of clinical data demonstrated that these patients had advanced disease reflected by severe diminished mean diffusion capacity (DLco) of 27% of predicted. Moreover, this could very well be an overestimation, since approximately 40% of the screened IPF patients were not able to perform diffusion test due to their poor clinical condition. These findings support the concern that a significant portion of IPF patients were listed for LTX too late.

To reduce waiting list mortality there are three essential elements to optimize: 1) the allocation of donor lungs; 2) the availability and management of donor lungs; and 3) bridging strategies of LTX recipients.

The allocation of donor lungs

In 2001 Eurotransplant introduced the possibility to list patients as 'high urgent' (HU). We found that after implementation of the HU-system in 2001 the waiting list mortality of IPF patients in the Netherlands decreased from 42% to 30%, which demonstrates that changes in allocation systems can have significant impact. The main goal of the introduction of the Lung Allocation Score (LAS) in May 2005 in the United States was also to decrease waiting list mortality. The LAS is a standardized numeric score ranging from 0 to 100 points that seeks to identify patients likely to benefit from LTX. The LAS gives priority to candidates who are most urgently in need of a transplant and who are expected to have the greatest benefit, implicating that the patient with the highest LAS will be the first one to receive the lung offer (1). After introduction of the LAS, waitlist deaths decreased significantly, from 500 per year to 300 per year (2). Furthermore, the distribution of recipient diagnoses changed dramatically, with significantly more patients with fibrotic lung disease receiving transplants (2-4). However, several investigations of transplant recipients also reported an association between high LAS and increased death after LTX, which indicates that the LAS needs further optimisation (5-7).

The availability and management of donor lungs

Currently, the number of patients waiting for LTX greatly exceeds the number of donors available and shortage of donors is an important factor that contributes to waiting list mortality. Two major components contribute to this deficit: 1) an overall low number of brain death donors and 2) a low rate (15-20%) of clinically acceptable lungs among those donors (8). To extend the donor pool different strategies have been adopted, such as the use of lungs from donors with extended criteria (high-risk donors) and/or donors from donation after cardiac death (DCD) (9). Furthermore, a new promising technique called *ex vivo* lung perfusion (EVLP) has emerged as a strategy to better assess extended criteria donor lungs prior to transplantation (10). EVLP-treated donor lungs can remain in a functional physiological state for several hours (11). During this period, lungs can be assessed, reconditioned, and repaired via a number of mechanisms:

dehydration of lung tissue; removal of harmful and toxic waste products; recruitment of atelectatic areas resulting in better ventilation/perfusion matching; and improved microcirculation (11, 12). Thus, EVLP has opened an important door for the potential assessment, diagnosis and repair of injured donor lungs that would otherwise be clinically discarded now hold promise to be included into the donor pool.

Bridging strategies of LTX recipients

It is important that the recipient obtains a relatively good clinical condition till LTX. IPF patients are at risk of developing acute respiratory failure (ARF). The management of ARF in IPF is difficult and intubation has been shown to be associated with a high mortality rate (13-16). In these patients extracorporeal membrane oxygenation (ECMO) or extracorporeal life support (ECLS) can be considered. In patients on ECMO, oxygen supply and decarboxylation are mainly separated from mechanical ventilation. This enables lung protective ventilation and might prevent ventilator-induced lung injury and further right ventricular dysfunction. Recent study demonstrated that patients with an interstitial lung disease (ILD) with an option for LTX benefit from ECMO therapy because of the time gained on ECMO (17).

Prognostic biomarkers

Prognostic markers are important for adequate timing of referral and listing of patients for LTX. Clinical factors like decline of pulmonary function, presence of pulmonary hypertension and the 6-minute walking test are established prognostic factors in IPF patients. Moreover, the use of prognostic biomarkers in blood or lavage fluid in IPF patients might be helpful to predict individual prognosis and provide a tool for optimisation of the timing of LTX.

The LAS is a calculated value designed to reflect disease severity and predict outcome after transplantation. Patients placed on the waiting list for lung transplantation are assigned a LAS value that has to be recalculated at least every 6 months. New factors that have been determined to be important predictors of death on the waiting list and have shown to be markers of disease severity can be incorporated into the algorithm, and the hazard ratios for established variables can be modified based on these analyses.

For example, biomarkers that can predict deterioration in patients may provide important information regarding the timing of listing. As newer information accrues, the algorithm can be modified to include these prognostic markers in the future.

In an attempt to predict disease course in IPF patients we studied IgA serum levels as a prognostic biomarker and found that the level of IgA in serum was significantly correlated with survival in IPF patients (chapter 4). Transforming growth factor-β (TGF-β) is upregulated as a result of repeated injury of alveolar epithelial cells and TGF-β is thought to be responsible for differentiation of fibroblasts to myofibroblasts and the formation of typical fibroblast foci in IPF (18). Moreover, TGF-β is involved in multiple signalling processes and one of the characterised effects of TGF-β is its ability to stimulate immunoglobulin (Ig) isotype switching to IgA (19, 20). We hypothesized that an increase in activated TGF-β is reflected by an increase in IgA in blood and therefore high levels of IgA in blood reflects the degree of fibrosis in the lungs. In chapter 5 we described significantly higher levels of IgA in serum in *TERT* mutation carriers compared to sporadic IPF patients and suggested that the poor survival in *TERT* mutation carriers is probably due to severe fibrotic active lung disease. IgA in serum is a common assay in almost all hospitals in the Netherlands and developed countries and is not an expensive test and therefore can be easily introduced as a biomarker in clinical practice.

Survival outcomes after LTX

Another major finding of this thesis is that the survival outcome of LTX in IPF patients in the Netherlands is relatively good. Survival analysis demonstrated a median survival of IPF patients after LTX of 10 years (chapter 3). In comparison, the last report from the ISHLT (International Society for Heart and Lung Transplantation) showed a mean survival after LTX in IPF of only 4.7 years (21). There are several possible explanations for this difference in survival outcome. At first, the ISHLT data included information of all transplants for whom any follow-up has been provided and the survival rate seems to be estimates rather than exact rates because the time of death is not known for all patients. Furthermore, country-specific factors could have contributed to the outstanding survival results.

Due to donor scarcity in the Netherlands, it is possible that stronger patient selection criteria were applied than in other countries. Indeed, our transplanted patients were slightly younger, had less comorbidities and therefore might have had a better clinical condition prior to LTX. Moreover, the better survival outcome might be explained by accurate follow up after LTX in the Netherlands, and also the fact that the patients visit the transplant centres on a regular basis, unhampered by long travel distances in the Netherlands.
Analysis of the ISHLT registry have shown that survival is better in bilateral LTX than single LTX recipients (median survival, 6.7 vs 4.6 years; $p < 0.001$), although this association is confounded by the large differences between these populations, particularly in respect to patients' underlying conditions (3). More focused analysis of the ISHLT and United Network for Organ Sharing (UNOS) registries have shown improved 10-year survival with bilateral LTX in patients with chronic obstructive pulmonary disease (COPD) but equivalent 5-years outcomes between bilateral and single LTX in patients with IPF (22, 23). In this thesis we demonstrated a significant survival benefit of bilateral LTX compared to single LTX in IPF patients for both short-term and long-term survival in the pre-LAS era (chapter 3). However, the use of the LAS has led to a change in the demographics of single and bilateral LTX-recipients. A recent study assessed the posttransplantation outcomes after implementation of the LAS and demonstrated that bilateral LTX was associated with better survival than single LTX in patients with IPF (24). Nevertheless, an important advantage of single LTX is that two patients can be accommodated by one donor and therefore provides a more efficient use of the limited donor pool. Future investigation should indicate whether the LAS effects the survival outcomes of IPF in the Netherlands.

Genetic and molecular characteristics

Recent studies indicate that mutations in different biologic pathways lead to the common phenotypes of familial interstitial pneumonia (FIP) and sporadic IPF. Recognition and further understanding of these subtypes can be of importance, because these patients might have a different course of disease, relevant comorbidities or are in need of different immunosuppressive regimes after LTX.

In this thesis we described clinical and molecular characteristics in patients with mutations in the genes encoding telomerase, lung surfactant proteins and lamellar bodies to advance our understanding of IPF and possible significance to lung transplant management.

Recognition of the clinical spectrum of the telomere syndromes and their various clinical manifestations might potentially influence available treatments, such as LTX (25). In affected patients symptoms related to pulmonary fibrosis predominate, however, in case of molecular defects in telomere maintenance, patients often have subclinical disease concurrently in other organs, because the telomere defect and reduced telomere length are systemic (26). For example, patients with IPF who have mutant telomerase genes are at increased risk of developing bone marrow failure and liver disease (27, 28). Three recent observational studies evaluated in total 31 patients with a mutation in the telomerase complex (*TERT* or *TR*) who underwent LTX (29-31). The most common complications after LTX were haematological, including thrombocytopenia, anaemia, leukopenia and a few patients developed myelodysplastic syndrome and/or bone marrow failure. Significant adjustment of the immunosuppressive regimen was required in almost all cases and most patients were able to tolerate only two drug immunosuppressive regimen. Other frequent observed complications were renal failure and respiratory infections or even sepsis, while three patients developed a malignancy.

In chapter 7 we report the genetic analysis of *SFTPA2* in a cohort of familial and sporadic patients with IIP. We described the clinical course of 3 *SFTPA2* mutation carriers of which 2 patients underwent LTX. Remarkably, 2 out of 3 patients developed a malignancy, both an adenocarcinoma of the lung (formerly known as bronchioloalveolar cell carcinoma). The underlying mechanism of tumorigenesis in *SFTPA2* mutation carriers is not clear, however, it is thought that transient increase of ER stress causes reduced senescence and promotion of tumorigenesis (32). Even though only limited data is available, *SFTPA2* mutation carriers seem to have an increased risk for lung cancer, especially when treated with immunosuppressive medication, and we suggest that transplantation should be bilateral in these patients. Furthermore, the potential role of ^{18}F-FDG-PET/CT in detecting malignancies in IIP and routinely use of the ^{18}F-FDG-PET/CT in the screening for LTX needs further investigation.

Dysfunctional lamellar bodies in type II alveolar epithelial cells were identified as a cause of pulmonary fibrosis in Hermansky-Pudlak syndrome (HPS) (33). In chapter 8 we described the occurrence of HPS in a cohort of IPF patients by screening medical records for clinical features of HPS. Recognition of HPS is particularly important in the context of LTX. LTX in patients with HPS was considered a contraindication due to bleeding diathesis, however successful LTX was reported for the first time in 2005 (34). To date, this is the only report that described LTX in a patient with HPS. Further investigation is needed to determine the occurrence of Hermansky-Pudlak syndrome in patients with pulmonary fibrosis and case series of HPS-patients who had a LTX are needed to establish if LTX is safe and to optimize LTX in these patients.

Future perspectives

This thesis has demonstrated that waiting list mortality, not exclusively in IPF patients, is a complex matter and we discussed several possibilities to improve this unsatisfactory situation. Extended use of ECMO as a bridge to transplantation and increasing competence when it comes to EVLP to improve the quality of the donor lungs prior to LTX are promising strategies to extend the current limited donor pool. Furthermore, this thesis enhanced the importance of timing of referral and listing for LTX and emphasized the difficulty of adequate timing due to the unpredictable course of IPF. The search for prognostic biomarkers in IPF should be continued and future research might determine the added value of IgA in serum as a prognostic biomarker for IPF in clinical practise. Ideally, new prognostic markers can be implemented in the LAS to improve the allocation of donor lungs in the future.

Furthermore, this thesis described and investigated several subtypes of IPF based on rare genetic mutations in telomere maintenance, lung surfactant proteins and lamellar bodies. Unravelling these subtypes of IPF is a challenge, however crucial since these genetic defects seems to have impact on prognosis, choice of examinations prior to LTX and immunosuppressive therapy after lung transplantation. Based on previous research and this thesis we plead that genetic evaluation of susceptible patients with pulmonary fibrosis should take place prior to LTX. Based on the current knowledge, we formulated mutation-specific recommendations for the work up of LTX.

LTX-related recommendations for patients with mutation in telomere maintenance

Possible transplant candidates with a *TERT* mutation and pulmonary fibrosis should be immediately referred to a transplant centre for further evaluation because of their poor prognosis and rapid listing is probably life-saving. The underlying *TERT* mutation should be approached as a systemic disease and patients have an increased risk of developing bone marrow failure or liver disease (25). Appropriate assessment in a centre of expertise prior to LTX is therefore recommended. However, further research is necessary to determine which investigations should be included in the standard work up in these patients. Moreover, evaluation of *TERT* mutation carriers after LTX demonstrated that adjustment of the immunosuppressive regimen was required in almost all cases and intensive management and follow up regarding hematologic problems is recommended. Future studies are warranted to investigate if reducing the intensity of the immunosuppressive regime should be standard care in *TERT* mutation carriers.

LTX-related recommendations for mutations in lung surfactant proteins

We recommend that all *SFTPA2* mutation carriers receive a bilateral LTX, because of the high risk of developing a malignancy. We promote the use of ^{18}F-FDG-PET/CT when there is the least doubt to distinguish between dense fibrosis and tumour.

LTX-related recommendations for mutations in lamellar bodies

Successful transplantation has been reported, however bleeding diathesis in these patients is a great concern. Adequate preparations and interventions to optimize haemostasis in the patient are needed.

Concluding remark

Since the first LTX in 1989 in the Netherlands many things have changed, however, there are still questions that need to be answered in order to optimize this complex treatment modality in the future. Accurate retrospective studies are needed to investigate LTX in specific patient groups like IPF. Because of the rarity of the disease and treatment modality cooperation between specialised centres is needed, inside and beyond the national borders. The establishment of a multicentre database with information of recipient and donor, would be of great value to improve LTX in specific patients groups and more importantly, in the individual patient.

References

(1) Egan TM, Murray S, Bustami RT, Shearon TH, McCullough KP, Edwards LB, Coke MA, Garrity ER, Sweet SC, Heiney DA, Grover FL. Development of the new lung allocation system in the United States. *Am J Transplant* 2006;6:1212-1227.

(2) Egan TM, Edwards LB. Effect of the lung allocation score on lung transplantation in the United States. *J Heart Lung Transplant* 2016;35:433-439.

(3) Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Dobbels F, Kirk R, Rahmel AO, Stehlik J, Hertz MI, International Society of Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report-2012. *J Heart Lung Transplant* 2012; 31:1073-1086.

(4) Gottlieb J, Greer M, Sommerwerck U, Deuse T, Witt C, Schramm R, Hagl C, Strueber M, Smits JM. Introduction of the lung allocation score in Germany. *Am J Transplant* 2014;14:1318-1327.

(5) Braun AT, Dasenbrook EC, Shah AS, Orens JB, Merlo CA. Impact of lung allocation score on survival in cystic fibrosis lung transplant recipients. *J Heart Lung Transplant* 2015;34:1436-1441.

(6) Weiss ES, Allen JG, Merlo CA, Conte JV, Shah AS. Lung allocation score predicts survival in lung transplantation patients with pulmonary fibrosis. *Ann Thorac Surg* 2009;88:1757-1764.

(7) Merlo CA, Weiss ES, Orens JB, Borja MC, Diener-West M, Conte JV, Shah AS. Impact of U.S. Lung Allocation Score on survival after lung transplantation. *J Heart Lung Transplant* 2009;28:769-775.

(8) Punch JD, Hayes DH, LaPorte FB, McBride V, Seely MS. Organ donation and utilization in the United States, 1996-2005. *Am J Transplant* 2007;7:1327-1338.

(9) Erasmus ME, van Raemdonck D, Akhtar MZ, Neyrinck A, de Antonio DG, Varela A, Dark J. DCD lung donation: donor criteria, procedural criteria, pulmonary graft function validation and preservation. *Transpl Int* 2015.

(10) Reeb J, Cypel M. Ex vivo lung perfusion. *Clin Transplant* 2016;30:183-194.

(11) Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M, Sato M, Harwood S, Pierre A, Waddell TK, de Perrot M, Liu M, Keshavjee S. Technique for prolonged normothermic ex vivo lung perfusion. *J Heart Lung Transplant* 2008;27:1319-1325.

(12) Van Raemdonck D, Neyrinck A, Cypel M, Keshavjee S. Ex-vivo lung perfusion. *Transpl Int* 2015;28:643-656.

(13) Fernandez-Perez ER, Yilmaz M, Jenad H, Daniels CE, Ryu JH, Hubmayr RD, Gajic O. Ventilator settings and outcome of respiratory failure in chronic interstitial lung disease. *Chest* 2008;133:1113-1119.

(14) Fumeaux T, Rothmeier C, Jolliet P. Outcome of mechanical ventilation for acute respiratory failure in patients with pulmonary fibrosis. *Intensive Care Med* 2001;27:1868-1874.

(15) Rangappa P, Moran JL. Outcomes of patients admitted to the intensive care unit with idiopathic pulmonary fibrosis. *Crit Care Resusc* 2009;11:102-109.

(16) Chiumello D, Coppola S, Froio S, Colombo A, Del Sorbo L. Extracorporeal life support as bridge to lung transplantation: a systematic review. *Crit Care* 2015;19:19-014-0686-7.

(17) Trudzinski FC, Kaestner F, Schafers HJ, Fahndrich S, Seiler F, Bohmer P, Linn O, Kaiser R, Haake H, Langer F, Bals R, Wilkens H, Lepper PM. Outcome of Patients with Interstitial Lung Disease Treated with Extracorporeal Membrane Oxygenation for Acute Respiratory Failure. *Am J Respir Crit Care Med* 2016;193:527-533.

(18) King TE,Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet* 2011;378:1949-1961.

(19) Banchereau J, Pascual V, O'Garra A. From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. *Nat Immunol* 2012;13:925-931.

(20) Stavnezer J, Kang J. The surprising discovery that TGF beta specifically induces the IgA class switch. *J Immunol* 2009;182:5-7.

(21) Yusen RD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Goldfarb SB, Levvey BJ, Lund LH, Meiser B, Rossano JW, Stehlik J. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report--2015; Focus Theme: Early Graft Failure. *J Heart Lung Transplant* 2015;34:1264-1277.

(22) Thabut G, Christie JD, Ravaud P, Castier Y, Brugiere O, Fournier M, Mal H, Leseche G, Porcher R. Survival after bilateral versus single lung transplantation for patients with chronic obstructive pulmonary disease: a retrospective analysis of registry data. *Lancet* 2008;371:744-751.

(23) Thabut G, Christie JD, Ravaud P, Castier Y, Dauriat G, Jebrak G, Fournier M, Leseche G, Porcher R, Mal H. Survival after bilateral versus single-lung transplantation for idiopathic pulmonary fibrosis. *Ann Intern Med* 2009;151:767-774.

(24) Schaffer JM, Singh SK, Reitz BA, Zamanian RT, Mallidi HR. Single- vs double-lung transplantation in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis since the implementation of lung allocation based on medical need. *JAMA* 2015;313:936-948.

(25) Armanios M, Blackburn EH. The telomere syndromes. *Nat Rev Genet* 2012;13:693-704.

(26) Armanios M. Syndromes of telomere shortening. *Annu Rev Genomics Hum Genet* 2009;10:45-61.

(27) Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, Vulto I, Xie M, Qi X, Tuder RM, Phillips JA,3rd, Lansdorp PM, Loyd JE, Armanios MY. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proc Natl Acad Sci U S A* 2008;105:13051-13056.

(28) Calado RT, Regal JA, Kleiner DE, Schrump DS, Peterson NR, Pons V, Chanock SJ, Lansdorp PM, Young NS. A spectrum of severe familial liver disorders associate with telomerase mutations. *PLoS One* 2009;4:e7926.

(29) Borie R, Kannengiesser C, Hirschi S, Le Pavec J, Mal H, Bergot E, Jouneau S, Naccache JM, Revy P, Boutboul D, Peffault de la Tour R, Wemeau-Stervinou L, Philit F, Cordier JF, Thabut G, Crestani B, Cottin V, Groupe d'Etudes et de Recherche sur les Maladies "Orphelines" Pulmonaires (GERM"O"P). Severe hematologic complications after lung transplantation in patients with telomerase complex mutations. *J Heart Lung Transplant* 2015;34:538-546.

(30) Silhan LL, Shah PD, Chambers DC, Snyder LD, Riise GC, Wagner CL, Hellstrom-Lindberg E, Orens JB, Mewton JF, Danoff SK, Arcasoy MO, Armanios M. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. *Eur Respir J* 2014;44:178-187.

(31) Tokman S, Singer JP, Devine MS, Westall GP, Aubert JD, Tamm M, Snell GI, Lee JS, Goldberg HJ, Kukreja J, Golden JA, Leard LE, Garcia CK, Hays SR. Clinical outcomes of lung transplant recipients with telomerase mutations. *J Heart Lung Transplant* 2015;34:1318-1324.

(32) Zhu B, Ferry CH, Markell LK, Blazanin N, Glick AB, Gonzalez FJ, Peters JM. The nuclear receptor peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) promotes oncogene-induced cellular senescence through repression of endoplasmic reticulum stress. *J Biol Chem* 2014;289:20102-20119.

(33) Brantly M, Avila NA, Shotelersuk V, Lucero C, Huizing M, Gahl WA. Pulmonary function and high-resolution CT findings in patients with an inherited form of pulmonary fibrosis, Hermansky-Pudlak syndrome, due to mutations in HPS-1. *Chest* 2000;117:129-136.

(34) Lederer DJ, Kawut SM, Sonett JR, Vakiani E, Seward SL,Jr, White JG, Wilt JS, Marboe CC, Gahl WA, Arcasoy SM. Successful bilateral lung transplantation for pulmonary fibrosis associated with the Hermansky-Pudlak syndrome. *J Heart Lung Transplant* 2005;24:1697-1699.

Nederlandse samenvatting / Dutch Summary

Longtransplantatie bij idiopathische pulmonale fibrose: oude & nieuwe concepten

Introductie

Idiopathische pulmonale fibrose

Interstitiële longziekten (ILD) is een verzamelnaam voor meer dan 100 verschillende longziekten die de alveoli (longblaasjes) en de ruimte gelegen tussen de alveoli (het interstitium) aantasten. Eén van de meest voorkomende en ernstigste vorm van ILD is idiopathische pulmonale fibrose (IPF).
IPF is een fibroserende longaandoening van onbekende oorzaak met een beperkte levensverwachting van ongeveer 3-4 jaar nadat de diagnose is gesteld. IPF treft voornamelijk mannen en een oudere leeftijd en roken zijn sterk geassocieerd met de ziekte. Patiënten presenteren zich vaak met langer dan 3 maanden bestaande klachten van progressieve kortademigheid en een (droge) hoest. IPF wordt gekarakteriseerd door een specifiek radiologisch en histopathologisch patroon genaamd *usual interstitial pneumonia* (UIP). Door de zeldzaamheid en complexiteit van de ziekte wordt het sterk aanbevolen dat de diagnose IPF gesteld wordt door een multidisciplinair team van longartsen, radiologen en pathologen in een hiervoor gespecialiseerd centrum.

Ontstaansmechanisme IPF

Het is nog onduidelijk hoe IPF ontstaat en de huidige gedachte is dat een complex samenspel van genetische aanleg, omgevingsfactoren en herhaaldelijke infecties van de long er voor zorgen dat het longweefsel keer op keer beschadigd. Deze continue beschadiging van de long leidt tot een buitensporige en oncontroleerbare genezingsreactie van de long in een poging de beschadiging te herstellen. Het oppervlakte van een alveoli bestaat voor 90% uit type I alveolaire epitheelcellen, waarover de gasuitwisseling van de long plaats vindt, en slechts uit enkele type II alveolaire epitheelcellen. Deze type II alveolaire epitheelcellen zijn multifunctioneel. Ze zorgen voor de uitscheiding van surfactant, maar kunnen ook functioneren als antigeen-presenterende cellen in het geval van een infectie en zijn ze in staat te regenereren in het geval van schade aan het epithelium van de alveoli. De huidige gedachte is dat continue beschadiging van het epitheel van de long leidt tot het 'lekken' van allerlei eiwitten (zoals fibrinogeen) in de alveolaire ruimte en het interstitium en daardoor ontstaat er een stolsel. In een poging deze beschadiging te herstellen zijn de alveolaire epitheelcellen geactiveerd, maar in het geval van IPF op een ongecontroleerde manier.

De buitensporig geactiveerde alveolaire epitheelcellen stimuleren migratie van fibrocyten en fibroblasten naar de plek van beschadiging en activeren onder andere *transforming growth factor-β* (TGF-β). TGF-β is een bijzonder veelzijdig cytokine die een bewezen belangrijke rol speelt in fibroserende processen. Er ontstaat een dusdanig pro-fibrotisch milieu waarin fibrosevorming continu wordt gestimuleerd. Dit leidt uiteindelijk tot een definitieve remodellering van het longweefsel door de vorming van honinggraatcysten en fibrose.

Genetische factoren in IPF

Bij een substantieel deel van de patiënten komt longfibrose in de familie voor. In 2 – 19% van de gevallen vermeldt de patiënt ten minste 1 eerstegraads familielid te hebben met een bepaalde vorm van een idiopathische interstitiële longziekte. Er wordt dan gesproken van familiaire longfibrose. Op basis van patiëntkarakteristieken is het niet goed mogelijk onderscheid te maken tussen patiënten met familiaire longfibrose en sporadische IPF, behalve dat de patiënten met familiaire longfibrose over het algemeen wat jonger zijn als de diagnose wordt gesteld. Het is ook nog niet duidelijk of patiënten met familiaire longfibrose een ander ziektebeloop hebben of anders reageren op bepaalde behandelingen, zoals longtransplantatie.
Wetenschappelijke studies hebben tot op heden verschillende genen geïdentificeerd die via verschillende ontstaansmechanismen (*pathways*) kunnen leiden tot longfibrose. Er worden onder andere mutaties gevonden in genen die coderen voor surfactant eiwitten A2 en C (respectievelijk *SFTPA2* en *SFTPC*), telomerase (*TERT* en *TERC*) en *lamellar body* eiwitten (*ABCA3* en *HPS1).*

Ziektebeloop IPF

Het ziektebeloop van patiënten met IPF wordt gekarakteriseerd door onherstelbare achteruitgang van de longfunctie met uiteindelijk de dood tot gevolg door respiratoire insufficiëntie danwel door complicerende comorbiditeiten (het tegelijkertijd voorkomende andere ziekten zoals infectie, longembolie of longkanker). Zoals eerder gezegd is de gemiddelde overleving van een patiënt met IPF 3 – 4 jaar, maar dit kan van patiënt tot patiënt erg verschillen.

De meeste patiënten tonen een langzaam progressief beloop over de jaren heen, maar in 10 – 15% van de patiënten is de ziekte grillig en kan er een plotselinge achteruitgang plaatsvinden die in een korte tijd van enkele maanden tot de dood leidt. In sommige gevallen is er een aanwijsbare oorzaak voor deze achteruitgang, maar in andere gevallen ook niet en dan spreekt men van een acute exacerbatie van IPF.
Het mechanisme van een acute exacerbatie van IPF is nog niet begrepen en helaas zijn er ook nog geen goede biomerkers (bepaalde stoffen in het lichaam die toestand van een ziekte weergeven) voorhanden om de het beloop van de individuele IPF-patiënt te voorspellen. Beiden zijn belangrijk voor het opstellen van een goed individueel behandelplan en voor het bepalen van het juiste tijdstip van verwijzen en aanmelden voor longtransplantatie.

Medicamenteuze behandeling van IPF

In de laatste 10 jaar is er veel progressie geboekt wat betreft klinische trials naar de medicamenteuze behandeling van IPF, met name bij patiënten met een milde vorm van IPF. In 2011 is er een nieuwe internationale richtlijn voor de management van IPF gepubliceerd door de ATS/ERS/JRS/ALAT waarin wetenschappelijk onderbouwde aanbevelingen worden gedaan en recentelijk is deze richtlijn vanwege nieuwe onderzoeken vernieuwd en aangepast. In deze richtlijn wordt behandeling met fibrose-remmende medicamenten zoals nintedanib en pirfenidon sterk aanbevolen en deze medicamenten vormen tegenwoordig dan ook de hoeksteen voor de medicamenteuze behandeling van IPF. Echter tot op heden is het niet mogelijk om patiënten met IPF te genezen. In een geselecteerde groep IPF-patiënten is dan de enige mogelijkheid om het leven eventueel te verlengen een longtransplantatie (LTX).

Longtransplantatie

LTX is een bijzonder complexe behandeling en alleen een behandeloptie voor patiënten met een eindstadium longziekten en niet reageren op medicamenteuze behandeling. Lang niet iedereen komt in aanmerking voor LTX en patiënten worden voorafgaand aan LTX uitgebreid gescreend om eventuele comorbiditeiten en/of contra-indicaties in kaart te brengen. Als een patiënt een geschikte transplantatie kandidaat is, wordt deze op de wachtlijst geplaatst.

Helaas zijn er in Nederland te weinig longdonoren ten opzichte van het aantal patiënten op de wachtlijst. Sinds de start van het longtransplantatieprogramma in Nederland worden de longen verdeeld op basis van de duur op de wachtlijst van de patiënt. In 2001 werd het ook mogelijk om patiënten met een zeer beperkte levensverwachting op een 'high-urgency' lijst te plaatsen. In 2014, in navolging van de Verenigde Staten en Duitsland, werd de Lung Allocation Score (LAS) in Nederland ingevoerd. De LAS is een gewogen score van 0 – 100 die de mogelijke 'benefit' van een LTX voor de individuele patiënt weergeeft (hoe hoger de score, hoe groter de voorspelde overlevingswinst) om zo de potentiële longdonoren zo eerlijk mogelijk te verdelen. Internationaal heeft de invoering van de LAS ervoor gezorgd dat er meer patiënten met IPF een LTX ondergingen en tegenwoordig is IPF de meest voorkomende indicatie voor LTX in de Verenigde Staten.
De internationale getallen ten aanzien van de overleving na LTX van IPF-patiënten is beperkt, en liggen gemiddeld rond de 4.5 jaar. De gerapporteerde overleving is ook slechter dan die van getransplanteerde patiënten met een andere longziekte, zoals COPD of cystic fibrosis. Hoe de overleving is van IPF-patiënten na LTX is in Nederland was vooralsnog niet eerder onderzocht. Het is ook nog niet bekend welke procedure, enkelzijdige of dubbelzijdige LTX, de voorkeur heeft bij IPF-patiënten. Om deze vraag in de toekomst goed te kunnen beantwoorden, is aanvullend onderzoek nodig.

Doel van dit proefschrift en beschrijving van de hoofdstukken

Gezien het onvoorspelbare ziektebeloop en slechte prognose van patiënten met idiopathische longfibrose (IPF) en de beperkte behandelingsmogelijkheden die voorhanden zijn (en longtransplantatie (LTX) slechts een mogelijkheid is voor een geselecteerd aantal patiënten), is het doel van dit proefschrift om de resultaten van LTX bij patiënten met IPF in Nederland te evalueren en mogelijke factoren te identificeren die de uitkomst van LTX in patiënten met IPF in de toekomst zouden kunnen verbeteren.

Hoofdstuk 2 is een historisch overzicht van alle patiënten met een interstitiële longziekte (ILD) verwezen voor LTX sinds de start van het longtransplantatieprogramma in Nederland in 1989. Het hoofdstuk beschrijft het traject van aanmelding tot aan LTX. Het hoofdstuk richt zich met name op de grootste groep verwezen patiënten binnen de ILD, namelijk patiënten met IPF. Klinische patiëntkarakteristieken, zoals leeftijd, geslacht en longfunctie van deze patiënten worden beschreven. Analyse van de wachtlijststerfte toont dat een derde van de IPF patiënten die op de wachtlijst zijn geplaatst zijn overleden. Een mogelijke oorzaak voor deze hoge wachtlijstmortaliteit kan verklaard worden door het tekort aan orgaandonoren in Nederland. Daarnaast blijkt uit een aanvullende analyse van de klinische gegevens dat deze patiënten een vergevorderd stadium van longfibrose hebben met een gemiddelde diffusiecapaciteit van slechts 27% van voorspeld als zij worden gescreend voor LTX. Deze getallen zijn verontrustend en wijzen er mogelijk op dat patiënten met IPF in Nederland vaak te laat worden verwezen voor longtransplantatie.

In aanvulling op hoofdstuk 2, wordt in **hoofdstuk 3** gekeken of het mogelijk is om aan de hand van bepaalde klinische karakteristieken van patiënten op de wachtlijst te voorspellen of patiënten komen te overlijden op de wachtlijst. Hieruit blijkt dat met name de aanwezigheid van pulmonale hypertensie (hoge bloeddruk van de longslagader) gecorreleerd is met overlijden op de wachtlijst.

Hoofdstuk 3 evalueert de overleving van patiënten met IPF nadat zij een LTX hebben ondergaan in de periode 1989 – juli 2011, dat wil zeggen voor de invoering van de Lung Allocation Score (LAS) in Nederland in 2014. De gemiddelde overleving na LTX van IPF-patiënten is internationaal slechts 4.7 jaar volgens het laatste rapport van de ISHLT (International Society for Heart and Lung Transplantation). Vergeleken met deze cijfers blijkt dat IPF-patiënten in Nederland een uitstekende overleving na LTX hebben van gemiddeld 10 jaar.

Aanvullend worden de uitkomsten na een enkelzijdige en dubbelzijdige LTX met elkaar vergeleken, waaruit blijkt dat de patiënten die een dubbelzijdige LTX hebben ondergaan gemiddeld langer leven. Echter, deze resultaten moeten met terughoudendheid worden geïnterpreteerd. Een enkelzijdige LTX heeft een belangrijk voordeel ten opzichte van dubbelzijdige LTX; namelijk dat je 2 patiënten kan voorzien van een nieuwe long in plaats van 1. Gezien de huidige schaarste van orgaandonoren in Nederland is dit een zwaarwegend argument om patiënten kritisch te selecteren voor enkelzijdig danwel dubbelzijdige LTX. Aanvullend onderzoek is nodig om te evalueren of de invoering van de LAS van invloed is op de keuze voor enkelzijdig en dubbelzijdige LTX en de overleving na LTX in Nederland.
Identificatie van prognostische biomarkers in bloed en/of longlavage in combinatie met klinische prognostische parameters zijn belangrijk, omdat zij de behandelend arts houvast kunnen bieden bij complexe beslissingen zoals het tijdstip van starten van medicamenteuze behandeling danwel het moment van verwijzing voor LTX. Het doel van predictiemodellen zoals de LAS is om de ernst van de ziekte te bepalen en mogelijke uitkomst na LTX te voorspellen. Vooralsnog is de LAS enkel gebaseerd op klinische factoren zoals geslacht, leeftijd, de uitslag van de 6-minuten looptest en de aanwezigheid van pulmonale hypertensie. Potentiële prognostische biomarkers, zoals IgA in serum, zouden in de toekomst in de LAS en andere predictiemodellen kunnen worden geïmplementeerd om zo beter inzicht te verkrijgen in het ziektebeloop van de individuele patiënt.
Hoofdstuk 4 beschrijft de analyse van immunoglobuline A (IgA) in serum als een potentiële biomarker in IPF-patiënten. In deze studie werd het cohort opgedeeld in 2 groepen op basis van de hoogte van de IgA serumwaarde gemeten op het moment dat de diagnose werd gesteld (IgA-waarde > 2.85 g/L en IgA-waarde < 2.85 g/L). Er wordt een significant slechtere overleving gevonden in de patiëntengroep met een IgA-waarden hoger dan 2.85 g/L. Deze resultaten worden bevestigd met een zelfde analyse in een duplicatiecohort. Toekomstig onderzoek zal moeten uitwijzen of herhaaldelijke metingen van IgA in serum over een bepaalde tijdsperiode ook prognostische waarde heeft.
De laatste hoofdstukken van dit proefschrift richten zich op de verschillende genetische mutaties die gevonden zijn in patiënten met sporadische en familiaire longfibrose. Herkenning van deze verschillende subtypen van longfibrose is belangrijk, omdat er (subtiele) verschillen kunnen zijn wat betreft beloop van de ziekte, danwel aanwezigheid van relevante comorbiditeiten.

Het is bijvoorbeeld goed mogelijk dat deze subtypen anders reageren op bepaalde medicamenteuze behandeling, zoals immunosuppressiva welke belangrijk zijn in het longtransplantatietraject. Het goed in kaart brengen van deze patiënten is essentieel om antwoord te kunnen geven op deze vragen. Patiënten met een mutatie in genen die betrokken zijn bij telomerase, *surfactant* eiwitten en *lamellar bodies* zijn onderzocht met als doel de klinische relevantie van deze genetische mutaties te bestuderen. De hoofdstukken 5 tot en met 8 richten zich op de prevalentie, klinische karakteristieken en prognose van patiënten met verschillende genetische mutaties en bespreekt de mogelijke consequenties van deze genmutaties voor een eventuele longtransplantatie.

In **hoofdstuk 5** worden de klinische karakteristieken en overleving van 26 longfibrose patiënten met een *TERT* mutatie vergeleken met patiënten met de sporadische vorm van IPF. Er wordt een significant slechtere overleving beschreven de eerste 18 maanden na het stellen van de diagnose in patiënten met een *TERT* mutatie. Dit is vooralsnog de eerste studie die een verschil in overleving tussen deze 2 groepen aantoont.

In het geval van ons cohort is longfibrose de dominante uiting van de onderliggende *TERT* mutatie, maar mogelijk hebben deze patiënten ook andere (sub)klinische uitingen van andere aangedane orgaansystemen. Het is beschreven dat deze patiënten een verhoogd risico hebben op het ontwikkelen van hematologische aandoeningen en leverziekten. Echter, op basis van klinische patiëntkarakteristieken zijn er vooralsnog nauwelijks verschillen te vinden tussen patiënten met een *TERT* mutatie en sporadische IPF-patiënten, behoudens subtiele afwijkingen in het bloedbeeld en longfunctie.

Aanvullend wordt IgA in serum en bronchoalveolaire lavage profielen geëvalueerd in patiënten met een *TERT* mutatie en vergeleken met die van sporadische IPF-patiënten. Er worden significant hogere IgA serumwaarden en een lager percentage lymfocyten in het lavagespoelsel gevonden in patiënten met een *TERT* mutatie.

Recent zijn er een aantal observationele studies verschenen die in totaal 31 patiënten met een *TERT* mutatie beschrijven die een LTX ondergingen. De meest voorkomende complicaties waren van hematologische aard, waardoor alle patiënten een aanpassing nodig hadden in de dosering van immunosuppressiva. Andere gerapporteerde complicaties waren nierfunctiestoornissen en longinfecties. Daarnaast ontwikkelden 3 patiënten een maligniteit na LTX.

Hoofdstuk 6 beschrijft het klinisch beloop na LTX van een Nederlandse patient met een *TERT* mutatie en mogelijke *TERT*-gerelateerde complicaties na LTX. **Hoofdstuk 7** beschrijft *surfactant protein A2 (SFTPA2)* mutaties in patiënten met een familiaire en sporadische vorm van longfibrose. Het klinisch beloop van patiënten met deze mutatie lijkt met name gekenmerkt door het voorkomen van maligniteiten. Gezien het hoge risico op de ontwikkeling van een maligniteit lijkt het aan te bevelen om patiënten met deze mutatie dan ook aan te melden voor dubbelzijdige LTX. Verder lijkt het raadzaam patiënten grondig te screenen en bij geringe verdenking op een maligniteit aanvullend onderzoek te verrichten. **Hoofdstuk 8** bestudeert het vóórkomen van het Hermansky Pudlak Syndroom (HPS) in een cohort met sporadische IPF patiënten. HPS is een syndroom waarbij patiënten een genetische mutatie hebben in 1 van de genen die coderen voor *lamellar bodies*. Niet goed functionerende *lamellar bodies* in type II alveolaire epitheelcellen kan leiden tot longfibrose. Het voorkomen van HPS is zeldzaam, maar herkenning van het syndroom is wel relevant. Lang werd HPS beschouwd als een contra-indicatie voor LTX gezien de gestoorde stolling in deze patiënten, echter succesvolle LTX bij patiënten met HPS is beschreven. Essentieel is dat het syndroom wordt herkend en de juiste maatregelen worden getroffen om de stolling in de patiënt rondom de LTX te optimaliseren.

Sinds de eerste longtransplantatie in 1989 is er veel veranderd, maar er zijn nog steeds veel vragen te beantwoorden om deze complexe behandeling verder te optimaliseren. Zorgvuldig opgezette studies, zowel retrospectief als prospectief, zijn nodig om LTX nader te onderzoeken in specifieke patiëntengroepen zoals IPF. Vanwege de zeldzaamheid van zowel de ziekte als de behandelingsmogelijkheden is een goede samenwerking tussen gespecialiseerde centra noodzakelijk, zowel nationaal als internationaal. Het opzetten van een multicenter database met informatie over alle getransplanteerde patiënten en donoren zou dan ook van grote waarde zijn. Beter inzicht in de huidige situatie is de eerste stap in het optimaliseren van LTX in specifieke patiëntengroepen, en belangrijker nog, in de individuele patiënt.

List of publications

van der Vis JJ, Snetselaar R, Kazemier KM, ten Klooster L, Grutters JC, van Moorsel CH. Effect of Muc5b promoter polymorphism on disease predisposition and survival in idiopathic interstitial pneumonias.
Respirology. 2015 Dec; 21(4): 712-7.

van Moorsel CH, ten Klooster L, van Oosterhout MF, de Jong PA, Adams H, van Es W, Ruven HJ, van der Vis JJ, Grutters JC. SFTPA2 mutations in familial and sporadic idiopathic interstitial pneumonia.
Am J Respir Crit Care Med. 2015 Nov 15; 192(10): 1249-52

ten Klooster L, Nossent GD, Kwakkel-van Erp JM, van Kessel DA, Oudijk EJ, van de Graaf EA, Luijk B, Hoek RA, van den Blink B, van Hal PT, Verschuuren EA, van der Bij W, van Moorsel CH, Grutters JC. Ten-year survival in patients with idiopathic pulmonary fibrosis after lung transplantation.
Lung 2015 Dec; 193(6): 919-26

ten Klooster L, van Moorsel CH, Kwakkel-van Erp JM, van Velzen-Blad H, Grutters JC. Immunoglobulin A in serum: an old acquaintance as a new prognostic biomarker in idiopathic pulmonary fibrosis.
Clin Exp Immunol. 2015 Aug; 181(2): 357-61

ten Klooster L*, van der Vis JJ*, van Oosterhout MFM, Grutters JC, van Moorsel CH. The occurrence of Hermansky Pudlak syndrome in patient with idiopathic pulmonary fibrosis – a cohort study.
Genet Syndr Gene Ther. vol.4: article 414, 2013

ten Klooster L, van Moorsel CH, van Hal PT, van den Blink B, Nossent GD, Verschuuren EA, Kwakkelvan Erp JM, van de Graaf EA, van Kessel DA, Grutters JC. High mortality in patients with idiopathic pulmonary fibrosis on Dutch lung transplant waiting list.
Ned Tijdschr Geneesk. 2012;156(2): A3752. Dutch.

Affiliations

H. Adams, Department of Nuclear Medicine, St. Antonius Hospital,
Nieuwegein, The Netherlands

W. van der Bij, University of Groningen, University Medical Center Groningen,
Department of Pulmonary Medicine and Tuberculosis, Groningen, The Netherlands

Dr. B. van den Blink, Department of Respiratory Medicine, Erasmus MC,
Rotterdam, The Netherlands

Dr. W. van Es, Department of Radiology, St. Antonius Hospital,
Nieuwegein, The Netherlands

Dr. E.A. van de Graaf, Division of Heart and Lungs, University Medical Center Utrecht,
Utrecht, The Netherlands

Prof. dr. J.C. Grutters, Centre of Interstitial Lung Diseases, Department of Pulmonology,
St. Antonius Hospital, Nieuwegein and Division of Heart and Lungs,
University Medical Center Utrecht, Utrecht, The Netherlands

Dr. P.T. van Hal, Department of Respiratory Medicine, Erasmus MC,
Rotterdam, The Netherlands

Dr. R.A.S. Hoek, Department of Respiratory Medicine, Erasmus MC,
Rotterdam, The Netherlands

P.A. de Jong, Department of Radiology, St. Antonius Hospital,
Nieuwegein, The Netherlands

D.A. van Kessel, Centre of Interstitial Lung Diseases, Department of Pulmonology,
St. Antonius Hospital, Nieuwegein and Division of Heart and Lungs,
University Medical Center Utrecht, Utrecht, The Netherlands

Prof. dr. J. Kuball, Department of Hematology and Immunology,
University Medical Center Utrecht, Utrecht, The Netherlands

Dr. J.M. Kwakkel-van Erp, Division of Heart and Lungs,
University Medical Center Utrecht, Utrecht, The Netherlands

Dr. B. Luijk, Division of Heart and Lungs, University Medical Center Utrecht,
Utrecht, The Netherlands

Dr. C.H.M. van Moorsel, Centre of Interstitial Lung Diseases, Department of Pulmonology,
St. Antonius Hospital, Nieuwegein and Division of Heart and Lungs,
University Medical Center Utrecht, Utrecht, The Netherlands

G.D. Nossent, University of Groningen, University Medical Center Groningen,
Department of Pulmonary Medicine and Tuberculosis, Groningen, The Netherlands

Dr. M.F. van Oosterhout, Department of Pathology, St. Antonius Hospital,
Nieuwegein, The Netherlands

Dr. E.J. Oudijk, Centre of Interstitial Lung Diseases, Department of Pulmonology,
St. Antonius Hospital, Nieuwegein and Division of Heart and Lungs,
University Medical Center Utrecht, Utrecht, The Netherlands

Dr. H.J. Ruven, Department of Clinical Chemistry, St. Antonius Hospital,
Nieuwegein, The Netherlands

R. Snetselaar, Centre of Interstitial Lung Diseases, Department of Pulmonology,
St. Antonius Hospital, Nieuwegein, The Netherlands

Dr. H. van Velzen-Blad, Department of Medical Microbiology and Immunology,
St. Antonius Hospital, Nieuwegein, The Netherlands

Dr. E.A. Verschuuren, University of Groningen, University Medical Center Groningen,
Department of Pulmonary Medicine and Tuberculosis, Groningen, The Netherlands

J.J. van der Vis, Centre of Interstitial Lung Diseases, Department of Pulmonology,
St. Antonius Hospital, Nieuwegein, The Netherlands

Dankwoord / Acknowledgement

Lang heb ik gedacht aan de allesomvattende tekst *"Iedereen heel erg bedankt!"*, maar voor de meest gelezen pagina's van een proefschrift en als een fervent dankwoord-lezer zelf, vond ik dat toch wat te gemakkelijk.

Mijn promotie is een bijzonder (en) leerzaam traject voor me geweest, met de bijbehorende pieken en dalen. Het heeft me gevormd als mens, persoonlijk en professioneel, maar heeft me bovenal laten inzien dat je het, eigenlijk net zoals in 'het echte leven', vaak niet alleen kan. Daarom wil ik iedereen die een steentje heeft bijgedragen aan de voltooiing van dit proefschrift heel erg bedanken en een aantal mensen hier in het bijzonder.

Allereerst gaat mijn dank uit naar wijlen prof. van den Bosch, pionier in de longziekten en specifiek in de longtransplantatie in Nederland. Bedankt dat u mij de mogelijkheid heeft geboden om onderzoek te doen naar dit bijzondere onderwerp binnen de geneeskunde. Onvergetelijk voor mij zijn de autoritten die we maakten langs de transplantatiecentra, waarbij u mij geschiedenisles gaf over het begin van longtransplantatie in Nederland. Hoe spijtig is het dan ook dat u de afronding van dit proefschrift niet meer hebt kunnen mee maken.

Geachte prof. Grutters, beste Jan. Ik heb veel bewondering voor jouw onuitputtelijke energie waarmee je je werk als onderzoeker en dokter doet. Bedankt voor je begeleiding door de jaren heen en na ontelbaar veel *"Komt goed, Liesbeth"*, is het dan daadwerkelijk goed gekomen met dit proefschrift!

Geachte dr. van Moorsel, beste Coline. Zonder jouw begeleiding was er waarschijnlijk nooit een kaft om dit proefschrift gekomen. Ik heb veel waardering gekregen voor jou als persoon en onderzoeker. En ik zal nog vaak glimlachen als ik de woorden '*ónzin!*' en '*excellent!*' ergens hoor!

Geachte dr. Kwakkel-van Erp, beste Hanneke. Bedankt voor je enthousiasme en steun, met name in de laatste fase van de voltooiing van dit proefschrift.

Ook wil ik hier alle co-auteurs van dit proefschrift bedanken voor het commentaar op de manuscripten. Ik ben er van overtuigd dat iedere kritische noot, hoe lastig soms ook, een stuk weer net iets beter maakt. En in het bijzonder wil ik alle longtransplantatieartsen van Nederland bedanken voor het vertrouwen dat ze mij hebben gegeven om de gezamenlijke nationale data te analyseren en op te schrijven.

Beste medewerkers van de secretariaten Longtransplantatie in het UMC Groningen, Erasmus MC en UMC Utrecht & St. Antonius ziekenhuis en met name Leonie Ooms en Joke van de Sluis, bedankt voor jullie hulp en gastvrijheid.
Pieter Zanen, wat fijn dat goed statistisch advies slechts een kopje koffie kost! Bedankt voor je hulp door de jaren heen.

Verreweg de meeste onderzoeksuren heb ik doorgebracht met mijn onderzoekcollega's. In een variabele samenstelling, maar met de constanten koffie en taart, goede discussies en meer of minder goede grappen. Daarbij heb ik waardevolle herinneringen aan onze congressen en uitjes samen.
Nicolien, Lisanne, Nicole, Renske, Bekir, Heleen, Reinier, Annemarie, Marjolijn, Thijs, Ingrid, Karin, Annette en natuurlijk ook Annelies; dank jullie wel!

Annette, ontzettend bedankt voor al je geduld met deze labkneus, maar meer nog voor je luisterend oor tijdens onze borrels en etentjes. Rens en Bekir: bij lange na geen hattrick maar wel bijna tijd voor écht lekkere koffie..!
En voor alle huidige onderzoekers: veel succes met jullie projecten!

Beste maatschap Longziekten van het St. Antonius, wat een voorrecht om door zo'n veelzijdige groep specialisten te worden opgeleid! Bedankt dat ik bij jullie mijn opleiding kan volgen.

Longcollega's, Antonianen en 'de Leesclub'; hoe fijn is het om dagelijks omringd te worden door hardwerkende, talentvolle en lieve mensen waarmee je als het werk gedaan is ook nog heel gezellig een drankje (of 2, 3) kan drinken!

Sander, als ik iemand de lay-out van mijn boekje toevertrouw dan ben jij dat! Bedankt, zowel voor deze klus als je vriendschap!

Ik wil op deze plek ook mijn lieve vrienden en (schoon)familie bedanken, gewoon omdat ik nu een keertje de kans heb om het op papier vast te leggen. Onze momenten samen (groot of klein/lief of leed) maken me bijzonder gelukkig!

Mijn paranimfen Sanne en Lidwien: Lieve Sanne, longmaatje van het eerste uur! Uit onze eerste periode als 'de lamme en de blinde' is een dierbare vriendschap ontstaan, waarbij we vaak maar een blik nodig hebben om elkaar te begrijpen. Lieve Lidwien, ik ken niemand waar ik zo mee kan lachen en huilen als met jou! Vanaf de eerste 'geneeskunde-minuut' waren we samen en dat is eigenlijk altijd zo gebleven. Superfijn dat jullie op deze dag achter me staan, dat geeft een goed gevoel!

Lieve Jos, wie had dat nou ooit gedacht, dat dat kleine dromerige zusje van jou een proefschrift zou schrijven? Jouw eigenzinnige kijk op de wereld is vaak verfrissend en helpt me om de dingen soms in een ander perspectief te zien. Bedankt voor wie je bent!

Lieve Pa & Ma, ik prijs me bijzonder gelukkig met jullie als mijn ouders. Jullie onvoorwaardelijke steun, liefde en interesse bij al mijn keuzes in het leven, geven me altijd het juiste vertrouwen om mijn hart te volgen. Daarbij zijn de opvoedkundige woorden *'als je geen zin hebt, dan maak je maar zin'* van grote waarde geweest bij de afronding van dit proefschrift!

Allerliefste Bas, de beste 'promotie' die ik ooit maakte: van jouw collega tot geliefde! Eindeloos gelukkig word ik van jou. Ik hou van je!

Curriculum Vitae

Liesbeth ten Klooster was born on March 24th in 1982 in Marknesse. She grew up in Kampen and finished Atheneum at the Ichthus College in 2000.

After graduating she attended the Hogeschool of Utrecht to become a remedial therapist Cesar in 2003. She worked for a short period of time at the department of physiotherapy at Bartimeus Doorn.

During her studies she generated a great interest for medicine and decided to study Medicine at the Utrecht University in 2003. During her studies she did an internship at the department of Internal Medicine at the Hospital Getúlio Vargas in Teresina, Brasil.

From February 2010 she worked as a research fellow in the Center of Interstitial Lung Diseases (head prof. dr. J.M.M. van den Bosch†, head prof. dr. J.C. Grutters) to study lung transplantation in patients with idiopathic pulmonary fibrosis, which was the foundation of this thesis. In January 2012 she started as a registrar in Respiratory Medicine at St. Antonius Hospital, Nieuwegein (head dr. F.M.N.H. Schramel), which commenced with two years of training in Internal Medicine (head dr. A.B.M. Geers). Her specialist training was interrupted for one year in 2013 to work on this thesis.

She is currently working as a registrar at the Pulmonary Vascular Disease Unit (head prof. D. Kiely) of the Royal Hallamshire Hospital in Sheffield, United Kingdom.