Features of a telomere syndrome in patients with idiopathic pulmonary fibrosis

T. W. Hoffman

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FEATURES OF A TELOMERE SYNDROME IN PATIENTS WITH IDIOPATHIC PULMONARY FIBROSIS

Kenmerken van een telomeersyndroom in patiënten met idiopathische pulmonale fibrose

(met een samenvatting in het Nederlands)

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Chapter 1

General introduction

Idiopathic pulmonary fibrosis (IPF) is a severe form of pulmonary fibrosis and is an interstitial lung disease (ILD). ILD are characterized by cellular proliferation, interstitial inflammation, or fibrosis in the pulmonary interstitium (Figure 1).[1] ILD are rare diseases, but IPF is one of the most prevalent ILD.[2] The incidence of IPF is estimated to be 3-9 cases per 100 000 per year in Europe and North America, and somewhat lower in East Asia and South America.[3] Risk factors for IPF include older age, male sex, having a family member with pulmonary fibrosis, cigarette smoking, and environmental exposures (Table 1).[2]

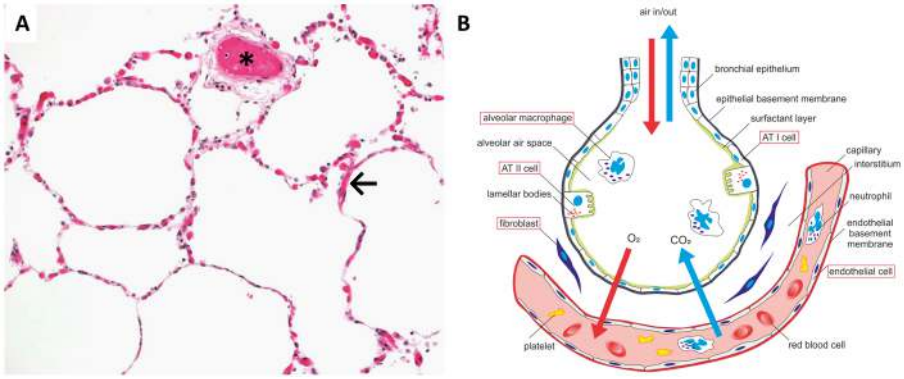

Figure 1: (A) histopathology slide of alveoli with nearby capillaries (↑) and pulmonary venule (*), haematoxin-eosin-staining, magnification 200x, courtesy of www.humpath.com. (B) schematic representation of an alveolus with associated capillary; alveolar walls consist of type I and II alveolar epithelial cells (AT I, AT II). The pulmonary interstitium is the space between the alveolar wall and the capillaries over which gas exchange takes place. Figure adapted from Nova and colleagues,[4] used under Creative Commons license.

Table 1: known risk factors for IPF

Male sex[5]
Older age[6]
Family member with pulmonary fibrosis[7]
Cigarette smoking[8]
Occupational history of farming or agriculture[8]
Exposure to metal dust[8]
Exposure to wood dust[8]
Exposure to pesticide[8]
Exposure to asbestos[9]

DIAGNOSIS OF IPF

The diagnosis of IPF is oftentimes made a long time after the onset of symptoms. In part this is caused by the indolent course of the disease. Most patients present with gradual-onset dyspnoea or cough, and this can be present for over five years prior to the final

diagnosis being made.[10] In a recent study, the time from onset of symptoms until the final diagnosis was over one year in about half of patients with IPF, and it was found that comorbid cardiac conditions can increase the diagnostic delay.[11] Furthermore, diagnostic delay also seems to be related to insufficient awareness of IPF in general practitioners and some pulmonologists.[12,13] Finally, it can be complicated to distinguish IPF from many other ILD, and this requires significant expertise (Figure 2).

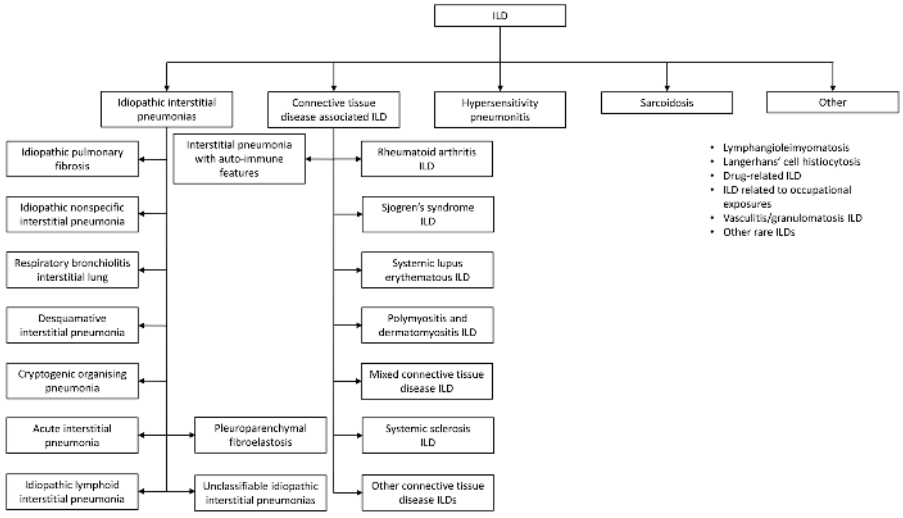

Figure 2: classification of interstitial lung disease, based on the article by Cottin and colleagues.[14] ILD, interstitial lung disease.

A diagnosis of IPF has to be made in accordance with international clinical practice guidelines. According to the 2011 ATS/ERS/JRS/ALAT guideline,[15] patients suspected of IPF should undergo high-resolution computed tomography (HRCT) scanning. When the HRCT shows a usual interstitial pneumonia (UIP) pattern (Figure 3A), and no other identifiable causes for the ILD can be found, a diagnosis of IPF can be made. When the HRCT shows a possible UIP pattern, or is inconsistent with UIP, a surgical lung biopsy should to be considered. Depending on the histopathological pattern of the lung biopsy specimen, a definite diagnosis of IPF, a probable diagnosis of IPF, or a possible diagnosis of IPF can be made. Alternatively, the diagnosis of IPF can be dismissed for specific combinations of HRCT and histopathology findings. In 2018 a new version of the international clinical practice guidelines was introduced.[16] According to the 2018 criteria, the HRCT patterns can be categorized into UIP, probable UIP, indeterminate for UIP, and alternative diagnosis (Table 2). A surgical lung biopsy is recommended when the HRCT pattern is probable UIP, indeterminate for UIP, or alternative diagnosis. The histopathology patterns can also be

categorized into UIP, probable UIP, indeterminate for UIP, and alternative diagnosis (Figure 4A, Table 2).

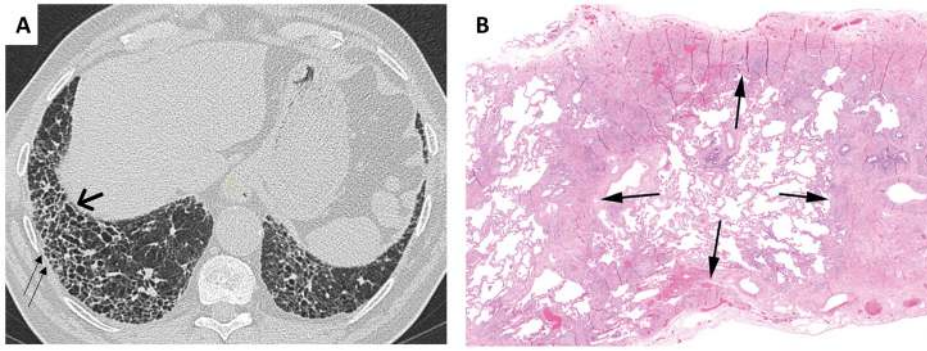

Figure 3: (A) Usual interstitial pneumonia (UIP) pattern on high-resolution computed tomography (HRCT) scan: the pattern is subpleural and basal predominant, and often heterogeneously distributed; there is honeycombing (↑↑) with peripheral traction bronchiectasis (↑).[16] (B) UIP pattern on histopathology: there is dense fibrosis with architectural distortion at the periphery of the lobule (↑), with sparing of the centrilobular regions.[16] Figure adapted from Smith[17], used under Creative Commons License.

Table 2: high-resolution computed tomography (HRCT) and histopathology patterns according to the 2018 ATS/ERS/JRS/ALAT clinical practice guidelines.[16]

	UIP	**Probable UIP**	**Indeterminate for UIP**	**Alternative diagnosis**
HRCT	Subpleural and basal predominant Often heterogeneously distributed Honeycombing with or without peripheral traction bronchiectasis or bronchiolectasis	Subpleural and basal predominant Often heterogeneously distributed Absence of honeycombing, reticular pattern with peripheral traction bronchiectasis or bronchiolectasis May have mild ground glass opacities	Subpleural and basal predominant Subtle reticulation May have mild ground glass opacities or distortion	Cysts Marked mosaic attenuation Predominant ground glass opacities Profuse micronodules Centrilobular nodules Nodules Consolidation Predominant peribronchovascular, perilymphatic, or upper or mid-lung distribution Pleural plaques Dilated oesophagus Distal clavicular erosions Extensive lymph node enlargement Pleural effusions or pleural thickening

Table 2: high-resolution computed tomography (HRCT) and histopathology patterns according to the 2018 ATS/ERS/JRS/ALAT clinical practice guidelines.[16] (continued)

	UIP	Probable UIP	Indeterminate for UIP	Alternative diagnosis
Histopathology	Dense fibrosis with architectural distortion Predominant subpleural and/or paraseptal distribution of fibrosis Patchy involvement of lung parenchyma by fibrosis Fibroblast foci	Some features of a UIP pattern are present, but to an extent that precludes a definitive diagnosis of UIP	Fibrosis with or without architectural distortion, with features favouring either a pattern other than UIP or features favouring UIP secondary to another cause Some features of a UIP pattern are present, but with other features suggesting an alternative diagnosis	Cellular inflammatory infiltrate away from areas of honeycombing Prominent lymphoid hyperplasia including secondary germinal centers A distinctly bronchiolocentric distribution

The diagnosis of IPF can be made in patients in whom the HRCT scan shows a UIP pattern when other causes of ILD are excluded. A diagnosis of IPF can also be made when the HRCT pattern is probable UIP and the histopathology is UIP or probable UIP, or when the HRCT pattern is indeterminate for UIP and the histopathology pattern is UIP. In patients who have a combination of probable UIP on HRCT and indeterminate for UIP on histopathology, or indeterminate for UIP on HRCT and probable UIP on histopathology, or alternative diagnosis on HRCT and UIP on histopathology, a diagnosis of IPF is considered to be likely (Table 3).

Table 3: diagnosis of IPF for specific combinations of HRCT patterns and histopathology patterns according to the 2018 ATS/ERS/JRS/ALAT clinical practice guidelines.[16]

		Histopathology pattern			
		UIP	**Probable UIP**	**Indeterminate for UIP**	**Alternative diagnosis**
HRCT pattern	**UIP**	IPF	IPF	IPF	Alternative diagnosis
	Probable UIP	IPF	IPF	IPF likely	Alternative diagnosis
	Indeterminate for UIP	IPF	IPF likely	Indeterminate for IPF	Alternative diagnosis
	Alternative diagnosis	IPF likely	Alternative diagnosis	Alternative diagnosis	Alternative diagnosis

In clinical practice, a somewhat different approach is often used, as has been described in a Fleischner Society White Paper from 2017.[18] This approach allows for a working diagnosis of IPF to be made in the absence of diagnostic tissue for patients who do not have a

UIP pattern on HRCT. A working diagnosis of IPF can only be made after multidisciplinary discussion, when the patient has a progressive fibrosing lung disease, and there is no alternative explanation.

PATHOGENESIS OF IPF

In the past decades significant progress has been made in our understanding of the pathogenesis of IPF. IPF is thought to be the result of aberrant repair and deposition of interstitial fibrosis in response to recurrent (sub)clinical epithelial injury.[2,19] Genome-wide association studies (GWAS) have identified variants in several genes involved in cell-adhesion and mechanotransduction that are overrepresented in patients with IPF.[20–22] Type II alveolar epithelial cells have emerged to be the key cells in IPF pathogenesis through several lines of evidence.[23] First, mutations in surfactant protein C, which is only expressed in type II alveolar epithelial cells, were found to underlie several cases of familial pulmonary fibrosis in adults.[24,25] Mutations in the genes encoding other surfactant proteins, as well as surfactant processing genes, have since been found to underlie other cases of familial pulmonary fibrosis.[26] Furthermore, GWAS identified associations between IPF and common variants in telomere-related genes,[20–22] and rare variants in telomere-related genes have been identified in both patients with familial and sporadic IPF.[27–29] Subsequent studies found that telomere dysfunction in type II alveolar cells alone leads to senescence and is sufficient to recapitulate a pulmonary fibrosis phenotype in mice.[30–32] In the lungs of patients with IPF, telomere shortening and accumulation of DNA damage mainly affects type II alveolar epithelial cells.[33]

The variant rs35705950 in the Mucin 5B (*MUC5B*) promoter has been identified as the strongest genetic risk factor for developing IPF.[20,34,35] The rs35705950 variant is associated with MUC5B overexpression in broncho-alveolar epithelia.[34,36] MUC5B is a component of normal mucus especially in the smaller airways, and is essential for mucociliary clearance and immune regulation in mice.[37] Excessive production of MUC5B is hypothesized to impair normal mucociliary clearance, and to lead to increased retention microorganisms and particles that damage the alveolar epithelium. In a mouse model higher Muc5b concentrations correlated with the degree of pulmonary fibrosis after a bleomycin challenge.[38]

TELOMERE SYNDROMES

Telomeres are repetitive hexanucleotide structures (TTAGGG) with their associated proteins at the end of chromosomes (Figure 4A). An overview of telomere-associated proteins

is presented in Table 4. Because chromosome ends cannot be fully replicated, telomeres shorten with every cell division. The shortening of telomeres in human embryonic and stem cells is counteracted by the activity of the telomerase enzyme, which can add nucleotides to the telomere. Nevertheless, the telomerase enzyme does not completely keep up with telomere shortening, and thus telomeres become increasingly short with age. When telomeres become critically short, replicative senescence or cellular apoptosis ensues. Telomere shortening can be accelerated when telomere maintenance is impaired.[39]

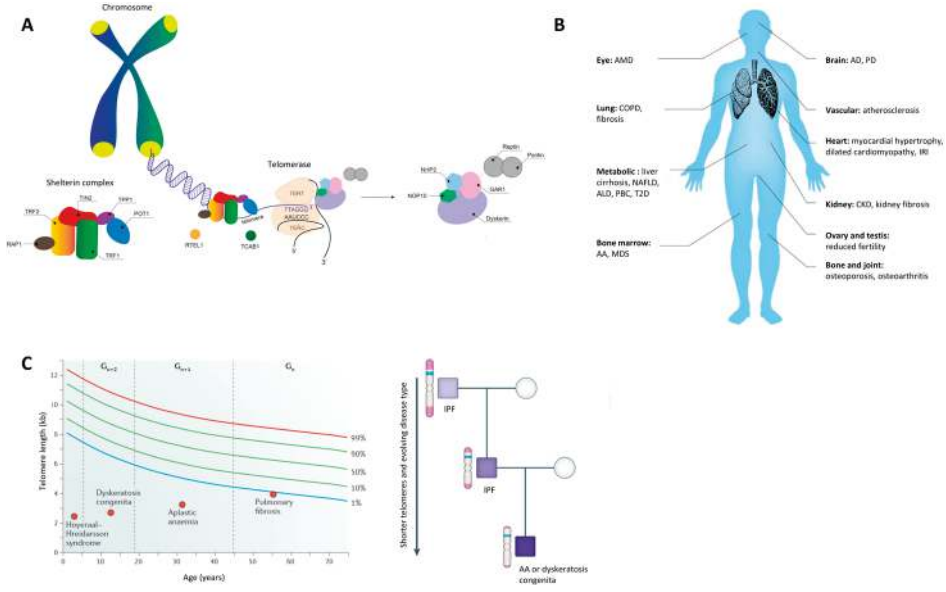

Figure 4: (A) telomere structure and representation of proteins involved in telomere maintenance. Telomeres are repetitive hexanucleotide sequences (TTAGGG), found at the ends of chromosomes in a duplex "D-loop-T-loop" configuration. RTEL1 is a helicase that disrupts T-loops for telomere replication and repair. Shelterin protects telomeres from DNA damage surveillance and comprises of six polypeptide components: TRF1, TRF2, RAP1, TIN2, TPP1 and POT1. Telomerase comprises of the essential components TERT and TERC, which synthesise telomeres and maintain telomere length. Dyskerin forms a complex with NHP2, NOP10 and GAR1. It binds to TERC to stabilise the telomerase complex. TCAB1 regulates the recruitment of telomerase to telomeres. Figure and description adapted from Kam and colleagues[40] under Creative Commons license. (B) potential manifestations of telomere dysfunction categorized by organ system, image adapted from Rossiello and colleagues,[41] with permission. (C) illustration of genetic anticipation, where telomeres become increasingly short in successive generations, leading to more severe disease earlier in life. On the left telomere length in individuals with four different clinical presentations across the age range is indicated. The dashed lines represent a typical age range in which these disorders may first manifest, and 'Gn', 'Gn + 1' and 'Gn + 2' designate three successive generations manifesting with earlier-onset and evolving disease type owing to progressive telomere shortening. On the right, a typical family tree is shown, wherein disease type evolves from lung-predominant to bone-marrow-failure-predominant. Figure and description adapated from Armanios and Blackburn,[39] with permission. AA, aplastic anaemia; AD, Alzheimer's disease; ALD, alcoholic liver disease; AMD, age-related macular degeneration; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; IRI, ischaemia–reperfusion injury; MDS, myelodysplastic syndrome; NAFLD, non-alcoholic fatty liver disease; PBC, primary biliary cirrhosis; PD, Parkinson's disease; T2D, type 2 diabetes mellitus.

Table 4: selected proteins involved in telomere maintenance.

Protein	Gene	Function
TRF1	*TERF1*	Part of the shelterin complex; binds double-strand telomeric DNA
TRF2	*TERF2*	Part of the shelterin complex; binds double-strand telomeric DNA
RAP1	*TERF2IP*	Part of the shelterin complex; accessory subunit of TRF2
TIN2	*TINF2*	Part of the shelterin complex; connects TRF1 and TRF2 dimers with POT-TPP1
POT1	*POT1*	Part of the shelterin complex; binds single-strand telomeric DNA
TPP1	*ACD*	Part of the shelterin complex; forms heterodimer with POT1; recruits telomerase to telomeres
CTC1	*CTC1*	Part of the CST complex; necessary for C-strand fill-in process during telomere replication
STN1	*STN1*	Part of the CST complex; necessary for C-strand fill-in process during telomere replication
TEN1	*TEN1*	Part of the CST complex; necessary for C-strand fill-in process during telomere replication
TERT	*TERT*	Part of telomerase complex
TR	*TERC*	Part of telomerase complex
Dyskerin	*DKC1*	Part of telomerase complex; subunit of H/ACA complex, required for stabilisation of TER
NHP2	*NHP2*	Part of telomerase complex; subunit of H/ACA complex, required for stabilisation of TER
NOP10	*NOP10*	Part of telomerase complex; subunit of H/ACA complex, required for stabilisation of TER
GAR1	*GAR1*	Part of telomerase complex; subunit of H/ACA complex, required for stabilisation of TER
NAF1	*NAF1*	Part of telomerase complex; subunit of H/ACA complex, required for stabilisation of TER; later replaced by GAR1
TCAB1	*WRAP53*	Facilitates telomerase localization to Cajal bodies
RTEL1	*RTEL1*	DNA helicase; facilitates telomerase function
PARN	*PARN*	Exoribonuclease, involved in maturation of TR
ZCCHC8	*ZCCHC8*	Involved in maturation of TR

More information on shelterin and the CST-complex can be found in a recent paper by Lim and Cech.[42] More information on telomerase can be found in a paper by Schmidt and Cech.[43] More information on RTEL1 functioning can be found in a paper by Vannier and colleagues.[44] More information on PARN can be found in a paper by Tummala and colleagues.[45] More information on ZCCHC8 can be found in a paper by Gable and colleagues.[46]

Conditions of impaired telomere maintenance are termed telomere syndromes. These syndromes can have a wide variety of manifestations throughout the body, including skin abnormalities, bone marrow failure, liver disease, gastrointestinal disease, early greying of hair, and pulmonary fibrosis or emphysema (Figure 4B). Specific genetic mutations can have a wide range of disease phenotypes, and specific phenotypes can be caused by various genetic mutations.[47] IPF is by far the most common manifestation of a telomere syndrome, but it is difficult to estimate the proportion of IPF patients in whom disease

is caused by telomere dysfunction.[28] Previous work has shown that leukocyte telomere length is shorter in patients with IPF compared to that in patients with other types of ILD.[48]

Telomere length is hereditary, and in families with a telomere syndrome, the phenomenon of genetic anticipation has been observed, where succeeding generations have increased disease severity and earlier onset of disease.[28] Depending on the severity of the telomere gene mutation, there can be a long time between the introduction of the mutation in a family and the first family member with overt telomere-related disease.[49] Recent work confirms that IPF can also present in patients who inherit short telomeres but not the pathogenic mutation of their ancestors.[50]

NATURAL COURSE AND PROGNOSIS OF IPF

In most patients, the natural course of IPF is characterized by gradually progressive fibrosis and a decline in lung function over several years. Some patients will remain stable for a longer period, and another minority of patients will experience a very rapid decline of lung function (Figure 5).[15] It is not well-known whether these distinct disease courses represent different phenotypes of IPF, and what factors underly disease progression. The prognosis of a patient with IPF can be estimated by the use of the Gender-Age-Physiology (GAP) Index, which includes information on the patient's sex, age, and lung function.[51] Factors associated with a worse prognosis include older age, male sex, having a family member with pulmonary fibrosis, lower lung function parameters, lower walking distance and increased desaturation during a 6-minute walking test, increased extent of pulmonary fibrosis on HRCT scan, exposure to air pollution, and the presence of comorbid conditions such as pulmonary hypertension, cardiovascular diseases and malignancy.[15,52–55] In addition, shorter leukocyte telomere length and mutations in telomere related genes, as well as increased serum concentrations of the biomarker Krebs von den Lungen 6 (KL-6) have been associated with shorter survival of patients with IPF.[15,56–59]

Between 5 and 20% of patients with IPF will experience an acute exacerbation of IPF each year.[60] An acute exacerbation of IPF is defined as an acute, clinically significant respiratory deterioration characterized by evidence of new widespread alveolar abnormality.[60] The diagnosis of an acute exacerbation of IPF requires a previous or concurrent diagnosis of IPF, acute worsening or development of dyspnoea usually for a duration of less than one month, new bilateral ground glass opacities and/or consolidations on HRCT, and the inability to fully explain the deterioration by cardiac failure or fluid overload.[60] The prognosis of patients with an acute exacerbation of IPF is poor, with a median survival of 3 months.[60] Risk factors for an acute exacerbation of IPF include increased disease severity, a UIP pat-

tern on HRCT, concurrent pulmonary hypertension, having never smoked cigarettes, and higher serum concentrations of KL-6.[61] There are several potential triggers for acute exacerbations of IPF, including infections, aspiration, air pollution, thoracic surgical procedures, bronchoalveolar lavage, lung cryobiopsy, chemotherapy, and thoracic radiotherapy.[55,61–63] However, a trigger cannot be identified in all cases.

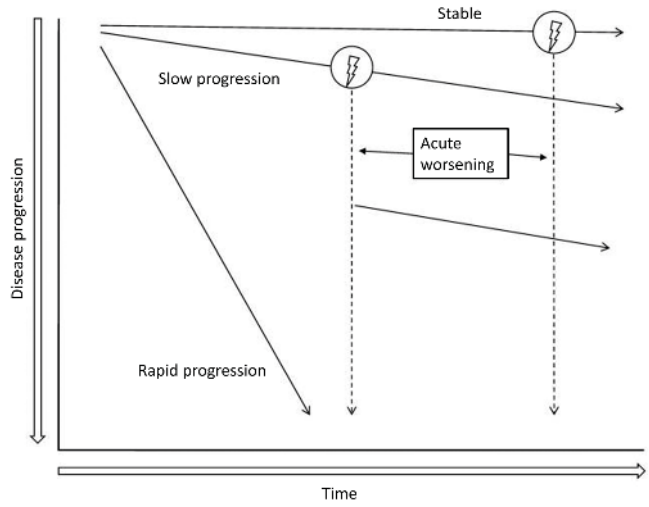

Figure 5: illustration of the natural course of IPF: most patients will have a slowly progressive course of disease over several years, but some patients will remain stable for years or have a rapidly progressive disease course. Lightning bolts represent an acute exacerbation of IPF, which can lead to acute and rapid worsening. Figure from Raghu and colleagues,[15] reprinted with permission of the American Thoracic Society.

TREATMENT OF IPF

Currently, there is no curative treatment available for IPF other than lung transplantation. However, in the past decade significant advances have been made in the pharmacologic management of patients with IPF. The antifibrotic medications pirfenidone and nintedanib have become available, and are now conditionally recommended for the treatment of patients with IPF.[2] Both medications have been shown to slow the rate of lung function decline,[64,65] and also seem to prolong survival.[66,67] The 2018 ATS/ERS/JRS/ALAT clinical practice guideline further includes a conditional recommendation for antacid therapy, conditional recommendations against the endothelin receptor antagonists macitentan and bosentan, the phosphodiesterase-5 inhibitor sildenafil, *N*-acetylcysteine monotherapy, and anti-pulmonary hypertension therapy for IPF-associated pulmonary hypertension, as well as strong recommendations against anticoagulation, combination prednisone,

azathioprine and *N*-acetylcysteine, the endothelin receptor antagonist ambrisentan, and the tyrosine kinase inhibitor imatinib (Table 5).[68] In addition, the 2011 clinical practice guidelines include strong recommendations for the use of supplemental oxygen therapy in patients with resting hypoxemia and lung transplantation in selected patients, as well as conditional recommendations for the use of pulmonary rehabilitation therapy and corticosteroids for patients with an acute exacerbation of IPF.[15]

Table 5: treatment recommendations for patients with IPF according to the 2015 ATS/ERS/JRS/ALAT clinical practice guideline.[68]

Treatment	Recommendation
Pharmacological treatment	
Ambrisentan	Strong recommendation against use
Anticoagulation	Strong recommendation against use
Bosentan	Conditional recommendation against use
Colchicine	Strong recommendation against use *
Combination prednisone, azathioprine, and *N*-acetylcysteine	Strong recommendation against use
Combined corticosteroid and immune-modulator therapy	Strong recommendation against use *
Corticosteroid monotherapy	Strong recommendation against use *
Cyclosporine A	Strong recommendation against use *
Etanercept	Strong recommendation against use *
Imatinib	Strong recommendation against use
Interferon gamma 1b	Strong recommendation against use *
Macitentan	Conditional recommendation against use
N-acetylcysteine monotherapy	Conditional recommendation against use
Nintedanib	Conditional recommendation **for** use
Pirfenidone	Conditional recommendation **for** use
Non-pharmacological treatment	
Lung transplantation	Strong recommendation **for** use * (in appropriate patients)
Mechanical ventilation	Conditional recommendation against use *
Pulmonary rehabilitation therapy	Conditional recommendation **for** use *
Supplemental oxygen therapy	Strong recommendation **for** use * (in patients with resting hypoxemia)
Management of comorbid conditions	
Antacid therapy	Conditional recommendation **for** use
Anti-pulmonary hypertension therapy for IPF-associated pulmonary hypertension	Conditional recommendation against use *
Management of acute exacerbations of IPF	
Corticosteroid therapy	Conditional recommendation **for** use *

* Not included in 2015 guideline and based on the 2011 clinical practice guideline.[15]

Other common measures that are not included in the guidelines include smoking cessation, avoidance of potentially toxic environmental exposures and drugs, yearly influenza vaccination and 5-yearly pneumococcal vaccination.[69] Furthermore, symptomatic treatment through both pharmacological and non-pharmacological interventions of cough, dyspnoea, depression and anxiety is important, as is timely initiation of palliative care.[69,70]

No specific treatments are available at present for patients with IPF in the context of a telomere syndrome and management of these patients is similar to that of other patients with IPF.[71] Recent studies indicate that morbidity after lung transplantation is higher in patients with a telomere syndrome, including a higher risk for haematological complications.[72] However, overall post-transplant survival is similar to that of patients without a telomere syndrome.[72,73] Several treatments that specifically target telomere dysfunction or shortening have been investigated,[74] including treatment with sex hormones such as danazol,[75] which seem to lengthen telomeres by increasing telomerase expression.

Recently there has been a shift in the treatment paradigm for other types of pulmonary fibrosis that have a similar disease course to that of IPF.[76] Historically, the focus has been on immunosuppressive therapy for patients with non-IPF ILD. However, recent studies indicate that patients with so-called progressive fibrosing ILD benefit from treatment with pirfenidone or nintedanib, regardless of the underlying lung disease.[77,78]

OUTLINE AND GOALS OF THIS THESIS

This thesis will explore clinical and genetic features of a telomere syndrome in patients with IPF, and their relation to leukocyte telomere length, acute exacerbations of IPF, and survival. In **Chapter 2** the prediagnostic trajectory of patients with IPF is explored, with a focus on imaging performed prior to the start of symptoms, as well as the identification of factors associated with a longer time to diagnosis. In **Chapter 3** the prevalence of potential extrapulmonary features of a telomere syndrome in patients with IPF is studied, including clinical history, family history, and laboratory features suggestive of a telomere syndrome. It is investigated whether these factors are associated with survival. In **Chapter 4** the focus is on immunological markers in patients with IPF, both in blood and in bronchoalveolar lavage fluid, and their association with leukocyte telomere length, acute exacerbations of IPF and survival. In **Chapter 5** coronary artery calcification score and bone mineral density, derived from HRCT scans, are described and their relation to leukocyte telomere length and survival are investigated.

In **Chapter 6** pulmonary phenotypes associated with common and rare variants in telomere-related genes are reviewed. In **Chapter 7** and **Chapter 8** pathogenic mutations in the telomere-related *ACD* and *TINF2* genes in patients with IPF are described. **Chapter 9** describes the effect of treatment with danazol, which is thought to elongate telomeres, on lung function and survival in patients with IPF. Finally, in **Chapter 10** a novel framework is proposed for the treatment of fibrosing ILD, including IPF. This framework is based on the treatable traits concept, which focusses on the treatment of clinically relevant traits that can be identified through the use of validated biomarkers.

REFERENCES

1. Sergew A, Brown KK. Idiopathic Pulmonary Fibrosis and Other Interstitial Lung Diseases. In: *Clinical Respiratory Medicine*; 2012:599-606.
2. Lederer DJ, Martinez FJ. Idiopathic Pulmonary Fibrosis. *N Engl J Med*. 2018;378(19):1811-1823. doi:10.1056/NEJMra1705751 [doi]
3. Hutchinson J, Fogarty A, Hubbard R, McKeever T. Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. *Eur Respir J*. 2015;46(3):795-806. doi:10.1183/09031936.00185114
4. Nova Z, Skovierova H, Calkovska A. Alveolar-Capillary Membrane-Related Pulmonary Cells as a Target in Endotoxin-Induced Acute Lung Injury. *Int J Mol Sci 2019, Vol 20, Page 831*. 2019;20(4):831. doi:10.3390/IJMS20040831
5. Kawano-Dourado L, Glassberg MK, Assayag D, et al. Sex and gender in interstitial lung diseases. *Eur Respir Rev*. 2021;30(162). doi:10.1183/16000617.0105-2021
6. Maher TM, Bendstrup E, Dron L, et al. Global incidence and prevalence of idiopathic pulmonary fibrosis. *Respir Res*. 2021;22(1):1-10. doi:10.1186/S12931-021-01791-Z/TABLES/3
7. García-Sancho C, Buendía-Roldán I, Fernández-Plata MR, et al. Familial pulmonary fibrosis is the strongest risk factor for idiopathic pulmonary fibrosis. *Respir Med*. 2011;105(12):1902-1907. doi:10.1016/J.RMED.2011.08.022
8. Park Y, Ahn C, Kim TH. Occupational and environmental risk factors of idiopathic pulmonary fibrosis: a systematic review and meta-analyses. *Sci Reports 2021 111*. 2021;11(1):1-10. doi:10.1038/s41598-021-81591-z
9. Abramson MJ, Murambadoro T, Alif SM, et al. Occupational and environmental risk factors for idiopathic pulmonary fibrosis in Australia: case–control study. *Thorax*. 2020;75(10):864-869. doi:10.1136/THORAXJNL-2019-214478
10. Hewson T, McKeever TM, Gibson JE, Navaratnam V, Hubbard RB, Hutchinson JP. Timing of onset of symptoms in people with idiopathic pulmonary fibrosis. *Thorax*. 2018;73(7):683-685. doi:10.1136/THORAXJNL-2017-210177
11. Snyder LD, Mosher C, Holtze CH, et al. Time to diagnosis of idiopathic pulmonary fibrosis in the IPF-PRO Registry. *BMJ open Respir Res*. 2020;7(1). doi:10.1136/BMJRESP-2020-000567
12. Hoyer N, Prior TS, Bendstrup E, Wilcke T, Shaker SB. Risk factors for diagnostic delay in idiopathic pulmonary fibrosis. *Respir Res*. 2019;20(1). doi:10.1186/S12931-019-1076-0
13. Mooney J, Chang E, Lalla D, et al. Potential Delays in Diagnosis of Idiopathic Pulmonary Fibrosis in Medicare Beneficiaries. *Ann Am Thorac Soc*. 2019;16(3):393-396. doi:10.1513/ANNALSATS.201806-376RL
14. Cottin V, Hirani NA, Hotchkin DL, et al. Presentation, diagnosis and clinical course of the spectrum of progressive-fibrosing interstitial lung diseases. *Eur Respir Rev*. 2018;27(150). doi:10.1183/16000617.0076-2018
15. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med*. 2011;183(6):788-824. doi:10.1164/rccm.2009-040GL [doi]

16. Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2018;198(5):e44-e68. doi:10.1164/rccm.201807-1255ST [doi]

17. Smith ML. The histologic diagnosis of usual interstitial pneumonia of idiopathic pulmonary fibrosis. Where we are and where we need to go. *Mod Pathol 2021 351*. 2021;35(1):8-14. doi:10.1038/s41379-021-00889-5

18. Lynch DA, Sverzellati N, Travis WD, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. *Lancet Respir Med*. 2018;6(2):138-153. doi:10.1016/S2213-2600(17)30433-2

19. Selman M, Pardo A. Revealing the Pathogenic and Aging-related Mechanisms of the Enigmatic Idiopathic Pulmonary Fibrosis. An Integral Model. *Am J Respir Crit Care Med*. 2014;189(10):1161-1172. doi:10.1164/rccm.201312-2221PP [doi]

20. Fingerlin TE, Murphy E, Zhang W, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. *Nat Genet*. 2013;45(6):613-620. doi:10.1038/ng.2609 [doi]

21. Noth I, Zhang Y, Ma SF, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. *Lancet Respir Med*. 2013;1(4):309-317. doi:10.1016/S2213-2600(13)70045-6

22. Allen RJ, Guillen-Guio B, Oldham JM, et al. Genome-wide association study of susceptibility to idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2020;201(5):564-574. doi:10.1164/RCCM.201905-1017OC

23. 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. doi:10.1155/2015/304186 [doi]

24. Nogee LM, Dunbar 3rd AE, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med*. 2001;344(8):573-579. doi:10.1056/NEJM200102223440805 [doi]

25. Van Moorsel CHM, Van Oosterhout MFM, Barlo NP, et al. Surfactant Protein C Mutations Are the Basis of a Significant Portion of Adult Familial Pulmonary Fibrosis in a Dutch Cohort. *Am J Respir Crit Care Med* 2012;182(11):1419-1425. doi:10.1164/RCCM.200906-0953OC

26. van Moorsel CHM, van der Vis JJ, Grutters JC. Genetic disorders of the surfactant system: focus on adult disease. *Eur Respir Rev*. 2021;30(159). doi:10.1183/16000617.0085-2020

27. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med*. 2007;356(13):1317-1326. doi:356/13/1317

28. Armanios M. Telomerase and idiopathic pulmonary fibrosis. *Mutat Res*. 2012;730(1-2):52-58. doi:10.1016/j.mrfmmm.2011.10.013 [doi]

29. Petrovski S, Todd JL, Durheim MT, et al. An Exome Sequencing Study to Assess the Role of Rare Genetic Variation in Pulmonary Fibrosis. *Am J Respir Crit Care Med*. January 2017. doi:10.1164/rccm.201610-2088OC [doi]

30. Alder JK, Barkauskas CE, Limjunyawong N, et al. Telomere dysfunction causes alveolar stem cell failure. *Proc Natl Acad Sci U S A*. 2015;112(16):5099-5104. doi:10.1073/pnas.1504780112 [doi]

31. Naikawadi RP, Disayabutr S, Mallavia B, et al. Telomere dysfunction in alveolar epithelial cells causes lung remodeling and fibrosis. *JCI insight*. 2016;1(14):e86704. doi:10.1172/jci.insight.86704 [doi]

32. Povedano JM, Martinez P, Flores JM, Mulero F, Blasco MA. Mice with Pulmonary Fibrosis Driven by Telomere Dysfunction. *Cell Rep*. 2015;12(2):286-299. doi:10.1016/j.celrep.2015.06.028 [doi]

33. Batenburg AA van, Kazemier KM, Oosterhout MFM van, et al. Telomere shortening and DNA damage in culprit cells of different types of progressive fibrosing interstitial lung disease. *ERJ Open Res*. 2021;7(2):00691-02020. doi:10.1183/23120541.00691-2020

34. Seibold MA, Wise AL, Speer MC, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1503-1512. doi:10.1056/NEJMoa1013660 [doi]

35. Moore C, Blumhagen RZ, Yang I V., et al. Resequencing study confirms that host defense and cell senescence gene variants contribute to the risk of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2019;200(2):199-208. doi:10.1164/rccm.201810-1891OC

36. Evans CM, Fingerlin TE, Schwarz MI, et al. Idiopathic pulmonary fibrosis: A genetic disease that involves mucociliary dysfunction of the peripheral airways. *Physiol Rev*. 2016;96(4):1567-1591. doi:10.1152/physrev.00004.2016

37. Roy MG, Livraghi-Butrico A, Fletcher AA, et al. Muc5b is required for airway defence. *Nature*. 2014;505(7483):412-416. doi:10.1038/nature12807

38. Hancock LA, Hennessy CE, Solomon GM, et al. Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice. *Nat Commun*. 2018;9(1). doi:10.1038/s41467-018-07768-9

39. Armanios M, Blackburn EH. The telomere syndromes. *Nat Rev*. 2012;13(10):693-704. doi:10.1038/nrg3246 [doi]

40. Kam MLW, Nguyen TTT, Ngeow JYY. Telomere biology disorders. *npj Genomic Med 2021 61*. 2021;6(1):1-13. doi:10.1038/s41525-021-00198-5

41. Rossiello F, Jurk D, Passos JF, d'Adda di Fagagna F. Telomere dysfunction in ageing and age-related diseases. *Nat Cell Biol 2022 242*. 2022;24(2):135-147. doi:10.1038/s41556-022-00842-x

42. Lim CJ, Cech TR. Shaping human telomeres: from shelterin and CST complexes to telomeric chromatin organization. *Nat Rev Mol Cell Biol*. 2021;22(4):283. doi:10.1038/S41580-021-00328-Y

43. Schmidt JC, Cech TR. Human telomerase: biogenesis, trafficking, recruitment, and activation. *Genes Dev*. 2015;29(11):1095-1105. doi:10.1101/gad.263863.115 [doi]

44. Vannier JB, Sarek G, Boulton SJ. RTEL1: functions of a disease-associated helicase. *Trends Cell Biol*. 2014;24(7):416-425. doi:10.1016/J.TCB.2014.01.004

45. Tummala H, Walne A, Collopy L, et al. Poly(A)-specific ribonuclease deficiency impacts telomere biology and causes dyskeratosis congenita. *J Clin Invest*. 2015;125(5):2151-2160. doi:10.1172/JCI78963

46. Gable DL, Gaysinskaya V, Atik CC, et al. ZCCHC8, the nuclear exosome targeting component, is mutated in familial pulmonary fibrosis and is required for telomerase RNA maturation. *Genes Dev*. 2019;33(19-20):1381-1396. doi:10.1101/GAD.326785.119

47. Holohan B, Wright WE, Shay JW. Cell biology of disease: Telomeropathies: An emerging spectrum disorder. *J Cell Biol*. 2014;205(3):289-299. doi:10.1083/jcb.201401012 [doi]

48. Snetselaar R, van Moorsel CH, Kazemier KM, et al. Telomere length in interstitial lung diseases. *Chest*. 2015;148(4):1011-1018. doi:10.1378/chest.14-3078 [doi]

49. van der Vis JJ, van der Smagt JJ, Hennekam FAM, Grutters JC, van Moorsel CHM. Pulmonary Fibrosis and a TERT Founder Mutation With a Latency Period of 300 Years. *Chest*. 2020;158(2):612-619. doi:10.1016/J.CHEST.2020.03.069

50. van der Vis JJ, van der Smagt JJ, van Batenburg AA, et al. Pulmonary fibrosis in non-mutation carriers of families with short telomere syndrome gene mutations. *Respirology*. 2021;26(12):1160-1170. doi:10.1111/RESP.14145

51. Ley B, Ryerson CJ, Vittinghoff E, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. *Ann Intern Med*. 2012;156(10):684-695. doi:10.7326/0003-4819-156-10-201205150-00004

52. Cutting CC, Bowman WS, Dao N, et al. Family History of Pulmonary Fibrosis Predicts Worse Survival in Patients With Interstitial Lung Disease. *Chest*. 2021;159(5):1913-1921. doi:10.1016/J.CHEST.2021.01.026

53. Poletti V, Ravaglia C, Buccioli M, et al. Idiopathic Pulmonary Fibrosis: Diagnosis and Prognostic Evaluation. *Respiration*. 2013;86(1):5-12. doi:10.1159/000353580

54. Caminati A, Lonati C, Cassandro R, et al. Comorbidities in idiopathic pulmonary fibrosis: an underestimated issue. *Eur Respir Rev*. 2019;28(153). doi:10.1183/16000617.0044-2019

55. Sesé L, Nunes H, Cottin V, et al. Role of atmospheric pollution on the natural history of idiopathic pulmonary fibrosis. *Thorax*. 2018;73(2):145-150. doi:10.1136/THORAXJNL-2017-209967

56. Stuart BD, Lee JS, Kozlitina J, et al. Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. *LancetRespiratory Med*. 2014;2(7):557-565. doi:10.1016/S2213-2600(14)70124-9 [doi]

57. Newton CA, Batra K, Torrealba J, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J*. 2016;48(6):1710-1720. doi:10.1183/13993003.00308-2016 [doi]

58. Snetselaar R, van Batenburg AA, van Oosterhout MFM, et al. Short telomere length in IPF lung associates with fibrotic lesions and predicts survival. *PLoS One*. 2017;12(12):e0189467. doi:10.1371/journal.pone.0189467 [doi]

59. Dai J, Cai H, Li H, et al. Association between telomere length and survival in patients with idiopathic pulmonary fibrosis. *Respirology*. 2015;20(6):947-952. doi:10.1111/resp.12566 [doi]

60. Collard HR, Ryerson CJ, Corte TJ, et al. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. *Am J Respir Crit Care Med*. 2016;194(3):265-275. doi:10.1164/rccm.201604-0801CI [doi]

61. Ryerson CJ, Cottin V, Brown KK, Collard HR. Acute exacerbation of idiopathic pulmonary fibrosis: Shifting the paradigm. *Eur Respir J*. 2015;46(2):512-520. doi:10.1183/13993003.00419-2015

62. Qiu M, Chen Y, Ye Q. Risk factors for acute exacerbation of idiopathic pulmonary fibrosis: A systematic review and meta-analysis. *Clin Respir J*. 2018;12(3):1084-1092. doi:10.1111/CRJ.12631

63. Tomos I, Dimakopoulou K, Manali ED, Papiris SA, Karakatsani A. Long-term personal air pollution exposure and risk for acute exacerbation of idiopathic pulmonary fibrosis. *Environ Heal A Glob Access Sci Source*. 2021;20(1):1-13. doi:10.1186/S12940-021-00786-Z

64. Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2071-2082. doi:10.1056/NEJMoa1402584 [doi]

65. King Jr TE, Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2083-2092. doi:10.1056/NEJMoa1402582 [doi]

66. Richeldi L, Cottin V, du Bois RM, et al. Nintedanib in patients with idiopathic pulmonary fibrosis: Combined evidence from the TOMORROW and INPULSIS((R)) trials. *Respir Med*. 2016;113:74-79. doi:10.1016/j.rmed.2016.02.001 [doi]

67. Nathan SD, Albera C, Bradford WZ, et al. Effect of pirfenidone on mortality: pooled analyses and meta-analyses of clinical trials in idiopathic pulmonary fibrosis. *LancetRespiratory Med*. 2017;5(1):33-41. doi:S2213-2600(16)30326-5

68. Raghu G, Rochwerg B, Zhang Y, et al. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2015;192(2):e3-19. doi:10.1164/rccm.201506-1063ST [doi]

69. Lee JS, McLaughlin S, Collard HR. Comprehensive care of the patient with idiopathic pulmonary fibrosis. *Curr Opin Pulm Med*. 2011;17(5):348-354. doi:10.1097/MCP.0B013E328349721B

70. Lindell K, Raghu G. Palliative care for patients with pulmonary fibrosis: symptom relief is essential. *Eur Respir J*. 2018;52(6). doi:10.1183/13993003.02086-2018

71. Courtwright AM, El-Chemaly S. Telomeres in Interstitial Lung Disease: The Short and the Long of It. *Ann Am Thorac Soc*. 2019;16(2):175-181. doi:10.1513/AnnalsATS.201808-508CME [doi]

72. Planas-Cerezales L, Arias-Salgado EG, Berastegui C, et al. Lung Transplant Improves Survival and Quality of Life Regardless of Telomere Dysfunction. *Front Med*. 2021;8:920. doi:10.3389/FMED.2021.695919

73. Alder JK, Sutton RM, Iasella CJ, et al. Lung transplantation for idiopathic pulmonary fibrosis enriches for individuals with telomere-mediated disease. *J Hear Lung Transplant*. 2022;41(5):654-663. doi:10.1016/J.HEALUN.2021.11.008

74. Martinez P, Blasco MA. Telomere-driven diseases and telomere-targeting therapies. *J Cell Biol*. 2017;216(4):875-887. doi:10.1083/jcb.201610111 [doi]

75. Townsley DM, Dumitriu B, Young NS. Danazol Treatment for Telomere Diseases. *N Engl J Med*. 2016;375(11):1095-1096. doi:10.1056/NEJMc1607752 [doi]

76. Wijsenbeek M, Cottin V. Spectrum of Fibrotic Lung Diseases. *N Engl J Med*. 2020;383:958-968. doi:10.1056/NEJMra2005230

77. Flaherty KR, Wells AU, Cottin V, et al. Nintedanib in progressive fibrosing interstitial lung diseases. *N Engl J Med*. 2019;381(18):1718-1727. doi:10.1056/NEJMoa1908681

78. Maher TM, Corte TJ, Fischer A, et al. Pirfenidone in patients with unclassifiable progressive fibrosing interstitial lung disease: a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Respir Med*. 2020;8(2):147-157. doi:10.1016/S2213-2600(19)30341-8

Chapter 2

Potential interstitial lung abnormalities on chest X-rays prior to symptoms of idiopathic pulmonary fibrosis

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ABSTRACT

Background:

Idiopathic pulmonary fibrosis (IPF) often has significant diagnostic delay. At present it is not well-known what factors associate with time to diagnosis and if this is associated with survival after the diagnosis. There has also been increasing attention for interstitial lung abnormalities on chest CT-scans. In this study we assessed what factors associate with time to diagnosis in patients with IPF, and whether early stages of pulmonary fibrosis can be seen on chest X-rays prior to the start of symptoms.

Methods:

In this retrospective study, 409 Dutch patients with IPF were included. Clinical characteristics, including patient demographics, medical history, time of start of symptoms, time of first visit to pulmonologist, and any previous radiographic imaging reports were collected from patient records.

Results:

In 96 patients (23%) a chest X-ray was available that had been made prior to the start of symptoms (median of 50.5 months (IQR 26.3-83.3 months)), and this showed potential interstitial lung abnormalities in 56 patients (58%). The median time from the start of symptoms to the final diagnosis was 24.0 months (interquartile range 9.0-48.0 months). In a multivariate model that corrected for diffusion capacity of the lung for carbon monoxide, forced vital capacity, sex, and age at diagnosis, time to diagnosis did not associate with survival (hazard ratio 1.051 (95% CI 0.800-1.380; p=0.72)).

Conclusions:

There is a significant diagnostic delay for patients with IPF, but longer time to diagnosis did not associate with survival. Interstitial lung abnormalities were seen in more than half of the patients in whom a chest X-ray had been made prior to the start of symptoms. This illustrates that a computed tomography scan should be strongly considered for analysis of unexplained abnormalities on a chest X-ray. This could facilitate early detection and possibly prevention of disease progression for patients with pulmonary fibrosis.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a severe fibrotic interstitial lung disease (ILD) with a median survival of 3-5 years after diagnosis. No curative treatment is available except for lung transplantation, but the antifibrotic agents pirfenidone and nintedanib have been shown to slow disease progression. Therefore, timely diagnosis and/or referral to an Interstitial Lung Diseases centre is important.[1]

Previous studies have shown that diagnostic delay for patients with IPF is common, and that consultations with a pulmonologist, as well as pulmonary function testing, chest CT-scans, and additional diagnostics are often performed in the years leading up to the eventual diagnosis.[2] Over half of all patients report having received another diagnosis prior to the diagnosis of IPF,[3] and the final diagnosis is oftentimes made more than one year after the onset of symptoms.[3–8] Factors that lead to an increased time to diagnosis in patients with IPF include underreporting of ILD features on diagnostic testing and prolonged time to pulmonology referral from a primary care setting,[9] as well as male sex, older age, and comorbid conditions such as cardiac and gastro-oesophageal disorders.[4,5]

In a study that was performed prior to the availability of antifibrotic agents, longer diagnostic delay was associated with worse survival, independent of disease severity at diagnosis.[7] However, in a more recent study, diagnostic delay, stratified by symptom onset >1 year or ≤1 year prior to diagnosis, did not associate with survival.[4] In another study, patients who were referred to an ILD centre within 12 months of symptom onset had significantly longer survival compared to patients referred later. Yet, when corrected for disease severity at diagnosis, diagnostic delay no longer associated with survival in that study either.[8]

Recently, there has been increased attention for interstitial lung abnormalities on chest computed tomography (CT) scans, as these likely represent an early stage of pulmonary fibrosis.[10] However, interstitial lung abnormalities are not defined on chest X-rays and it is not known if early stages of pulmonary fibrosis can be seen on chest X-rays of patients with IPF prior to the start of symptoms.

In the present study we investigated the patient trajectory prior to diagnosis in a large Dutch cohort of patients with IPF, including radiographic imaging that was performed prior to the start of symptoms, as well as factors associated with time to diagnosis and the relation between time to diagnosis and mortality.

PATIENTS AND METHODS

This was a retrospective study. All patients with a diagnosis of IPF between January 2011 and October 2017 that were included in the Biobank for ILD of St. Antonius Hospital were included. Participants of the Biobank have given permission for the use of their clinical data in scientific research (approved by the local ethics committee (MEC-U) under study number R05-08A). The study was performed in accordance with the declaration of Helsinki.

The diagnosis of IPF was always made by a multidisciplinary team, and this was done in accordance with the Fleischner Society recommendations and ATS/ERS/JRS/ALAT guidelines.[11,12] Diagnoses were classified as either a consensus or a working diagnosis of IPF. A consensus diagnosis of IPF can be made when, in the appropriate clinical context of IPF, a definite pattern of usual interstitial pneumonia (UIP) is seen on the chest CT-scan, or after integration of clinical, radiographic and histopathologic findings during multidisciplinary discussion. All biopsy results included were from surgical lung biopsies. A working diagnosis of IPF can be made when a patient has a progressive fibrosing interstitial pneumonia in the absence of an alternative explanation, and IPF is thought to be the most likely diagnosis by the multidisciplinary team.

Clinical characteristics were retrieved from patient records. This included patient demographics, medical history, time of start of symptoms, time of first visit to pulmonologist, and, when available, any previous radiographic imaging reports. When the imaging report mentioned reticular abnormalities, interstitial abnormalities, infiltrative abnormalities, or pulmonary fibrosis, this was considered to represent potential interstitial lung abnormalities.

Patient follow up was completed up to December 2018. Study data were collected and managed using REDCap electronic data capture tools hosted at St. Antonius Hospital.[13] Data analyses were performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA). Median values with interquartile range (IQR) are reported for non-normally distributed continuous variables. For comparing two groups, Student's t-test, Mann-Whitney U-test, Kruskall-Wallis test, Fisher's exact test, or chi-squared test were used where appropriate. For survival analyses, Kaplan-Meier curves were made, and Log-rank tests and Cox-regression analyses were performed where appropriate. Patients were censored when lost to follow up, when they underwent lung transplantation, or at the time of data collection. A p-value <0.05 was considered to represent statistical significance.

RESULTS

Four-hundred-and-nine patients were included. Baseline characteristics are provided in Table 1. Three-hundred-and-thirteen patients were male (77%), the median age at diagnosis was 68.2 years (IQR 62.0-74.5), and 78 patients (19%) were under 60 years of age at the time of diagnosis. Presenting complaints included cough in 284 patients (69%) and dyspnoea in 343 patients (84%). Nine patients (2%), did not have any respiratory complaints, of whom five (1%) were screened for pulmonary fibrosis because they had a family member with pulmonary fibrosis.

Table 1: baseline characteristics for 409 patients with idiopathic pulmonary fibrosis.

Total patients	409
Male (%)	313 (77)
Median age at diagnosis (IQR)	68.2 (62.0-74.5)
First-degree family member with pulmonary fibrosis (%)	116 (28)
Other family member with pulmonary fibrosis (%)	24 (6)
Current smoker (%)	33 (8)
Former smoker (%)	298 (73)
Never smoker (%)	78 (19)
Significant exposure to asbestos, dusts, fumes, or radiation (%)	79 (9)
Gastro-oesophageal reflux (%)	149 (36)
Cardiovascular disease (%)	188 (46)
Initial symptoms	
Cough	284 (69)
Dyspnoea	343 (84)
No respiratory complaints	9 (2)
Evaluation in context of screening of family members of patients with familial pulmonary fibrosis	5 (1)
Chest X-ray done prior to start of symptoms (%)	96 (23)
Showing potential interstitial lung abnormalities (%)	56 (58)
CT-imaging done prior to start of symptoms (%)	44 (11)
Showing interstitial lung abnormalities or pulmonary fibrosis (%)	34 (77)
Symptoms started directly after infectious episode (%)	40 (10)
Symptoms started directly after surgery (%)	5 (1)
Treatment with steroids prior to diagnosis (%) #	103 (25)
Treatment with other immunosuppressant prior to diagnosis (%)	23 (6)
Other ILD diagnosis prior to diagnosis of IPF (%)	67 (16)

Table 1: baseline characteristics for 409 patients with idiopathic pulmonary fibrosis. (continued)

Cardiology workup for symptoms prior to pulmonology visit (%)	45 (11)
Time from start of symptoms to first visit with pulmonologist, median months (IQR)	5.0 (2.0-12.0)
Time from first visit with pulmonologist to start of symptoms, median months (IQR)	10.0 (4.0-33.0)
Time from start of symptoms to diagnosis, median months (IQR)	24.0 (9.0-48.0)
Forced vital capacity at diagnosis, median percentage of predicted (IQR)	78.1 (64.0-91.3)
Diffusion capacity for carbon monoxide at diagnosis, median percentage of predicted (IQR)	41.0 (32.0-51.0)
Radiographic pattern at diagnosis	
UIP pattern (%)	238 (58)
Probable UIP (%)	101 (25)
Inconsistent with UIP or indeterminate for UIP (%)	70 (17)
Histology pattern at diagnosis	99 (24)
UIP pattern (%) *	70 (70)
Probable UIP (%) *	16 (16)
Inconsistent with UIP or indeterminate for UIP (%) *	13 (13)
Consensus diagnosis of IPF (%)	190 (47)
Received treatment with antifibrotic therapy after diagnosis (%)	331 (81)

prednisolone equivalent >=10mg/daily for >4 weeks. * percentages represent the percentage of the patients in whom lung biopsy specimens were available.

In 98 patients (23%) a chest X-ray was available that had been made prior to the start of symptoms, and this showed potential interstitial lung abnormalities in 56 patients (58%). Two examples are provided in Figure 1. The indications for the chest X-rays are shown in Supplementary Table 1. Chest X-ray findings are described in more detail in Supplementary Table 2. The median time between the chest X-ray and the start of symptoms was 50.5 months (IQR 26.3-83.3 months). In 91 patients, the time between the chest X-ray and the start of symptoms was one year or more. The time between the chest X-ray and the start of symptoms did not significantly differ between patients in whom the chest X-ray did or did not show potential interstitial lung abnormalities (median 44.5 months (IQR 24.0-73.0 months) compared to 58.0 months (IQR 32.3-97.5 months); *p*-value 0.16). CT-imaging that had been done prior to the start of symptoms was available in 44 patients (11%), and showed interstitial lung abnormalities or pulmonary fibrosis in 34 patients (77%). Details on CT-imaging findings are provided in Supplementary Tables 2 and 3. In 23 of those 34 patients with abnormalities seen on CT-imaging a chest X-ray had also been made, and this showed potential interstitial lung abnormalities in seventeen patients (74%). A CT-scan, done prior to the start of symptoms, was available for 19 patients with potential interstitial

lung abnormalities on chest X-ray, and showed abnormalities consistent with pulmonary fibrosis or interstitial lung abnormalities in 17 (89%). Symptoms had started directly after a respiratory tract infection in 40 patients (10%) and directly after surgery in five patients (1%).

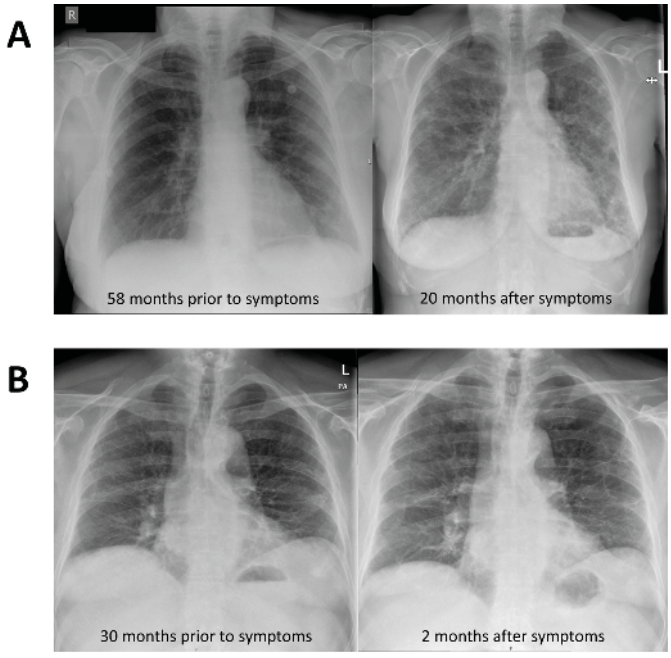

Figure 1: examples of patients who had potential interstitial lung abnormalities on chest X-rays that were made prior to the start of symptoms. (A) patient who presented with temporary dyspnoea after a wasp sting 58 months prior to developing a cough and exertional dyspnoea. The chest X-ray at the time showed minimal reticular changes in the left lower lung. The chest X-ray that was made 20 months after symptoms started shows bilateral reticular changes in the lower fields, especially on the left. (B) patient who was evaluated for an episode of chest pain 30 months prior to developing a cough. The chest X-ray at the time showed bilateral reticular abnormalities. The chest X-ray that was made 2 months after symptoms started showed progressive reticular changes mainly in the lower fields.

The median time from the start of symptoms to the first visit with a pulmonologist was 5.0 months (IQR 2.0-12.0 months), and 45 patients (11%) had undergone evaluation by a cardiologist prior to their visit to a pulmonologist. The median time from the first visit with a pulmonologist to the final diagnosis was 10 months (IQR 4.0-33.0) and the median time from the start of symptoms to the final diagnosis was 24.0 months (IQR 9.0-48.0). In 209 patients (51%), the time from start of symptoms to the final diagnosis was less than 24 months.

The association between clinical characteristics and time to diagnosis is shown in Table 2. Factors that associated with longer time from the start of symptoms to diagnosis included having any family member with pulmonary fibrosis (median time to diagnosis 19.0 months (IQR 6.0-42.5 months) compared to 24.5 months (IQR 10.3-49.0); p=0.03), no chest X-ray having been made prior to the start of symptoms (median time to diagnosis 17.0 months (IQR 7.0-34.5), compared to 26.0 months (IQR 10.0-50.8); p-value 0.01), symptoms that did not start directly after a respiratory tract infection (median time to diagnosis 25.0 months (IQR 10.0-49.0), compared to 9.5 months (IQR 5.0-27.8); p<0.001), having received treatment with prednisolone prior to diagnosis (median time to diagnosis 37.0 months (IQR 19.0-78.0), compared to 18.5 months (IQR 8.0-41.3); p<0.001), having received treatment with other immunosuppressive medication prior to diagnosis (median time to diagnosis 48.0 months (IQR 26.0-84.0), compared to 23.0 months (IQR 9.0-45.5); p=0.002), and having received another ILD diagnosis prior to the diagnosis of IPF (median time to diagnosis 47.0 months (IQR 26.0-83.0), compared to 19.0 months (IQR 8.0-42.0); p<0.001). Patients who had received another ILD diagnosis prior to the diagnosis of IPF had received treatment with prednisolone or other immunosuppressive medication significantly more often (69% versus 17%, p<0.001; 15% versus 4%, p=0.001).

Table 2: clinical characteristics associated with time to diagnosis in 409 patients with IPF.

	Time from symptom onset to diagnosis, months (IQR)		***p*-value**
	Factor present	**Factor absent**	
Male sex (n=313)	24.0 (9.0-47.5)	24.5 (8.3-49.8)	0.70
Age at diagnosis <60 years (n=78)	17.0 (6.8-45.0)	25.0 (10.0-49.0)	0.10
First-degree family member with pulmonary fibrosis (n=116)	19.5 (6.3-41.8)	24.0 (10.0-49.0)	0.05
Other family member with pulmonary fibrosis (n=24)	11.5 (5.3-57.8)	24.0 (9.0-48.0)	0.12
Any family member with pulmonary fibrosis (n=125)	19.0 (6.0-42.5)	24.5 (10.3-49.0)	0.03
Current smoker (n=33)	19.0 (5.5-39.0)		0.07
Former smoker (n=298)	26.0 (10.0-52.0)		
Never smoker (n=78)	23.0 (7.8-37.8)		
Significant exposure to asbestos, dusts, fumes, or radiation (n=79)	26.0 (10.0-49.0)	23.0 (8.0-47.0)	0.18
Gastro-oesophageal reflux (n=149)	24.0 (9.5-57.0)	24.0 (9.0-46.5)	0.49

Table 2: clinical characteristics associated with time to diagnosis in 409 patients with IPF. (continued)

	Time from symptom onset to diagnosis, months (IQR)		***p*-value**
	Factor present	**Factor absent**	
Cardiovascular disease (n=188)	24.5 (8.0-52.0)	24.0 (9.0-44.0)	0.35
Chest X-ray done prior to start of symptoms (n=96)	17.0 (7.0-34.5)	26.0 (10.0-50.8)	0.01
Chest X-ray showing potential interstitial lung abnormalities (n=56) *	17.0 (7.0-27.8)	21.0 (7.0-45.0)	0.34
Symptoms started directly after infectious episode (n=40)	9.5 (5.0-27.8)	25.0 (10.0-49.0)	<0.001
Symptoms started directly after surgery (n=5)	12.0 (7.5-88.5)	24.0 (9.0-48.0)	0.99
Treatment with steroids prior to diagnosis (n=103) #	37.0 (19.0-78.0)	18.5 (8.0-41.3)	<0.001
Treatment with other immunosuppressant prior to diagnosis (n=23)	48.0 (26.0-84.0)	23.0 (9.0-45.5)	0.002
Other ILD diagnosis prior to diagnosis of IPF (n=67)	47.0 (26.0-83.0)	19.0 (8.0-42.0)	<0.001
Cardiology workup for symptoms prior to pulmonology visit (n=45)	23.0 (8.0-60.5)	24.0 (9.0-48.0)	0.59
Consensus diagnosis of IPF (n=190)	23.0 (9.0-49.0)	24.0 (9.0-47.0)	0.75
Received treatment with antifibrotic therapy after diagnosis	25.0 (10.0-49.0)	19.5 (7.0-42.0)	0.17

* compared to patients in whom a chest X-ray was performed but did not reveal potential interstitial lung abnormalities. # prednisolone equivalent >=10mg/daily for >4 weeks.

Time to diagnosis did not significantly associate with survival after diagnosis. In patients with time to diagnosis >24 months, median survival after diagnosis was 1012 days (IQR 530-1863), compared to 1322 days (IQR 610-2476) in patients with time to diagnosis ≤24 months (Figure 2; p=0.05). In a multivariate model that corrected for diffusion capacity of the lung for carbon monoxide, forced vital capacity, sex, and age at diagnosis, time to diagnosis did not associate with survival (hazard ratio 1.051 (95% CI 0.800-1.380; p=0.72)). When using time to diagnosis as a continuous variable in the same multivariate model, this did not associate with survival either (hazard ratio 1.000 (95% CI 0.997-1.004; p=0.97)). Factors that associated with survival in the same multivariate model included being a current smoker (hazard ratio 0.46 (95% CI 0.21-0.97); p=0.04), a history of cardiovascular

disease (hazard ratio 1.41 (95% CI 1.07-1.86); p=0.01), and treatment with antifibrotic therapy (hazard ratio 0.52 (95% CI 0.37-0.75; p<0.001) (Table 3).

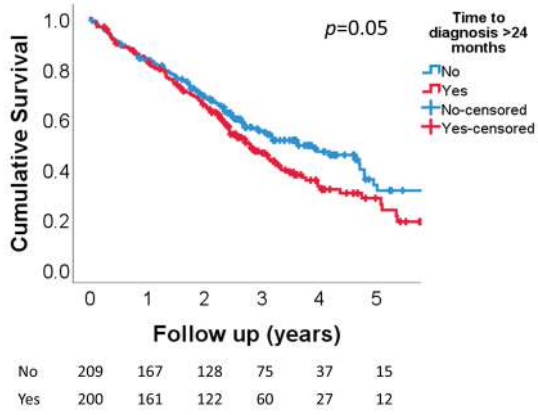

Figure 2: survival after diagnosis in 409 patients with IPF, stratified by time to diagnosis >24 months or ≤24 months. Median survival after diagnosis was 1012 days in patients with time to diagnosis >24 months (interquartile range (IQR) 530-1863), compared to 1322 days in patients with time to diagnosis ≤24 months (IQR 610-2476) (p=0.05). In a multivariate model that corrected for diffusion capacity of the lung for carbon monoxide, forced vital capacity, sex, and age at diagnosis, time to diagnosis did not associate with survival (hazard ratio 1.051 (95% confidence interval 0.800-1.380; p=0.72)). At risk tables represent the number of patients at the start of every year during follow up.

Table 3: factors associated with survival.

	Hazard ratio (95% confidence interval)	***p*-value**
First-degree family member with pulmonary fibrosis (n=116)	1.18 (0.86-1.63)	0.30
Other family member with pulmonary fibrosis (n=24)	1.35 (0.69-2.63)	0.38
Any family member with pulmonary fibrosis (n=125)	1.18 (0.86-1.62)	0.29
Current smoker (n=33) *	0.46 (0.21-0.97)	0.04
Former smoker (n=298) *	1.01 (0.68-1.50)	0.97
Significant exposure to asbestos, dusts, fumes, or radiation (n=79)	1.12 (0.85-1.47)	0.44
Gastro-oesophageal reflux (n=149)	0.98 (0.75-1.30)	0.91
Cardiovascular disease (n=188)	1.41 (1.07-1.86)	0.01
Chest X-ray prior to start of symptoms showing potential interstitial lung abnormalities (n=56) #	1.65 (0.88-3.08)	0.56

Table 3: factors associated with survival. (continued)

	Hazard ratio (95% confidence interval)	***p*-value**
CT-scan prior to start of symptoms showing potential interstitial lung abnormalities (N=34) $	0.98 (0.37-2.58)	0.97
Symptoms started directly after infectious episode (n=40)	0.82 (0.51-1.34)	0.43
Symptoms started directly after surgery (n=5)	0.65 (0.16-2.64)	0.55
Treatment with steroids prior to diagnosis (n=103) ^	1.10 (0.81-1.49)	0.55
Treatment with other immunosuppressant prior to diagnosis (n=23)	0.94 (0.53-1.65)	0.82
Other ILD diagnosis prior to diagnosis of IPF (n=67)	1.01 (0.71-1.44)	0.96
Cardiology workup for symptoms prior to pulmonology visit (n=45)	0.96 (0.64-1.44)	0.83
Time from start of symptoms to first visit with pulmonologist (n=409)	1.00 (0.99-1.01)	0.81
Time from start of symptoms to diagnosis (n=409)	1.00 (1.00-1.00)	0.97
Consensus diagnosis of IPF (n=190)	0.89 (0.68-1.17)	0.40
Received treatment with antifibrotic therapy after diagnosis	0.52 (0.37-0.75)	<0.001

Hazard ratios are derived from a multivariate model that corrected for diffusion capacity of the lung for carbon monoxide, forced vital capacity, sex, and age at diagnosis. * compared to never smokers as a reference category. # compared to patients in whom a chest X-ray was performed but did not reveal potential interstitial lung abnormalities. $ compared to patients in whom a CT-scan was performed but did not reveal potential interstitial lung abnormalities. ^ prednisolone equivalent >=10mg/daily for >4 weeks.

DISCUSSION

In this cohort of 409 patients with IPF from the Netherlands, a long time between the start of symptoms and the final diagnosis was common. The median time between the start of symptoms and the final diagnosis was 24 months, and the time to diagnosis was longer than 24 months in 49% of the patients. Most patients had an insidious onset of the disease, but in 10% of patients the first symptoms became apparent after a respiratory tract infection, and in 1% of patients after surgery. The insidious disease onset is also evidenced by the fact that in 96 patients a chest X-ray had been done for other reasons prior to the start of symptoms, and that 58% of those chest X-rays showed potential interstitial lung abnormalities. In the 44 patients for whom CT-imaging of the lungs was done prior to the start of symptoms, interstitial lung abnormalities were seen on 77% of the scans. The median time between the first visit to a pulmonologist and the final diagnosis was 10

months, indicating a significant doctor's delay in addition to a patient's delay in arriving at the final diagnosis.

Factors that associated with longer time to diagnosis were having no family member with pulmonary fibrosis, treatment with immunosuppressive medication prior to the diagnosis, having received another ILD diagnosis prior to the diagnosis of IPF, and symptoms not having started directly after a respiratory tract infection. In contrast to previous studies,[4] we did not find an association between a history of cardiovascular disease or gastro-oesophageal reflux and longer time to diagnosis, nor was longer time to diagnosis associated with a cardiology consultation prior to the first visit to a pulmonologist.

Somewhat counterintuitively, longer time to diagnosis was not significantly related to shorter survival after diagnosis, also in a multivariate model that corrected for sex, age and lung function at diagnosis. This is in contrast to a previous study that was done prior to the availability of antifibrotic therapy.[7] However, this is in line with the findings from two more recent studies. [4,8] Other than a clinical history of cardiovascular disease and being a current smoker, no other clinical factors associated with survival in our study. The absence of an association between longer time to diagnosis and survival is sobering, as this illustrates that despite our best efforts, we still have little to offer to IPF patients in order to prevent disease progression and prolong survival. In this cohort, it needs to be taken into account that approximately 80% of patients was treated with antifibrotic medication after they were diagnosed with IPF. Notably, pirfenidone and nintedanib do seem improve survival in patients with IPF according to meta-analyses of randomized controlled trials, but the actual magnitude of this effect is uncertain.[14] In our cohort treatment with antifibrotic therapy was also associated with better survival in a multivariate model, but we cannot exclude that this is due to selection bias.

The fact that a significant proportion of patients in whom either a chest X-ray or a CT-scan had been done for some other reason, prior to the start of symptoms related to IPF, had potential interstitial lung abnormalities, is of interest. Interstitial lung abnormalities are found on CT-scans of 2-9% of older adults. [10] When followed up, interstitial lung abnormalities are oftentimes progressive, and they associate with respiratory symptoms and increased mortality.[10] However, as far as we are aware, it is not known what proportion of patients with IPF had potential interstitial lung abnormalities on previous imaging. The proportion of 58% that we found on chest X-rays is very high. Furthermore, these chest X-rays were done a median of 44.5 months prior to the start of symptoms, which indicates that subclinical disease can be present for a long time in many patients. This would indicate that screening of certain populations at high risk for IPF might be worthwhile, as there would be ample time for intervention and halting disease progression.[15] However, it is im-

portant to acknowledge that it is presently unknown whether treatment with antifibrotic medication can prevent progression of interstitial lung abnormalities.[15] Furthermore, it is not known whether the abnormalities seen on the chest X-rays represent the same clinical entity as interstitial lung abnormalities seen on chest CT-scans. The latter are often subtle changes that are not visible on chest X-rays, and might represent a much earlier stage of fibrosis.

We were not able to explore precisely how the radiology reports that mentioned potential interstitial lung abnormalities were interpreted by the requesting physician at the time that the imaging was done. In any case, the absence of (persisting) respiratory symptoms might have caused the physician at that time to refrain from further investigations or follow-up. Unfortunately, in these cases, this has meant that a rather easy opportunity for earlier diagnosis of pulmonary fibrosis or interstitial lung abnormalities was missed. A potential solution to be considered is that the radiologist report specifically advises to perform a chest CT-scan in case of potential interstitial lung abnormalities on a chest X-ray. Considering that 23% of this cohort had a chest X-ray done and 58% of chest X-rays showed abnormalities in this cohort, this would facilitate earlier diagnosis in at least one in eight patients with IPF. However, this might cause unnecessary CT-scans to be performed, and it is not known if this would lead to more timely diagnosis or recognition of patients with interstitial lung abnormalities that are a precursor to IPF.

Despite our findings, we do not think that chest X-ray is a useful screening tool for patients with suspected IPF, such as first-degree relatives of patients with pulmonary fibrosis.[16,17] This is partially because chest X-ray is a cruder diagnostic tool, and some subtle abnormalities may be missed. Furthermore, in the context of recent studies in relatives of patients with pulmonary fibrosis, as well as lung cancer screening programs, more information will be available on the prognostic significance of interstitial lung abnormalities, as seen on CT-scan, than abnormalities seen on chest X-ray.[10,16,17]

The present study has several limitations. First, this was a retrospective study, and this can always lead to missing data or incorrect categorization. Second, because patients estimated the time since the start of symptoms at the time of the first visit to a pulmonologist, this could be subject to recall bias, and may not be a completely reliable value. We are however confident that in most if not all cases, the imaging was done before the start of symptoms. In the majority of patients, the time between the chest X-ray and the reported symptom onset was one year or more, and for the patients in whom we were able to assess the reason for requesting the chest X-ray, this was not related to symptoms of IPF. Third, because radiographic imaging was oftentimes performed at another hospital, we are not certain that we were able to assess all chest X-rays and CT-scans that were done

prior to the start of symptoms in our patients. In addition, we were not able to view the original images, and had to determine whether potential interstitial lung abnormalities were present based on the radiologist's report. This could have led to underreporting of interstitial lung abnormalities.

CONCLUSION

In this cohort of 409 Dutch patients with IPF, the median time between the start of symptoms and the final diagnosis was 24 months, with half of the patients having a time to diagnosis of over two years. In 23% of the patients, a chest X-ray was made a median of 50.5 months prior to the start of symptoms, and this showed potential interstitial lung abnormalities in 58%, which demonstrates the potential for earlier diagnosis in a proportion of patients with IPF. Time to diagnosis did not associate with survival, illustrating that more work needs to be done on prevention of disease progression in patients with pulmonary fibrosis.

REFERENCES

1. Oldham JM, Noth I. Idiopathic pulmonary fibrosis: early detection and referral. *Respir Med*. 2014;108(6):819-829. doi:10.1016/J.RMED.2014.03.008
2. Mooney J, Chang E, Lalla D, et al. Potential Delays in Diagnosis of Idiopathic Pulmonary Fibrosis in Medicare Beneficiaries. *Ann Am Thorac Soc*. 2019;16(3):393-396. doi:10.1513/ANNALSATS.201806-376RL
3. Cosgrove GP, Bianchi P, Danese S, Lederer DJ. Barriers to timely diagnosis of interstitial lung disease in the real world: the INTENSITY survey. *BMC Pulm Med*. 2018;18(1). doi:10.1186/S12890-017-0560-X
4. Snyder LD, Mosher C, Holtze CH, et al. Time to diagnosis of idiopathic pulmonary fibrosis in the IPF-PRO Registry. *BMJ open Respir Res*. 2020;7(1). doi:10.1136/BMJRESP-2020-000567
5. Hoyer N, Prior TS, Bendstrup E, Wilcke T, Shaker SB. Risk factors for diagnostic delay in idiopathic pulmonary fibrosis. *Respir Res*. 2019;20(1). doi:10.1186/S12931-019-1076-0
6. van der Sar IG, Jones S, Clarke DL, et al. Patient Reported Experiences and Delays During the Diagnostic Pathway for Pulmonary Fibrosis: A Multinational European Survey. *Front Med*. 2021;8. doi:10.3389/FMED.2021.711194
7. Lamas DJ, Kawut SM, Bagiella E, Philip N, Arcasoy SM, Lederer DJ. Delayed access and survival in idiopathic pulmonary fibrosis: a cohort study. *Am J Respir Crit Care Med*. 2011;184(7):842-847. doi:10.1164/RCCM.201104-0668OC
8. Brereton CJ, Wallis T, Casey M, et al. Time taken from primary care referral to a specialist centre diagnosis of idiopathic pulmonary fibrosis: an opportunity to improve patient outcomes? *ERJ Open Res*. 2020;6(2):00120-02020. doi:10.1183/23120541.00120-2020
9. Pritchard D, Adegunsoye A, Lafond E, et al. Diagnostic test interpretation and referral delay in patients with interstitial lung disease. *Respir Res*. 2019;20(1). doi:10.1186/S12931-019-1228-2
10. Hatabu H, Hunninghake GM, Richeldi L, et al. Interstitial lung abnormalities detected incidentally on CT: a Position Paper from the Fleischner Society. *Lancet Respir Med*. 2020;8(7):726-737. doi:10.1016/S2213-2600(20)30168-5
11. Lynch DA, Sverzellati N, Travis WD, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. *Lancet Respir Med*. 2018;6(2):138-153. doi:10.1016/S2213-2600(17)30433-2
12. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med*. 2011;183(6):788-824. doi:10.1164/rccm.2009-040GL [doi]
13. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010 [doi]
14. Rochwerg B, Neupane B, Zhang Y, et al. Treatment of idiopathic pulmonary fibrosis: a network meta-analysis. *BMC Med*. 2016;14:18-016-0558-x. doi:10.1186/s12916-016-0558-x [doi]
15. Hunninghake GM. Interstitial lung abnormalities: erecting fences in the path towards advanced pulmonary fibrosis. *Thorax*. 2019;74(5):506-511. doi:10.1136/THORAXJNL-2018-212446

16. Salisbury ML, Hewlett JC, Ding G, et al. Development and Progression of Radiologic Abnormalities in Individuals at Risk for Familial Interstitial Lung Disease. *Am J Respir Crit Care Med*. 2020;201(10):1230-1239. doi:10.1164/RCCM.201909-1834OC

17. Hunninghake GM, Quesada-Arias LD, Carmichael NE, et al. Interstitial Lung Disease in Relatives of Patients with Pulmonary Fibrosis. *Am J Respir Crit Care Med*. 2020;201(10):1240-1248. doi:10.1164/RCCM.201908-1571OC

SUPPLEMENTARY DATA

Supplementary Table 1: Indications for chest X-rays done prior to the start of symptoms in 96 patients with IPF

Indication	N patients
Prior to/after thoracic surgery	12
Cough (temporary)	10
Chest pain	6
Dyspnoea (temporary)	5
Follow-up after pneumonia	5
Trauma	3
Crackles	2
Follow-up for malignancy	2
Haemoptysis	1
Family history	1
Heart murmur	1
Tuberculosis screening	1
Tiredness	1
Recurrent infections	1
Unknown	45

Supplementary Table 2: additional information on radiographic findings in 56 patients with potential interstitial lung abnormalities detected on chest X-ray prior to start of symptoms of idiopathic pulmonary fibrosis.

Patient	Time between chest X-ray and start of symptoms (months)	Chest X-ray description	Time between chest X-ray and CT-scan (months)	CT-scan findings
1	101	Increased interstitial markings bilaterally	12	Fibrotic changes
2	34	Interstitial abnormalities		
3	12	Fibrotic changes		
4	60	Fibrotic abnormalities		
5	24	Interstitial abnormalities	1	Fibrotic changes
6	56	Fibrotic abnormalities	1	Fibrotic abnormalities
7	51	Increased subpleural reticulation		
8	73	Increased interstitial markings	11	Some fibrotic changes
9	12	Fibrotic changes		
10	27	Increased reticular markings in the right lung		
11	45	Interstitial abnormalities	1	Fibrotic abnormalities

Supplementary Table 2: additional information on radiographic findings in 56 patients with potential interstitial lung abnormalities detected on chest X-ray prior to start of symptoms of idiopathic pulmonary fibrosis. (continued)

Patient	Time between chest X-ray and start of symptoms (months)	Chest X-ray description	Time between chest X-ray and CT-scan (months)	CT-scan findings
12	58	Some interstitial abnormalities		
13	120	Fibrotic abnormalities		
14	35	Interstitial abnormalities		
15	33	Fibrotic abnormalities	4	Interstitial lung abnormalities
16	44	Interstitial abnormalities, especially in the righ lung	1	Fibrotic abnormalities
17	4	Interstitial abnormalities	-5	Minimal reticulation
18	106	Fibrotic abnormalities	75	Fibrotic abnormalities
19	33	Fibrotic abnormalities		
20	104	Increased interstitial markings in lower and middle fields as well as right upper field		
21	64	Increased interstitial markings		
22	92	Somewhat increased interstitial markings		
23	67	Somewhat increased interstitial markings		
24	20	Interstitial abnormalities bilaterally		
25	74	Interstitial abnormalities, possibly redistribution	63	Fibrotic abnormalities
26	50	Reticular abnormalities		
27	6	Interstitial abnormalities, especially in the right lung		
28	10	Interstitial abnormalities		
29	7	Increased markings in both pleural sinuses		
30	15	Interstitial abnormalities	2	Fibrotic abnormalities
31	15	Increased interstitial markings, somewhat more on the right		
32	37	Interstitial abnormalities		
33	65	Fibrotic changes	48	Fibrotic changes
34	34	Fibrotic changes bilaterally		
35	23	Increased interstitial markings peripherally	1	Fibrosis

Supplementary Table 2: additional information on radiographic findings in 56 patients with potential interstitial lung abnormalities detected on chest X-ray prior to start of symptoms of idiopathic pulmonary fibrosis. (continued)

Patient	Time between chest X-ray and start of symptoms (months)	Chest X-ray description	Time between chest X-ray and CT-scan (months)	CT-scan findings
36	3	Fibrotic changes in the lower zones peripherally		
37	70	Somewhat increased interstitial markings	2	Small airways disease
38	40	Interstitial abnormalities		
39	86	Fibrotic abnormalities		
40	73	Increased interstitial markings	20	Subpleural fibrotic changes
41	212	Bilateral reticular and nodular abnormalities	1	No abnormalities
42	24	Increased interstitial markings		
43	12	Increased interstitial markings bilaterally in the lower fields		
44	24	Interstitial abnormalities		
45	126	Fibrotic abnormalities		
46	78	Bronchopathic and fibrotic abnormalities		
47	71	Somewhat increased interstitial markings		
48	91	Fibrotic abnormalities	3	Fibrotic changes
49	26	Increased interstitial markings especially peripherally	1	Bilateral subpleural reticulation
50	73	Increased interstitial markings		
51	30	Increased interstitial markings, especially in the left lower zone		
52	101	Some fine changes, especially in the left lower zone		
53	51	Fibrotic abnormalities	1	Fibrotic changes
54	53	Reticular abnormalities		
55	43	Interstitial abnormalities		
56	43	Diffusely increased interstitial markings		

Note that the chest X-ray and CT-scan description was based on radiology reports and not on revision of the images. Negative time between chest X-ray and CT-scan indicates that the CT-scan was made before the chest X-ray.

Supplementary Table 3: additional information on radiographic findings in 17 patients with potential interstitial lung abnormalities detected on CT-scan prior to start of symptoms of idiopathic pulmonary fibrosis.

Patient	Time between CT-scan and start of symptoms	CT-scan findings	Time between CT-scan and chest X-ray	Potential interstitial lung abnormalities on chest X-ray
1	40	Some fibrotic abnormalities		
2	82	Some fibrotic abnormalities	-2	No
3	44	Mild fibrosis		
4	52	Normal		
5	9	Mild fibrosis		
6	43	Mild fibrotic abnormalities in lower fields (abdominal scan)		
7	43	Some fibrosis		
8	108	Normal		
9	60	Normal		
10	62	Normal	1	No
11	142	Some bronchiectasis and alveolar infiltrates	12	No
12	29	Mild fibrosis		
13	68	Normal	0	No
14	110	Some fibrotic abnormalities	-30	No
15	33	Normal	28	No
16	18	Fibrotic abnormalities in lower fields (abdominal scan)		
17	48	Normal	-240	No
18	34	Subtle interstitial lung abnormalities	1	No
19	99	Some fibrotic abnormalities in right upper lobe		
20	40	Fibrotic abnormalities	-108	No
21	84	Minimal fibrotic changes	2	No
22	84	Normal	47	No
23	46	Subpleural reticulation		
24	54	Bilateral fibrotic abnormalities		
25	42	Some fibrosis		

Seventeen other patients with potential interstitial lung abnormalities on CT-scan that are included in Supplementary Table 2 are not included here, so no patients in this table have abnormalities on chest X-ray. Note that the chest X-ray and CT-scan description was based on radiology reports and not on revision of the images. Negative time between CT-scan and chest X-ray indicates that the chest X-ray was made before the CT-scan.

Chapter 3

Extrapulmonary manifestations of a telomere syndrome in patients with idiopathic pulmonary fibrosis are associated with decreased survival

T.W. Hoffman, J.J. van der Vis, D.H. Biesma, J.C. Grutters, and C.H.M. van Moorsel

ABSTRACT

Background:

Idiopathic pulmonary fibrosis (IPF) is a heterogenous disease with a median survival of 3-4 years. Patients with mutations in telomere-related genes were shown to exhibit extrapulmonary signs and symptoms of a telomere syndrome and represent a distinct phenotype of IPF with worse survival. As genetic analyses are not available for most patients with IPF, we sought to determine the predictive value of extrapulmonary signs and symptoms of a telomere syndrome in patients with IPF.

Methods:

We retrospectively studied 409 patients with IPF. Clinical characteristics, laboratory results, and family history suggestive of a telomere syndrome were related to leukocyte telomere length measured by quantitative polymerase chain reaction and patient outcomes.

Results:

The cohort included 293 patients with sporadic IPF and 116 patients with background of familial pulmonary fibrosis. Any or a combination of a clinical history (haematological disease, liver disease, early greying of hair, nail dystrophy, skin abnormalities), a family history, or haematological laboratory abnormalities (macrocytosis, anaemia, thrombopenia or leukopenia) suggestive of a telomere syndrome was present in 27% of IPF patients and associated with shorter leukocyte telomere length and shorter survival (p=0.002 in a multivariate model). In sporadic IPF, having either a clinical history, family history, or haematological laboratory abnormalities was not significantly associated with decreased survival (p=0.07 in a multivariate model).

Conclusions:

Taking a careful clinical and family history focused on extrapulmonary manifestations of a telomere syndrome can provide important prognostic information in patients with IPF, as this is associated with shorter survival.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a severe interstitial lung disease. It is characterized by progressive fibrosis and decline in pulmonary capacity, with a median survival of 3-4 years after diagnosis.[1] Current treatments include the antifibrotic agents pirfenidone and nintedanib, which are not curative but have been shown to slow pulmonary function decline in clinical trials.[1] Risk factors for IPF include male sex, smoking, other fibrogenic exposures (e.g. wood dust, irritant inhalants, etc.), and having a family member with pulmonary fibrosis.[2] Up to 10% of patients with IPF have one or more family members with pulmonary fibrosis. In case of two or more first-degree family members with pulmonary fibrosis, it is termed familial idiopathic pulmonary fibrosis (fIPF).[1]

Telomere dysfunction plays a key role in disease pathogenesis in a part of IPF and fIPF patients.[3] Telomere dysfunction can be caused by decreased functionality of telomere-related proteins or (inheritance of) short telomeres, and causes replicative senescence or cellular apoptosis.[4] Conditions of impaired telomere maintenance are termed telomere syndromes. These syndromes can have various manifestations throughout the body, including skin abnormalities, bone marrow failure, liver disease, gastrointestinal disease, early greying of hair, and pulmonary fibrosis or emphysema.[4] Mutations in telomere-related genes can have a wide range of disease phenotypes, and specific phenotypes can be caused by various genetic mutations.[5] IPF is by far the most common manifestation of a telomere syndrome, but it is difficult to estimate the proportion of IPF patients in whom disease is caused by telomere dysfunction.[3] Up to 25% of patients with fIPF and up to 10% of patients with sporadic IPF have mutations in telomere-related genes.[6] These patients seem to represent a distinct clinical phenotype of IPF, characterized by shorter survival after diagnosis. [7,8]

Some, but not all patients with pulmonary fibrosis who have proven mutations in telomere-related genes show extrapulmonary manifestations of a telomere syndrome. Previous studies that investigated possible extrapulmonary manifestations of telomere syndromes in IPF patients studied highly selected populations (i.e. only lung transplant recipients, or only patients with proven telomere-related gene mutations), and might not be representative of the general IPF population.[7–9] Furthermore, genetic analyses are not always available in clinical practice. The aim of this study was to determine the prevalence of extrapulmonary manifestations suggestive of a telomere syndrome in the general IPF population and their association with prognosis and leukocyte telomere length.

PATIENTS AND METHODS

Patients were retrospectively included from the St. Antonius Hospital Interstitial Lung Diseases Biobank. This study was approved by the local medical ethics committee (Medical Resarch Ethics Committees United; number R05-08A). Participants have given informed consent for the use of their clinical data in scientific research, and have provided blood to be stored and used for later analyses. This study involves all patients with IPF who were included in the Biobank from January 2011 to October 2017. Only patients in whom a DNA sample was available were included. Patients were followed up to June 2019.

The diagnosis of IPF was always made by a multidisciplinary team using clinical, radiological, and if necessary histopathological data, in accordance with the Fleischner Society recommendations, as well as ATS/ERS/JRS/ALAT guidelines.[10,11] Patients were given either a consensus or a working diagnosis of IPF. A consensus diagnosis of IPF was given in the appropriate clinical context of IPF (age >60 years, absence of significant exposure, no evidence of collagen vascular disease), with a definite CT-pattern of usual interstitial pneumonia (UIP), or after integration of clinical, imaging and histologic features. A working diagnosis of IPF was given to patients with probable UIP on CT or progressive fibrosing interstitial pneumonia, in the absence of an alternative diagnosis, and when IPF was thought to be the most likely diagnosis.

Clinical data were retrieved from patient records. Retrieved information included comorbid conditions, full clinical history and physical examination. Family history up to third-degree family members and history of pulmonary exposures were retrieved from standardized forms that patients filled in at time of referral. Results from pulmonary function testing (in accordance with European Respiratory Society standards) at time of diagnosis were automatically retrieved from patient records. High-resolution computed-tomography (HRCT) scans at time of diagnosis were retrieved from the hospital's radiology database. Scan reports were reviewed for the radiological fibrosis pattern. Histopathological information, when available, was retrieved from histopathology reports generated at the time of diagnosis. Laboratory test results were retrieved from the hospital laboratory system. Only test results within 6 months of the date of diagnosis were included, and in all cases values as close to the date of diagnosis as possible were used.

Familial pulmonary fibrosis was defined as two or more first-degree family members with pulmonary fibrosis. Clinical features that were deemed suggestive of a telomere syndrome included anaemia or thrombocytopenia, bone marrow failure, haematological malignancies, liver cirrhosis or liver cancer, greying of hair before 30 years, oral leucoplakia, nail dystrophy, and skin hyperpigmentation. Features were not counted if a clear alternative

aetiology was apparent from the patient records. Features in the family history that were deemed suggestive of a telomere syndrome included first-, second-, or third-degree family members with aplastic anaemia, bone marrow failure, liver cirrhosis, liver cancer, or dyskeratosis congenita. Laboratory features deemed suggestive of a telomere syndrome included anaemia, thrombocytopenia, leukopenia, and macrocytosis. Local laboratory cut-off values were used as lower limits of normal. Patients with any one or more of these features (i.e. clinical features in the patient history, family history, or laboratory features suggestive of a telomere syndrome) are referred to as patients in whom there is a clinical suspicion of a telomere syndrome.

Blood samples were taken around time of diagnosis. The samples were stored at -80 degrees Celsius until use. DNA was isolated from white blood cells using a magnetic beads-based method (chemagic DNA blood 10k kit; PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany). Telomere length was determined using a previously described quantitative polymerase chain reaction (PCR) method.[12] Briefly, telomere length was estimated for each sample from the ratio of telomere repeat copy number to a single gene (human β-globin gene) copy number (T/S ratio). Measurements were performed on the Bio-Rad CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) in duplicate, with additional measurements if the duplicates differed more than 0.05; the mean value of the measured samples was used. Furthermore, quality control samples were included on each PCR-run, with a <0.05 margin of variance to reference values allowed. The control cohort included 164 healthy adults (71 male) who were between 20-70 years of age. Age-adjusted normal values for the T/S-ratio were calculated by determining the best-fitting linear regression line through the data, and percentiles were derived from the regression line.

Study data were collected and managed using REDCap electronic data capture tools hosted at St. Antonius Hospital.[13] Data analyses were performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). For comparing two groups, Student's t-test, Mann-Whitney U-test, Fisher's exact test, or chi-squared test were used where appropriate. For the relation between telomere length and other continuous variables, univariate and multivariate generalized linear regression analyses were used. For survival analyses, Kaplan-Meier curves were calculated and Cox-regression analyses were performed, with the latter adjusting for age and lung function (forced vital capacity and diffusion capacity of the lung for carbon monoxide) at diagnosis in multivariate analyses. We also explored the effect of adding smoking status to the multivariate analyses, and did a multivariate analyses that corrected for the GAP-score, a composite index that uses data on sex, lung function, and age, and can be used to determine the prognosis for patients

with idiopathic pulmonary fibrosis.[14] Patients were censored when lost to follow up, when they underwent lung transplantation, or at the time of data collection.

RESULTS

Baseline characteristics for the included patients are shown in Table 1. The cohort comprised 190 patients with a consensus diagnosis of IPF, and 219 patients with a working diagnosis of IPF. There were no significant differences between patients with a working and consensus diagnosis, except that having familial pulmonary fibrosis was more common in patients with a working diagnosis (p=0.02), and a UIP pattern on radiological imaging or histopathology was more common in patients with a consensus diagnosis (p<0.001). Seventy-seven percent of the patients was male, and the median age at diagnosis was 68 years. There were 116 patients with familial pulmonary fibrosis.

Table 1: Baseline characteristics for 409 patients with idiopathic pulmonary fibrosis (IPF)

Clinical characteristics	
Male (%)	313 (77)
Median age at diagnosis (range)	68 (21-89)
Median years between start of symptoms and diagnosis (IQR)	2.0 (0.8-4.0)
Consensus diagnosis of IPF (%)	190 (46)
1st-degree relative with pulmonary fibrosis (%)	116 (28)
Smoking status	
≥5 packyears (%)	302 (74)
≥20 packyears (%)	190 (47)
Lung function at diagnosis	
Median FVC% (IQR)	78 (64-91)
Median FEV1% (IQR)	82 (69-96)
Median DLCOc% (IQR)	41 (32-51)
Radiology findings	
UIP pattern (%)	238 (58)
Probable UIP (%)	101 (25)
Inconsistent with UIP or indeterminate for UIP (%)	70 (17)
Emphysema (%)	111 (27)
Histopathology findings	99 (24)
UIP pattern (%)#	70 (70)
Probable UIP (%)#	16 (16)
Inconsistent with UIP or indeterminate for UIP (%)#	13 (13)

percentages represent the percentage of the patients in whom lung biopsy specimens were available. FVC = forced vital capacity; FEV1 = forced expiratory volume in one second; DLCOc = diffusion capacity of the lung for carbon monoxide, corrected for haemoglobin level; UIP = usual interstitial pneumonia

In total, 35 patients (9%) had a clinical history suggestive of a telomere syndrome (Table 2 and Table 3). The most common finding was haematological disease in 23 patients, followed by liver disease in twelve patients. Thirty patients had one finding suggestive of a telomere syndrome in their clinical history, and five had two. In addition, 41 patients (10%) had a family history suggestive of a telomere syndrome (Supplementary Table 1). Seventy-seven patients (19%) had any haematological laboratory abnormality at time of diagnosis. These characteristics are presented in more detail in Table 3. A family history suggestive of a telomere syndrome was significantly more common in patients with familial pulmonary fibrosis (p<0.001), as was a clinical history of haematological disease (p=0.03).

Table 2: extrapulmonary clinical features suggestive of a telomere syndrome in 409 patients with idiopathic pulmonary fibrosis (IPF), stratified by sporadic and familial background.

	Sporadic idiopathic pulmonary fibrosis (n=293)	**IPF with familial background of pulmonary fibrosis (n=116)**	***p*-value**
Any extrapulmonary clinical feature suggestive of a telomere syndrome	23 (8)	12 (10)	0.44
History of anaemia (%)	4 (1)	3 (3)	0.41
History of thrombocytopenia (%)	2 (1)	3 (3)	0.14
History of bone marrow failure (%)	4 (1)	4 (3)	0.23
Haematological malignancy (%)	1 (0)	2 (2)	0.20
Liver cirrhosis (%)	10 (3)	2 (2)	0.52
Liver malignancy (%)	1 (0)	0 (0)	1.00
Early greying (%)	3 (1)	0 (0)	0.56
Nail dystrophy (%)	0 (0)	1 (1)	0.28
Skin hyperpigmentation (%)	0 (0)	1 (1)	0.28

Familial pulmonary fibrosis was defined as two or more first-degree family members with pulmonary fibrosis. Early greying was defined as grey hair before the age of 30.

Table 3: clinical manifestations suggestive of a telomere syndrome in patients with IPF stratified by sporadic and familial disease.

	Idiopathic pulmonary fibrosis (n=293)	**Familial pulmonary fibrosis (n=116)**	***p*-value**
Family history suggestive of telomere syndrome (%)	16 (5)	25 (22)	<0.001
Liver disease (%)	4 (1)	7 (12)	0.009
Haematological disease (%)	13 (4)	19 (16)	<0.001
Clinical history suggestive of telomere syndrome (%)	23 (8)	12 (10)	0.42
Early greying (%)	3 (1)	0 (0)	0.56
Liver disease (%)	10 (3)	2 (2)	0.52

Table 3: clinical manifestations suggestive of a telomere syndrome in patients with IPF stratified by sporadic and familial disease. (continued)

	Idiopathic pulmonary fibrosis (n=293)	**Familial pulmonary fibrosis (n=116)**	***p*-value**
Haematological disease (%)	12 (4)	11 (9)	0.03
Skin manifestations (%)	0 (0)	2 (2)	0.08
Any haematological laboratory abnormality (%)	50 (17)	27 (23)	0.15
Anaemia (%)#	5 (2)	2 (2)	1.00
Macrocytosis (%)#	36 (12)	21 (19)	0.12
Leukopenia (%)	0 (0)	0 (0)	-
Thrombocytopenia (%)#	15 (5)	10 (9)	0.18
Number of domains with any clinical manifestations suggestive of a telomere syndrome (family history, clinical, history, or laboratory abnormalities)			
One domain (%)	48 (16)	29 (25)	0.06
Two domains (%)	19 (6)	10 (9)	0.59
Three domains (%)	1 (0)	5 (4)	0.008
Any domain (%)	68 (23)	44 (38)	0.003

Familial pulmonary fibrosis was defined as two or more first-degree family members with pulmonary fibrosis. Early greying was defined as grey hair before the age of 30. Anaemia was defined as haemoglobin level <7.0 mmol/L, macrocytosis was defined as mean corpuscular volume >98 fL, leukopenia was defined as leukocytes <2.5 x10^9/L, thrombocytopenia was defined as thrombocytes <150 x10^9/L. # unavailable in 5 patients

One-hundred-and-twelve patients had any of or a combination of: clinical history suggestive of a telomere syndrome, family history suggestive of a telomere syndrome, or any haematological laboratory abnormality. This group included 68 patients with sporadic IPF as well as 44 patients with fIPF (23% versus 38%; *p*=0.003). Supplementary Table 2 shows additional clinical characteristics of the patients with sporadic IPF. Patients with sporadic IPF in whom there was a clinical suspicion of a telomere syndrome were significantly less likely to have been exposed to asbestos (*p*=0.04; 18% versus 30%) or other potentially fibrogenic substances (*p*=0.04; 12% versus 24%). Other clinical characteristics were not significantly different between patients with sporadic IPF in whom there was or was no clinical suspicion of a telomere syndrome.

Leukocyte telomere length was below the 10th percentile for age in 122 patients with sporadic IPF (42%) and 48 patients with fIPF (41%) (Figure 1). The percentage of patients with age-adjusted telomere length below the 1st percentile was 12% for sporadic IPF patients and 19% for fIPF patients (*p*=0.08). For the whole cohort, 26% of patients in whom there was a clinical suspicion of a telomere syndrome had telomere length below the 1st percentile for age, compared to 10% of patients who did not have a clinical suspicion of a telomere syndrome (*p*<0.001; Supplementary Table 3). When restricting analyses to pa-

tients with sporadic IPF, leukocyte telomere length of patients with a clinical suspicion of a telomere syndrome did not differ from patients without clinical suspicion of a telomere syndrome (Supplementary Table 2).

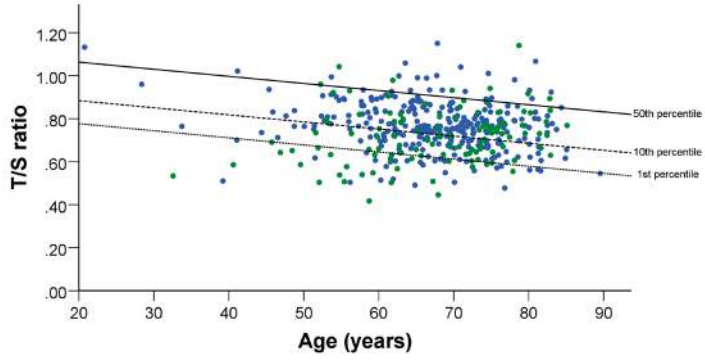

Figure 1: Leukocyte telomere length in sporadic and familial idiopathic pulmonary fibrosis patients. Green dots represent patients with any clinical manifestation or family history suggestive of a telomere syndrome, blue dots represent patients who had no suggestions of a telomere syndrome. The mean T/S-ratio was 0.75 (standard deviation 0.12). The solid line represents the 50th percentile for the age-adjusted normal value. The course dashed line represents the 10th percentile and the fine dashed line represents the 1st percentile.

Survival was significantly longer in patients with fIPF (median (IQR) 1448 days (674-NA) compared to 990 days in patients with sporadic IPF (496-1863); *p*=0.02), but this was not significant in a multivariate model that corrected for age and lung function at diagnosis (hazard ratio 1.17 (95% confidence interval 0.85-1.61); *p*=0.33). Univariate survival analyses for clinical characteristics suggestive of a telomere syndrome are shown in Supplementary Table 4. Patients in whom there was a clinical suspicion of a telomere syndrome had significantly shorter survival (*p*=0.002; Figure 2A). In a multivariate model that corrected for age and lung function at diagnosis (Supplementary Table 5), survival was significantly shorter in patients in whom there was a clinical suspicion of a telomere syndrome (*p*=0.002), as well as in a multivariate model that corrected for GAP-score (p=0.0004).[14] When restricting the analyses to patients with sporadic IPF, patients in whom there was a clinical suspicion of a telomere syndrome had significantly shorter survival (*p*=0.01; Figure 2B). This was not significant in a multivariate model that corrected for age and lung function at diagnosis (*p*=0.07).

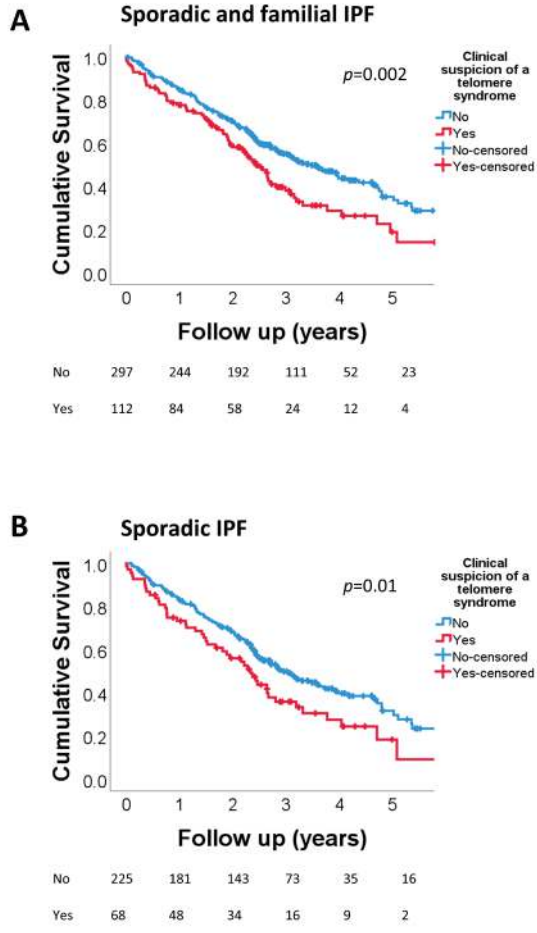

Figure 2: Association between clinical characteristics suggestive of a telomere syndrome and survival in 409 patients with idiopathic pulmonary fibrosis (IPF). (A) patients with sporadic IPF or familial idiopathic pulmonary fibrosis (fIPF) in whom there was a clinical suspicion of a telomere syndrome (either a family history suggestive of a telomere syndrome, clinical characteristics suggestive of a telomere syndrome, or haematological laboratory abnormalities), had significantly shorter survival. Median survival was 898 days (interquartile range (IQR) 416-1718), compared to 1267 (592-2476); *p*=0.002. This was still significant in a multivariate model that corrected for diffusion capacity of the lung for carbon monoxide, forced vital capacity, and age at diagnosis (hazard ratio (HR) 1.58 (95% confidence interval (95% CI) 1.19-2.11); *p*=0.002), and also in a multivariate model that corrected for GAP-score[13] (HR 1.68 (95% CI 1.26-2.24); *p*=0.0004), or when adding smoking status to the first multivariate model (HR 1.59 (95% CI 1.19-2.12); *p*=0.002). (B) patients with sporadic IPF in whom there was a clinical suspicion of a telomere syndrome (either a family history suggestive of a telomere syndrome, clinical characteristics suggestive of a telomere syndrome, or haematological laboratory abnormalities), had significantly shorter survival. Median survival was 852 days (IQR 279-1475) compared to 1071 days (IQR 548-1960); p=0.01. This was not significant in a multivariate model that corrected for diffusion capacity of the lung for carbon monoxide, forced vital capacity and age at diagnosis (HR 1.37 (95% CI 0.97-1.94); *p*=0.07), or in a multivariate model that corrected for GAP-score (HR 1.39 (95% CI 0.99-1.96); *p*=0.06).[13] or when adding smoking status to the first multivariate model (HR 1.38 (95% CI 0.98-1.96); *p*=0.07). At risk tables represent the number of patients at the start of every year during follow up.

DISCUSSION

In this cohort of 409 patients with IPF, extrapulmonary manifestations suggestive of a telomere syndrome, including known haematological disease, liver disease, early greying of hair, and nail dystrophy, were present in 9% of patients. These manifestations were not significantly more common in patients with fIPF compared to sporadic IPF. A family history suggestive of a telomere syndrome, with family members having liver or haematological disease, was noted in 10% of patients, and was significantly more common in patients with fIPF. Haematological laboratory abnormalities were seen in 19% of patients, with macrocytosis being the most common abnormality, seen in 14%. Twenty-seven percent of patients had one or more of: extrapulmonary manifestations suggestive of a telomere syndrome, a family history suggestive of a telomere syndrome or haematological laboratory abnormalities. These patients had leukocyte telomere length below the 10th or 1st percentile for age significantly more often and had significantly shorter survival. When only analysing patients with sporadic IPF, there was a clinical suspicion of a telomere syndrome in 23% of patients. In these patients, leukocyte telomere length below the 10th or 1st percentile for age was not more common, and survival was significantly shorter.

In previous reports, the prevalence of clinical features suggestive of a telomere syndrome in patients with IPF was higher than 40% or even higher than 70%.[7,8] The relative paucity of extrapulmonary manifestations suggestive of a telomere syndrome in our cohort could be explained by the fact that our cohort consists of unselected IPF patients, whereas previous studies focussed on patients with mutations in telomere-related genes.

The relation between leukocyte telomere length and survival has been studied in several cohorts of pulmonary fibrosis patients. Most studies found that shorter leukocyte telomere length at time of diagnosis was associated with shorter survival,[15–17] but some did not.[8] A recent study in Spanish patients with IPF found that short leukocyte telomere length in young patients (<60 years old) who also had haematological laboratory abnormalities was associated with worse survival, but this was not seen in older IPF patients.[18] In the current study there was no association between survival and leukocyte telomere length, even though patients with a clinical suspicion of a telomere syndrome did have shorter leukocyte telomere length and shorter survival. We have no clear explanation why the association between leukocyte telomere length and survival could not be confirmed, as our cohort seems to be comparable to those of previous studies. However, in general leukocyte telomere length may not be a perfect marker for telomere dysfunction in the lung, as a previous study found no correlation between leukocyte telomere length and telomere length measured by qPCR in lung tissue of patients with IPF.[19]

Our study has some limitations. First, this was a retrospective study and the availability of data was limited by what was recorded in patient files and there was no standard template for the history focussing on possible manifestations of a telomere syndrome. This might lead to an underestimation of the prevalence of certain clinical characteristics, such as skin abnormalities and early greying of hair. However, our cohort was large and all patients underwent an otherwise standardized and thorough evaluation, meaning that the results are representative of the information that is available in clinical practice. Second, telomere length was measured using a qPCR technique. It has been suggested that this technique is less reliable for measuring telomere length than other techniques.[20] The main concern is a relatively high level of variation between replication samples. Also, differences between labs in the precise techniques used mean that absolute telomere length values cannot be translated to other populations.[21] To ameliorate this, we used strict quality control measures in measuring telomere length, and telomere length in controls was measured in the same manner. This allowed us to reliably estimate which patients had short telomeres for age, although the absolute values cannot be compared to external cohorts. Third, we did not have genetic testing results available for the present study, and were not able to relate clinical manifestations to the presence of telomere gene mutations. This would have been valuable information given that the interest in using telomere length as well as the presence of telomere gene mutations in clinical decision making is growing.[22] Finally, this was a single centre study, and the results will need to be confirmed in another cohort.

CONCLUSION

In conclusion, we found features suggestive of a telomere syndrome in 27% of patients with IPF. This included extrapulmonary clinical manifestations such as liver disease and haematological disease in 9% of patients, a family history of liver or haematological disease in 10% of patients, and haematological laboratory abnormalities in 19% of patients. The presence of any of these features was associated with significantly shorter leukocyte telomere length and decreased survival. When excluding patients with fIPF from the analyses, 23% had any feature suggestive of a telomere syndrome. In these patients this was however not associated with shorter leukocyte telomere length, but there was a trend towards shorter survival. Clinical features suggestive of a telomere syndrome are easily identified and have prognostic relevance for patients with IPF in the absence of genetic test results.

REFERENCES

1. Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2018;198(5):e44-e68. doi:10.1164/rccm.201807-1255ST [doi]
2. Lederer DJ, Martinez FJ. Idiopathic Pulmonary Fibrosis. *N Engl J Med*. 2018;378(19):1811-1823. doi:10.1056/NEJMra1705751 [doi]
3. Armanios M. Telomerase and idiopathic pulmonary fibrosis. *Mutat Res*. 2012;730(1-2):52-58. doi:10.1016/j.mrfmmm.2011.10.013 [doi]
4. Armanios M, Blackburn EH. The telomere syndromes. *Nat Rev*. 2012;13(10):693-704. doi:10.1038/nrg3246 [doi]
5. Holohan B, Wright WE, Shay JW. Cell biology of disease: Telomeropathies: An emerging spectrum disorder. *J Cell Biol*. 2014;205(3):289-299. doi:10.1083/jcb.201401012 [doi]
6. Hoffman TW, van Moorsel CHM, Borie R, Crestani B. Pulmonary phenotypes associated with genetic variation in telomere-related genes. *Curr Opin Pulm Med*. 2018;24(3):269-280. doi:10.1097/MCP.0000000000000475 [doi]
7. Borie R, Tabeze L, Thabut G, et al. Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. *Eur Respir J*. 2016;48(6):1721-1731. doi:10.1183/13993003.02115-2015 [doi]
8. Newton CA, Batra K, Torrealba J, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J*. 2016;48(6):1710-1720. doi:10.1183/13993003.00308-2016 [doi]
9. Diaz de Leon A, Cronkhite JT, Katzenstein AL, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. *PLoS One*. 2010;5(5):e10680. doi:10.1371/journal.pone.0010680 [doi]
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(6):788-824. doi:10.1164/rccm.2009-040GL [doi]
11. Lynch DA, Sverzellati N, Travis WD, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. *Lancet Respir Med*. 2018;6(2):138-153. doi:10.1016/S2213-2600(17)30433-2
12. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;30(10):e47.
13. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010 [doi]
14. Ley B, Ryerson CJ, Vittinghoff E, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. *Ann Intern Med*. 2012;156(10):684-695. doi:10.7326/0003-4819-156-10-201205150-00004
15. Stuart BD, Lee JS, Kozlitina J, et al. Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. *Lancet Respir Med*. 2014;2(7):557-565. doi:10.1016/S2213-2600(14)70124-9 [doi]

16. Dai J, Cai H, Li H, et al. Association between telomere length and survival in patients with idiopathic pulmonary fibrosis. *Respirology*. 2015;20(6):947-952. doi:10.1111/resp.12566 [doi]

17. Newton CA, Oldham JM, Ley B, et al. Telomere length and genetic variant associations with interstitial lung disease progression and survival. *Eur Respir J*. 2019;53(4):10.1183/13993003.01641-2018. Print 2019 Apr. doi:10.1183/13993003.01641-2018

18. Planas-Cerezales L, Arias-Salgado EG, Buendia-Roldan I, et al. Predictive factors and prognostic effect of telomere shortening in pulmonary fibrosis. *Respirology*. 2019;24(2):146-153. doi:10.1111/resp.13423 [doi]

19. Snetselaar R, van Batenburg AA, van Oosterhout MFM, et al. Short telomere length in IPF lung associates with fibrotic lesions and predicts survival. *PLoS One*. 2017;12(12):e0189467. doi:10.1371/journal.pone.0189467 [doi]

20. Khincha PP, Dagnall CL, Hicks B, et al. Correlation of Leukocyte Telomere Length Measurement Methods in Patients with Dyskeratosis Congenita and in Their Unaffected Relatives. *Int J Mol Sci*. 2017;18(8):1765. doi:10.3390/ijms18081765

21. Montpetit AJ, Alhareeri AA, Montpetit M, et al. Telomere length: a review of methods for measurement. *Nurs Res*. 2014;63(4):289-299. doi:10.1097/NNR.0000000000000037 [doi]

22. Adegunsoye A, Vij R, Noth I. Integrating Genomics Into Management of Fibrotic Interstitial Lung Disease. *Chest*. 2019;155(5):1026-1040. doi:S0012-3692(19)30001-7

SUPPLEMENTARY DATA

Supplementary Table 1: Family history suggestive of a telomere syndrome in 409 patients with idiopathic pulmonary fibrosis

Any family history suggestive of a telomere syndrome (%)	**41 (10)**
First-degree relative with aplastic anaemia or bone marrow failure (%)	9 (2)
Second-degree relative with aplastic anaemia or bone marrow failure (%)	1 (0)
Third-degree relative with aplastic anaemia or bone marrow failure (%)	4 (1)
First-degree relative with haematological malignancy (%)	15 (4)
Second-degree relative with haematological malignancy (%)	6 (1)
First-degree relative with liver cirrhosis (%)	5 (1)
Second-degree relative with liver cirrhosis (%)	1 (0)
Third-degree relative with liver cirrhosis (%)	2 (0)
First-degree relative with liver malignancy (%)	1 (0)
Second-degree relative with liver malignancy (%)	3 (1)
First-degree relative with dyskeratosis congenita (%)	1 (0)
Second-degree relative with dyskeratosis congenita (%)	0 (0)
Third-degree relative with dyskeratosis congenita (%)	1 (0)

Supplementary Table 2: Clinical characteristics of 293 sporadic IPF patients, stratified for the presence or absence of clinical signs, family history, or laboratory abnormalities suggestive of a telomere syndrome.

	Clinical suspicion of telomere syndrome (n=68)	**No clinical suspicion of telomere syndrome (n=225)**	***p*-value**
Male (%)	60 (88)	179 (80)	0.11
Median age at diagnosis (range)	71.9 (62.9-76.1)	68.9 (63.9-74.5)	0.37
Median years between start of symptoms and diagnosis (IQR)	2.2 (0.8-4.8)	2.0 (0.9-4.0)	0.58
Consensus diagnosis of IPF (%)	36 (53)	111 (49)	0.60
Lung function at diagnosis			
Median FVC% (IQR)	71.1 (62.0-85.5)	77.0 (65.5-90.0)	0.14
Median FEV1% (IQR)	76.0 (66.0-88.3)	80.2 (70.0-94.5)	0.14
Median DLCOc% (IQR)	35.0 (28.7-47.4)	39.0 (32.0-49.1)	0.23
Smoking status			
≥5 packyears (%)	53 (78)	170 (76)	0.69
≥20 packyears (%)	37 (54)	108 (48)	0.35
Other fibrogenic exposures			
Asbestos	12 (18)	68 (30)	0.04
Other	8 (12)	53 (24)	0.04
Comorbidities			
Malignancy (%)	16 (24)	33 (15)	0.09
Auto-immune disease (%)#	4 (6)	23 (10)	0.35

Supplementary Table 2: Clinical characteristics of 293 sporadic IPF patients, stratified for the presence or absence of clinical signs, family history, or laboratory abnormalities suggestive of a telomere syndrome. (continued)

	Clinical suspicion of telomere syndrome (n=68)	**No clinical suspicion of telomere syndrome (n=225)**	***p*-value**
Chronic obstructive pulmonary disease (%)	4 (6)	17 (8)	0.79
Obstructive sleep apnea (%)	5 (7)	19 (8)	1.00
Hypothyroidism (%)	4 (6)	10 (4)	0.75
Diabetes mellitus type II (%)	15 (22)	44 (20)	0.65
Leukocyte telomere length			
<10th percentile for age (%)	32 (47)	90 (40)	0.30
<1st percentile for age (%)	9 (13)	27 (12)	0.79

IQR = interquartile range. # please note that all patients were diagnosed with IPF after multidisciplinary discussion, and that no patients were diagnosed with connective-tissue disease associated interstitial lung disease.

Supplementary Table 3: Clinical characteristics of 409 patients with idiopathic pulmonary fibrosis in relation to leukocyte telomere length, stratified by percentile for age.

	Leukocyte telomere length ≥10th percentile	**Leukocyte telomere length <10th percentile**	***p*-value**	**Leukocyte telomere length <1st percentile**	***p*-value**
All patients (%)	239 (58)	170 (42)	-	58 (14)	-
Family history suggestive of telomere syndrome (%)	16 (39)	25 (61)	0.008	15 (37)	<0.001
Liver disease (%)	2 (18)	9 (82)	0.01	6 (55)	0.002
Haematological disease (%)	14 (44)	18 (56)	0.08	11 (34)	0.001
Clinical history suggestive of telomere syndrome (%)	17 (49)	18 (51)	0.22	8 (23)	0.12
Early greying (%)	2 (67)	1 (33)	1.00	0 (0)	1.00
Liver disease (%)	3 (25)	9 (75)	0.03	5 (42)	0.02
Haematological disease (%)	12 (52)	11 (48)	0.53	5 (22)	0.35
Skin manifestations (%)	0 (0)	2 (100)	0.17	1 (50)	0.26
Any haematological laboratory abnormality (%)	36 (47)	41 (53)	0.02	18 (23)	0.01
Anaemia (%)	5 (71)	2 (29)	0.70	0 (0)	0.60
Macrocytosis (%)	22 (39)	35 (61)	0.001	16 (28)	0.001
Thrombocytopenia (%)	12 (48)	13 (52)	0.28	7 (28)	0.04
Any feature suggestive of telomere syndrome (%)	53 (47)	59 (53)	0.005	29 (26)	<0.001

P-values were calculated compared to patients in whom a certain feature was not present.

Supplementary Table 4: univariate survival analyses for clinical characteristics suggestive of a telomere syndrome in 409 patients with idiopathic pulmonary fibrosis.

	Median survival if factor present, days (IQR)	**Median survival if factor absent, days (IQR)**	***p*-value**
Family history suggestive of telomere syndrome (N=41)	990 (666-1809)	1125 (536-2135)	0.98
Liver disease (N=11)	814 (709-968)	1125 (547-2135)	0.52
Haematological disease (N=32)	990 (635-NA)	1125 (545-1960)	1.00
Clinical history suggestive of telomere syndrome (N=35)	974 (347-1475)	1130 (565-2135)	0.13
Early greying (N=3)	-	1112 (547-1960)	0.18
Liver disease (N=12)	898 (279-NA)	1125 (548-2135)	0.38
Haematological disease (N=23)	898 (347-1475)	1125 (565-2135)	0.09
Skin manifestations (N=2)	47 (NA)	1122 (548-1960)	0.25
Any haematological laboratory abnormality (N=77)	852 (406-1377)	1195 (586-2476)	0.001
Anaemia (N=7)	406 (47-1164)	1125 (565-2135)	0.03
Macrocytosis (N=57)	864 (545-1475)	1161 (565-2135)	0.02
Leukopenia (N=0)	-	1122 (548-1960)	-
Thrombocytopenia (N=25)	864 (518-1475)	1138 (554-2135)	0.07
Elevated liver enzymes (N=101)	1112 (496-2476)	1125 (566-1960)	0.66
Clinical suspicion of a telomere syndrome (N=112)	898 (416-1718)	1267 (592-2476)	0.002
Leukocyte telomere length <10th percentile for age (N=170)	1071 (565-NA)	1122 (536-2135)	0.52
Leukocyte telomere length <1st percentile for age (N=58)	1267 (645-NA)	1091 (532-1954)	0.31

P-values were calculated using a log-rank test. Early greying was defined as grey hair before the age of 30. Anaemia was defined as haemoglobin level <7.0 mmol/L, macrocytosis was defined as mean corpuscular volume >98 fL, leukopenia was defined as leukocytes <2.5 $x10^9$/L, thrombocytopenia was defined as thrombocytes <150 $x10^9$/L. Elevated liver enzymes were defined as AST level >31 u/L or ALT >34 u/L. IQR = interquartile range, NA = not available. Note that 75th percentile cannot be provided for subgroups where cumulative survival did not get below 25% at the end of follow up.

Supplementary Table 5: multivariate survival analyses for clinical characteristics suggestive of a telomere syndrome in 409 patients with idiopathic pulmonary fibrosis.

	Hazard-ratio (95% confidence interval)	***p*-value**
Family history suggestive of telomere syndrome (N=41)	1.14 (0.89-2.25)	0.15
Liver disease (N=11)	1.17 (0.51-2.68)	0.72
Haematological disease (N=32)	1.53 (0.92-2.56)	0.10
Clinical history suggestive of telomere syndrome (N=35)	1.49 (0.93-2.40)	0.10
Liver disease (N=12)	1.38 (0.66-2.86)	0.39
Haematological disease (N=23)	1.68 (0.964-2.93)	0.07
Any haematological laboratory abnormality (N=77)	1.50 (1.10-2.06)	0.01
Anaemia (N=7)	2.48 (0.91-6.74)	0.08
Macrocytosis (N=57)	1.33 (0.93-1.90)	0.12
Thrombocytopenia (N=25)	1.38 (0.83-2.31)	0.22
Elevated liver enzymes (N=101)	1.09 (0.81-1.49)	0.56
Clinical suspicion of a telomere syndrome (N=112)	1.58 (1.19-2.11)	0.002
Leukocyte telomere length <10th percentile for age (N=170)	1.20 (0.91-1.57)	0.20
Leukocyte telomere length <1st percentile for age (N=58)	0.98 (0.64-1.51)	0.93

P-values were calculated using a Cox-regression analysis, correcting for age, forced vital capacity, and diffusion capacity of the lung for carbon monoxide, corrected for haemoglobin level, at diagnosis. Anaemia was defined as haemoglobin level <7.0 mmol/L, macrocytosis was defined as mean corpuscular volume >98 fL, leukopenia was defined as leukocytes <2.5 $x10^9$/L, thrombocytopenia was defined as thrombocytes <150 $x10^9$/L. Elevated liver enzymes were defined as AST level >31 u/L or ALT >34 u/L.

Chapter 4

Humoral immune status in relation to outcomes in patients with idiopathic pulmonary fibrosis

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ABSTRACT

Background:

Idiopathic pulmonary fibrosis (IPF) is a severe fibrotic lung disease, in which inflammation is thought to only play a secondary role. Several factors associated with acute exacerbations of IPF (AE-IPF) have been identified, including infections. This study investigated whether humoral immunodeficiency or increased inflammatory markers at diagnosis were associated with AE-IPF and survival.

Methods:

Four-hundred-and-nine patients diagnosed with IPF between 2011 and 2017 were retrospectively included. Immune status investigations at diagnosis included measurement of serum immunoglobulins (available in 38%), leukocyte and lymphocyte subsets in blood and bronchoalveolar lavage (BAL) fluid (available in 58%), as well as response to pneumococcal vaccination (available in 64%).

Results:

Serum immunoglobulins or IgG subclass levels were below the lower limit of normal in 6%. The response to pneumococcal vaccination was severely impaired in 1%. Thirteen percent of patients developed an AE-IPF (4.7% per year). AE-IPF were associated with elevated lymphocytes in BAL-fluid at diagnosis (p=0.03). Higher serum IgA and IgG at diagnosis were associated with worse survival (p=0.01; and p=0.04), as were an increased BAL lymphocyte percentage (p=0.005), and higher blood leukocytes and neutrophils (p=0.01; and p=0.0005). In a multivariate model, only BAL lymphocyte count retained statistical significance (p=0.007).

Conclusions:

The prevalence of humoral immunodeficiencies was low in patients with IPF and not associated with AE-IPF or survival. Elevated lymphocytes in BAL were associated with the development of AE-IPF and worse survival. Higher serum immunoglobulins and immune cells in blood were also associated with worse survival. The local immune response in the lungs may be a target for future therapies.

INTRODUCTION

In the past, idiopathic pulmonary fibrosis (IPF) was thought to be an inflammatory disease, and immunosuppressive drugs were the mainstay of treatment.[1] Since the PANTHER-trial showed that mortality was increased in patients treated with prednisolone, azathioprine and N-acetylcysteine compared to placebo, immunosuppressive therapy has been strongly discouraged for patients with IPF.[2] Standard treatment currently consists of antifibrotic drugs.[3] However, in recent years, there has been a renewed interest in the role of inflammatory processes in the pathogenesis of IPF.

Although inflammation is not considered to be the primary process responsible for the development of IPF, it does seem to modify disease development.[4,5] Several measures of increased inflammation have been associated with survival of patients with IPF. There is also increasing evidence that infections modify the disease course in patients with IPF.[6] Furthermore, IPF is known to be caused by dysfunction of telomeres in at least a part of the patients.[7] Telomere dysfunction can also lead to disease in other organ systems, and patients with a so-called telomere syndrome can have an immunodeficiency.[8] In addition, leukocyte telomere length has been associated with the robustness of B-cell responses.[9]

Some patients will experience acute exacerbations (AE) of IPF. An AE-IPF is an acute deterioration of respiratory function, which is associated with new widespread alveolar abnormalities.[10] Histopathologically, AE-IPF are characterized by a pattern of diffuse alveolar damage (DAD) superimposed on pulmonary fibrosis. AE-IPF are associated with a very poor prognosis, and there is no effective treatment available.[10] Potential triggers for AE-IPF include infections, gastro-oesophageal reflux, surgical interventions, and bronchoscopy with bronchoalveolar lavage.[10] Risk factors for AE-IPF include more severe lung function impairment, and never having smoked.[11]

The role of the immune system in AE-IPF is not yet clear. In this study we have investigated the humoral immune status of patients with IPF. Immune status investigations included several immunological markers in blood, response to pneumococcal vaccination, and immunological markers in bronchoalveolar lavage fluid. We have explored two hypotheses. First, we hypothesized that the humoral immune system is impaired in part of the patients with IPF due to telomere dysfunction, which leads to increased risk of infections and AE-IPF. We therefore examined the prevalence of humoral immunodeficiency in patients with IPF and related this to leukocyte telomere length, AE-IPF and overall survival. Second, we hypothesized that some patients with IPF have an increased tendency to inflammation, which increases the risk of AE-IPF. We therefore investigated whether increased immune status markers at diagnosis were associated with AE-IPF and overall survival.

PATIENTS AND METHODS

This was a retrospective study. Patients were included from the Biobank for interstitial lung diseases of St. Antonius Hospital. Participants of the Biobank have given informed consent for the use of their clinical data in scientific research, and have provided blood and/or other samples to be stored and used for later analyses (approved by the local ethics committee (MEC-U) under study number R05-08A). The study was performed in accordance with the Declaration of Helsinki. We studied patients included in the Biobank from January 2011 to October 2017 who had a consensus diagnosis or a working diagnosis of IPF. The diagnosis was always made by a multidisciplinary team using clinical, radiological, and histopathological data, in accordance with the Fleischner Society recommendations and ATS/ERS/JRS/ALAT guidelines.[12,13] A consensus diagnosis of IPF is made when there is an appropriate clinical context of IPF (age >60 years, absence of significant exposure, no evidence of collagen vascular disease), and a definite CT-pattern of usual interstitial pneumonia (UIP), or after integration of clinical, imaging and histologic features during multidisciplinary discussion. A working diagnosis of IPF is made during multidisciplinary discussion in the presence of a progressive fibrosing interstitial pneumonia, in the absence of an alternative explanation, and when IPF was thought to be the most likely diagnosis by the expert clinicians that were present.

Clinical characteristics were retrieved from patient records. Data was collected until December 2018. Acute exacerbations of IPF were diagnosed based on the definition of a 2016 International Working Group Report.[10] An AE-IPF was always verified by the investigators, and was only diagnosed in patients who had new widespread areas of ground glass on a chest computed tomography scan. When findings on post-mortem examination were concordant with an AE-IPF, a computed tomography scan was not required. The GAP-score was calculated as has previously been described.[14]

Leukocyte subsets, immunoglobulin levels and IgG subclass levels were routinely measured at diagnosis for patients with pulmonary fibrosis that were primarily evaluated at our center or referred for lung transplantation. The results were retrieved from the hospital laboratory system or from the referring hospital. In the patients who had developed an AE-IPF, leukocyte subsets from the time of the AE-IPF were retrieved as well. Local laboratory reference values were used for interpretation of these values.

Bronchoalveolar lavage (BAL) was performed at the discretion of the treating physicians, mainly to exclude alternative diagnoses. Leukocyte counts, leukocyte subset counts, lymphocyte subset counts, and albumin in BAL fluid were retrieved from the hospital laboratory database or from correspondence of the referring hospital. Blood lymphocyte subset

counts and serum albumin were measured on the same day and were retrieved from the laboratory database as well. Immunoglobulin A and G levels were measured in BAL fluid samples using enzyme-linked immunosorbent assay kits (Invitrogen, Carlsbad, CA., USA).

All new patients with pulmonary fibrosis who had not yet received a pneumococcal vaccination in the previous five years were vaccinated with the pneumococcal polysaccharide vaccine (23vPPV). If possible, blood samples were drawn prior to vaccination and three weeks after vaccination with 23vPPV. Serum samples were stored at −80°C until use. Serum IgG antibody concentrations against 13 pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F (Danish nomenclature)) were measured using a Luminex platform.[15]

The 2015 AAAAI/ACAAI classification was used for overall categorization of the response to pneumococcal vaccination.[16] Responses are categorized as (1) normal, (2) mildly impaired (antibody levels >1.3 g/mL for ≥70% of serotypes, but twofold or greater increase between prevaccination and postvaccination antibody titers for <70% of serotypes), (3) moderately impaired (antibody levels >1.3µg/mL for <70% of serotypes), or (4) severely impaired (antibody levels >1.3 g/mL for ≤2 serotypes). As we evaluated 13 pneumococcal serotypes, we used a cutoff value of <69% instead of <70%: in case of adequate antibody levels against ≥9 of 13 serotypes, the response was categorized as normal.

Blood samples were taken around time of diagnosis. The samples were stored at -80 degrees Celsius until use. DNA was isolated from white blood cells using a magnetic beads-based method (chemagic DNA blood 10k kit; PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany). Telomere length was determined using a previously described quantitative polymerase chain reaction (PCR) method.[17] Briefly, telomere length was estimated for each sample from the ratio of telomere repeat copy number to a single gene (human β-globin gene) copy number (T/S ratio). Measurements were performed on the Bio-Rad CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). All sample measurements were performed in duplicate, with additional measurements if the duplicates differed more than 0.05; the mean value of the measured samples was used (in case of suspected faulty measurements, that value was excluded when calculating the mean). Quality control samples were included on each PCR-run, with a <0.05 margin of variance to reference values allowed. The control cohort included 164 healthy adults. Age-adjusted normal values for the T/S-ratio were calculated by determining the best-fitting linear regression line through the data, and percentiles were derived from the regression line.

Study data were collected and managed using REDCap electronic data capture tools hosted at St. Antonius Hospital.[18] Data analyses were performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA). Median values with interquartile range (IQR) or mean values with 95% confidence intervals (95% CI) are reported for non-normally and normally distributed continuous variables. For comparing two groups, Student's t-test, Mann-Whitney U-test, Kruskall-Wallis test, Fisher's exact test, or chi-squared test were used where appropriate. For correlation between two non-normally distributed continuous variables, Kendall's Tau correlation coefficient was used. For survival analyses, Kaplan-Meier curves were made, and Log-rank tests and Cox-regression analyses were performed where appropriate. Patients were censored when lost to follow up, when they underwent lung transplantation, or at the time of data collection. For analyses on the relation of certain variables to AE-IPF or survival, only patients in whom that variable was available were used. A p-value <0.05 was considered to represent statistical significance.

RESULTS

The cohort comprised 409 patients. Seventy-seven percent of all patients was male, and the median age was 68.2 years (range 21-89 years). Baseline characteristics are shown in Table 1. Response to pneumococcal vaccination, BAL fluid samples, and immunoglobulin levels were not available in all patients (Supplementary Table 1). Baseline characteristics were not significantly different between patients in whom response to pneumococcal vaccination or BAL fluid was or was not available. Patients in whom immunoglobulin levels were available, were significantly younger compared to patients in whom these were not available (p<0.001).

Serum immunoglobulin levels are shown in Supplementary Table 2. Ten patients had one or more immunoglobulins or IgG subclasses below the lower limit of normal. The response to pneumococcal vaccination was normal in 165 patients (64%), while 18 patients (7%) had a mildly impaired response, 74 patients (29%) had a moderately impaired response, and three patients (1%) had a severely impaired response. There were no significant differences in clinical characteristics between patients with a normal response compared to patients with an impaired response (Table 2).

Table 1: patient characteristics at diagnosis in 409 patients with multidisciplinary team diagnosis of idiopathic pulmonary fibrosis.

	Total (n=409)
Male (%)	313 (77)
Median age (IQR)	68.2 (62.0-74.5)
Age <60 (%)	78 (19)
Ever smoker (%)	331 (81)
Smoked >20 packyears (%)	190 (47)
First-degree family member with pulmonary fibrosis (%)	116 (28)
Consensus diagnosis IPF (%)	190 (47)
Lung function	
Median FVC % of predicted (IQR)	78.0 (64.0-91.0)
Median DLCO % of predicted (IQR)	41.0 (32.0-51.0)
Leukocyte telomere length <1st percentile	58 (14)
Leukocyte telomere length <10th percentile	170 (42)
Leukocyte subsets in blood available	406 (99)
Serum immunoglobulins available	157 (38)
Response to pneumococcal vaccination available	260 (64)
Bronchoalveolar lavage available	237 (58)

Immune status investigations were not available for all patients, and in 77 patients all four were available. There were no significant differences in clinical characteristics between patients in whom response to pneumococcal vaccination or BAL fluid cell counts were available and patients in whom these were not available. Patients in whom immunoglobulin levels were available, were significantly younger compared to patients in whom these were not available (p<0.001). IQR = interquartile range.

Table 2: clinical characteristics in pulmonary fibrosis patients with a normal response to 23-valent pneumococcal polysaccharide, compared to patients with an impaired response.

	Normal response (n=165)	**Impaired response (n=95)**	***p*-value**
Male (%)	121 (73)	74 (78)	0.41
Median age (IQR)	67.7 (62.1-72.4)	70.3 (62.9-76.3)	0.09
Age <60 (%)	25 (15)	18 (19)	0.43
Ever smoker (%)	132 (80)	81 (85)	0.31
Smoked <20 packyears (%)	93 (56)	50 (53)	0.56
First-degree family member with pulmonary fibrosis (%)	48 (29)	28 (30)	0.95
Consensus diagnosis IPF (%)	81 (49)	56 (59)	0.13
Leukocyte telomere length <1st percentile	22 (13)	10 (11)	0.51
Leukocyte telomere length <10th percentile	73 (44)	34 (36)	0.18

Vaccination responses were categorized as normal or impaired (mildly, moderately, or severely impaired), according to AAAAI/ACAAI criteria. IQR = interquartile range

Blood leukocyte subsets and lymphocyte subsets at the time of diagnosis are shown in Supplementary Table 3, Figure 1A, and Figure 1B. Leukocyte subset percentages and lymphocyte subset counts and percentages are shown in Supplementary Table 4 and Figure 1C. BAL lymphocyte percentage but not eosinophil or neutrophil percentage was associated with smoking status (median (IQR) 7.0 (2.6-12), 4.6 (2.5-8.9), and 1.8 (0.9-2.5) for never, former, and current smokers, respectively; p=0.005). Immunoglobulin A and G levels in BAL fluid had a poor correlation to serum immunoglobulin levels (Supplementary Figure 1).

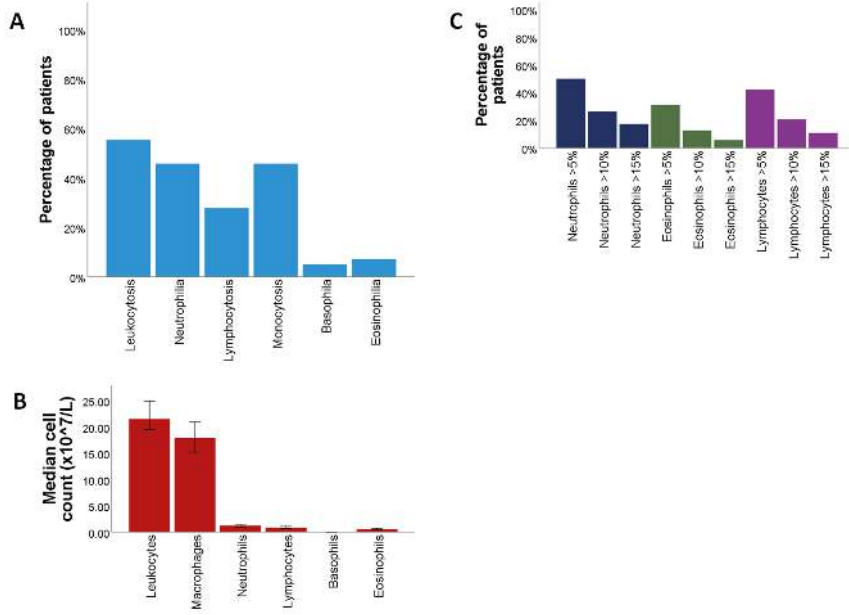


Figure 1: leukocyte subsets in blood and bronchoalveolar lavage fluid. (A) percentage of patients with blood levels of leukocytes and leukocyte subsets above the upper limit of normal. Upper limits of normal were as follows: leukocytes 8.2 x 10^9/L; neutrophils 5.45 x 10^9/L; lymphocytes 2.40 x10^9/L; monocytes 0.70 x 10^9/L; basophils 0.10 x 10^9/L; and eosinophils 0.50 x 10^9/L. Leukocytosis was present in 227 patients (56%). Neutrophilia was present in 186 patients (46%), monocytosis in 186 patients (46%), eosinophilia in 28 patients (7%), and lymphocytosis in 113 patients (28%). (B) leukocyte subset percentages in bronchoalveolar lavage fluid from patients with pulmonary fibrosis at time of diagnosis. Bars represent 95% confidence intervals. (C) neutrophil percentage, eosinophil percentage, and lymphocyte percentage above 15%, 10% and 5% in bronchoalveolar lavage fluid from patients with pulmonary fibrosis at time of diagnosis.

Patients were followed up for a median of 877 days (IQR 482-1256) after the diagnosis. Two hundred and twenty-six patients (55%) died, 29 patients (7%) underwent lung transplantation and 37 patients were lost to follow up (9%). Forty-seven patients (13%) experienced an AE-IPF during the follow-up, corresponding to a rate of 4.7% per year. AE-IPF was preceded by an infection in 28 patients (60%). Of the patients with an AE-IPF,

42 (89%) had died at the end of follow-up, and three (6%) underwent lung transplantation. Clinical characteristics did not significantly differ between patients with and without an AE-IPF (Table 3), except that patients with AE-IPF were more likely to have smoked <20 packyears (p=0.006).

Table 3: clinical characteristics in pulmonary fibrosis patients with and without AE-IPF.

	AE-IPF (n=47)	**No AE-IPF (n=362)**	***p*-value**
Male (%)	37 (79)	276 (76)	0.71
Median age, years (IQR)	66.1 (60.1-71.2)	68.5 (62.2-74.7))	0.09
Age <60 (%)	11 (23)	67 (19)	0.42
Ever smoker (%)	37 (79)	194 (81)	0.57
Smoked <20 packyears (%)	34 (72)	185 (51)	0.006
Gastro-oesophageal reflux (%)	19 (40)	130 (36)	0.55
Pulmonary hypertension	4 (9)	56 (15)	0.27
First-degree family member with pulmonary fibrosis	15 (32)	101 (28)	0.57
Consensus diagnosis IPF (%)	21 (45)	169 (47)	0.80
CT-imaging			
UIP (%)	30 (64)	208 (58)	0.79
Possible UIP (%)	11 (23)	90 (25)	
Inconsistent with UIP (%)	64 (18)	6 (13)	
Lung function			
Median FVC % of predicted (IQR)	74.9 (63.2-85.3)	78.6(64.2-92.1)	0.15
Median DLCO % of predicted (IQR)	42.8 (32.1-56.5)	41.0 (32.0-51.8)	0.58
Leukocyte telomere length <1st percentile	6 (13)	52 (14)	0.77
Leukocyte telomere length <10th percentile	24 (51)	146 (40)	0.16

IQR = interquartile range

The results of immune status investigations in patients who developed or did not develop an AE-IPF are shown in Figure 2, Supplementary Table 5 and Supplementary Table 6. A BAL lymphocyte percentage >10% was significantly more common in patients who developed an AE-IPF (p=0.03), as was a lymphocyte percentage >15% (p=0.05). A BAL neutrophil percentage >10% was significantly less common in patients who developed an AE-IPF (p=0.02). The median percentage of T-lymphocytes in BAL fluid was significantly higher in patients who developed an AE-IPF (p=0.01), as was the CD4/CD8-ratio (p=0.03). Immunoglobulin A and G levels in BAL fluid did not differ between patients who developed or did not develop an AE-IPF, also when corrected for BAL albumin concentration.

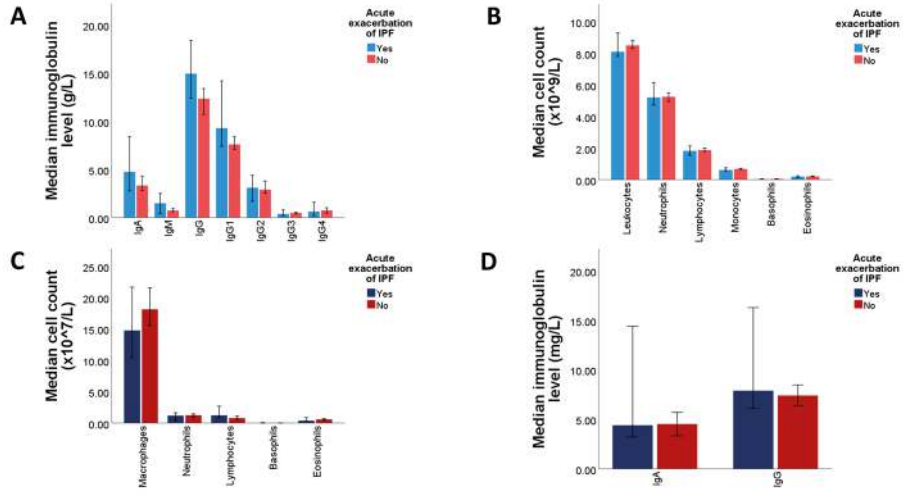


Figure 2: Immune status investigations at diagnosis in patients with and without acute exacerbations of IPF (AE-IPF). (A) serum immunoglobulin levels and IgG subclass levels. (B) blood leukocyte subset levels at diagnosis. (C) bronchoalveolar lavage fluid (BAL) leukocyte subset counts. (D) immunoglobulin A and G levels in BAL fluid. Patients who developed an AE-IPF more often had a BAL lymphocyte percentage >10%, and a higher percentage of CD3+- lymphocytes, as well as a higher CD4-CD8-ratio (Table S4 and Table S5). Bars represent 95% confidence intervals.

Blood leukocyte subsets at the time of an AE-IPF were available for 37 patients (Figure 3). Compared to the time of diagnosis, blood neutrophil and monocyte counts were significantly higher (p<0.001), whereas lymphocyte counts were significantly lower (p=0.01).

Median survival was 1122 days (IQR 548-1960), and was not significantly different between patients with a consensus and working diagnosis of IPF. Patients who developed an AE-IPF had worse survival than patients who did not develop an AE-IPF (median survival 711 days (IQR 155-1012), versus 1195 days (IQR 611-2360) p<0.001 compared to patients without an AE-IPF). Leukocyte telomere length <10th percentile for age was not associated with shorter survival (HR 1.09 (95%-CI 0.84-1.32); p=0.52).

A multivariate model was built that corrected GAP-score and smoking status (Table 4). Higher serum IgA and IgG levels at diagnosis were both associated with increased mortality (p=0.001 and p=0.04, respectively). Higher blood leukocytes and neutrophils at diagnosis were associated with worse survival (p=0.01, and p=0.0005, respectively). Increased lymphocyte count and percentage in BAL were associated with worse survival (p=0.005). BAL lymphocyte percentage >5% was significantly associated with shorter survival (p=0.001; Figure 4). Higher BAL basophil percentage was also associated with worse survival (p=0.03). In a model that included GAP-score, smoking status, blood leukocytes

and neutrophils at diagnosis, serum IgA and IgG at diagnosis, and lymphocyte count in BAL at diagnosis, only GAP-score and BAL lymphocyte count retained statistical significance (p<0.0001 and p=0.007, respectively; 109 patients available for the model).

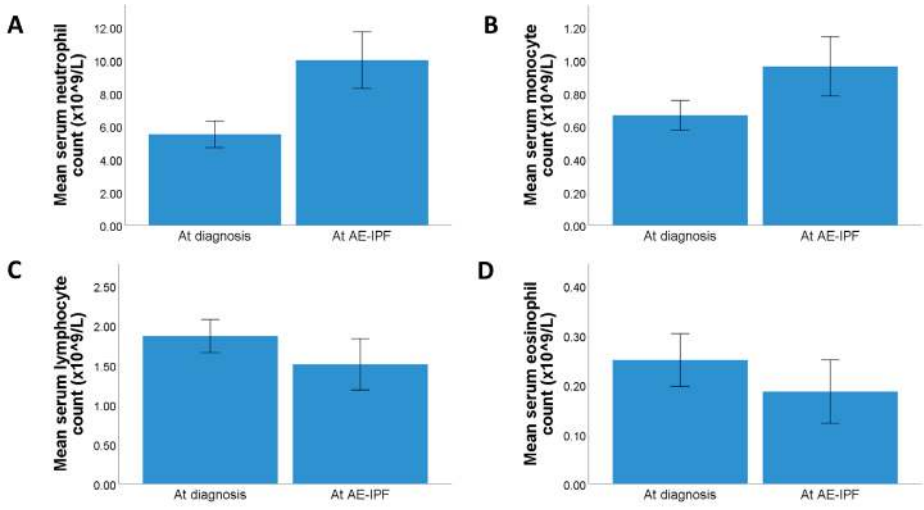


Figure 3: leukocyte subsets in blood at diagnosis and at time of an acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF). (A) mean blood neutrophil counts were significantly higher at the time of AE-IPF compared to diagnosis (mean 10.0 x10^9/L (95%-CI 8.29-11.72) compared to 5.51 x10^9/L (95%-CI 4.7-6.31); p<0.001). (B) mean blood monocyte counts were significantly higher at the time of AE-IPF compared to diagnosis (mean 0.96 x10^9/L (95%-CI 0.78-1.14) compared to 0.67 x10^9/L (95%-CI 0.58-0.76); p<0.001). (C) mean blood lymphocyte counts were significantly lower at the time of an AE-IPF (mean 1.51 x10^9/L (95%-CI 1.18-1.83) compared to 1.87 x10^9/L (95%-CI 1.66-2.07); p=0.01). (D) mean blood eosinophil counts were not significantly different between the time of AE-IPF (mean 0.19 x10^9/L (95%-CI 0.12-0.25) compared to 0.25 x10^9/L (95%-CI 0.20-0.30); p=0.09) Bars represent 95% confidence intervals.

Table 4: immune status investigations at time of diagnosis in relation to survival in a multivariate model.

Variable	Hazard ratio (95%-confidence interval)	*p*-value
Serum IgM	0.97 (0.69-1.35)	0.85
Serum IgA	1.15 (1.03-1.29)	0.01
Serum IgG	1.05 (1.00-1.10)	0.04
Impaired response to pneumococcal vaccination	1.00 (0.70-1.43)	1.00
Blood leukocyte count	1.07 (1.02-1.14)	0.01
Blood neutrophil count	1.11 (1.05-1.17)	0.0005
Blood lymphocyte count	0.87 (0.73-1.03)	0.10
Blood lymphocytes > 2.40 x 10^9/L	0.74 (0.55-1.01)	0.06
Blood monocyte count	0.94 (0.53-1.67)	0.83

Table 4: immune status investigations at time of diagnosis in relation to survival in a multivariate model. (continued)

Variable	Hazard ratio (95%-confidence interval)	*p*-value
Blood monocytes > 0.70 x 10^9/L	0.99 (0.76-1.30)	0.96
Blood monocytes > 0.95 x 10^9/L	0.98 (0.69-1.39)	0.90
Blood basophil count	0.26 (0.00-16.79)	0.53
Blood eosinophil count	0.63 (0.27-1.47)	0.28
BAL leukocyte count	1.01 (0.99-1.02)	0.08
BAL macrophage percentage	0.99 (0.98-1.00)	0.12
BAL neutrophil percentage	0.99 (0.98-1.02)	0.89
BAL lymphocyte percentage #	1.03 (1.01-1.06)	0.005
BAL lymphocyte percentage >5 #	1.87 (1.03-2.71)	0.001
BAL lymphocyte percentage >10 #	1.49 (0.97-2.30)	0.07
BAL lymphocyte percentage >15 #	1.40 (0.79-2.47)	0.25
BAL basophil percentage	1.76 (1.06-2.94)	0.03
BAL eosinophil percentage	1.01 (0.99-1.04)	0.30
BAL IgA	1.01 (0.97-1.05)	0.66
BAL IgG	1.01 (0.97-1.06)	0.54
BAL IgA corrected for BAL albumin	2.03 (0.18-23.59)	0.57
BAL IgG corrected for BAL albumin	2.59 (0.15-43.91)	0.51

The model (Cox-regression) corrects for GAP-score at diagnosis. # also corrected for smoking status. BAL = bronchoalveolar lavage

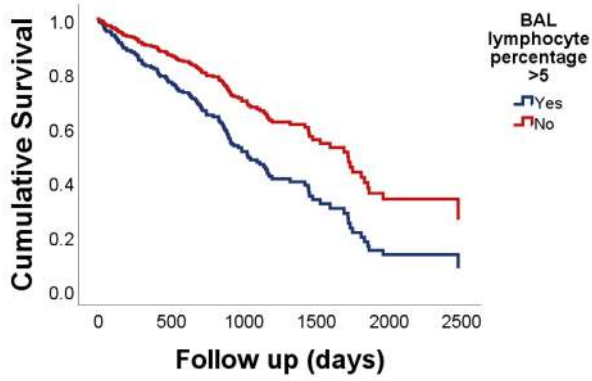


Figure 4: survival of patients with idiopathic pulmonary fibrosis in relation to lymphocyte percentage in bronchoalveolar lavage fluid. Survival in patients with pulmonary fibrosis stratified by lymphocyte percentage >5% or ≤5% in bronchoalveolar lavage fluid at time of diagnosis. Multivariate cox-regression correcting for GAP-score at diagnosis, smoking status, blood leukocytes and neutrophils at diagnosis, and serum IgA and IgG at diagnosis, showed significantly shorter survival in patients with BAL fluid lymphocyte percentage >5%.

DISCUSSION

In this study we explored the humoral immune status of 409 patients with IPF. We investigated whether humoral immunodeficiency was associated with an increased risk of AE-IPF and reduced survival. We found a low prevalence of humoral immunodeficiencies at the time of diagnosis. Only 6% of the patients had one or more immunoglobulin or IgG subclass levels below the lower limit of normal, and only 1% of patients had a severely impaired response to pneumococcal polysaccharide vaccination. The response to pneumococcal vaccination was comparable to that in healthy elderly.[19] No patients with low serum immunoglobulins or a severely impaired response to pneumococcal vaccination developed an AE-IPF, even though the majority of AE-IPF were preceded by an infection. We therefore do not see a place for measurement of the response to pneumococcal vaccination in the standard evaluation of patients with IPF.

We then investigated whether increased inflammatory markers at diagnosis were associated with development of AE-IPF and decreased survival. Patients who developed an AE-IPF had significantly higher lymphocytes in BAL at baseline, with a higher percentage of T-lymphocytes and a higher CD4/CD8-ratio. Blood neutrophils and monocytes were significantly higher at the time of AE-IPF compared to at the time of diagnosis, and blood lymphocyte levels were significantly lower. Several immunological parameters at diagnosis were found to be associated with survival. This included higher serum IgG and IgA levels, which were significantly associated with decreased survival. In addition, higher blood neutrophils and leukocytes and higher BAL lymphocytes were associated with decreased survival as well. In a multivariate model that included all the aforementioned factors, only BAL lymphocyte count retained statistical significance. We were unable to confirm a previously reported association between blood monocytes and survival, also when adopting the cut-off level (0.95 x 10^9/L) that was used in that study.[20]

This study expands on previous work and has several strengths. First, we studied multiple factors in the same population, and were able to provide an overall characterization of the humoral immune system, instead of only investigating individual factors. Second, the response to pneumococcal vaccination and immunoglobulin levels in BAL have not been investigated in patients with IPF before. Third, the diagnoses in this cohort were made in accordance with the Fleischner Society recommendations,[12] which are often used in practice, and differ from the diagnostic criteria used in older studies. And fourth, we were able to study leukocyte subsets both at time of diagnosis and at time of AE-IPF.

The combined findings of this study point to an association between increased lymphocyte inflammation and AE-IPF and decreased survival. The role of lymphocytes in the

pathogenesis of IPF is not completely clear.[4] Previous studies have shown that autoreactive T-cells, as well as IgG autoantibodies are present in the blood of patients with IPF.[21] Circulating T-helper and B-cell populations in patients with IPF are different from those in healthy controls.[22] The association between increased serum IgA and decreased survival has been reported before,[23] and patients with IPF have higher levels of auto-reactive IgA compared to controls.[24] Lymphocytes are present in patches of induced bronchus associated lymphoid tissue in IPF lungs, also in end-stage disease.[25] It has been found that B-cells from patients with IPF produce higher amounts of inflammatory mediators compared to those of healthy controls, and the supernatants of these B-cells stimulated proliferation and migration of fibroblasts.[26] In another study on IPF patients who underwent a lung transplantation, the amount of inflammatory cells, including neutrophils, macrophages, and lymphocytes, in explanted lungs was associated with faster disease progression prior to transplantation.[27]

Despite the evidence that inflammation plays an important role in AE-IPF, treatment with corticosteroids or cyclophosphamide does not seem to improve outcomes.[28–30] The optimal treatment for patients with an AE-IPF will need to be the subject of further studies as mortality after AE-IPF is very high, also in our cohort. The results from one pilot study suggest that auto-antibody-targeted treatment, in the form of rituximab, plasma exchange, and intravenous immunoglobulins, might be beneficial for patients that are experiencing an AE-IPF.[31]

The rate of AE-IPF reported in the literature is 5-20%.[10,32] In this study the rate was 4.7% per year, which is on the low end of this range. This is probably because not all patients were admitted to the hospital prior to death. Patients who died at home with an AE-IPF were not diagnosed accordingly in the absence of CT-imaging. Underdiagnosis of AE-IPF might have influenced our results, but this would probably have made it less likely that any true associations could be identified. An alternative explanation would be that most previous studies were done prior to the availability of antifibrotic treatment for IPF, whereas the majority of this study population received treatment with antifibrotic medication. Nintedanib in particular has been associated with a reduction of the frequency of AE-IPF.[33]

This study has several limitations. The immune status investigations, including BAL analysis, were not available for all patients, and this might influence the generalisability of our findings to all patients with IPF. However, there were no significant differences between patients with certain immune status investigation results available or not available, except for a younger age in patients in whom immunoglobulins levels were measured. This is probably because immunoglobulin levels are included in the screening investigations for lung transplantation. The missing data also leads to a smaller patient group being avail-

able for multivariate analyses, which might have lowered the chance of confirming any true associations. In addition, our cohort consists of patients with consensus and working diagnoses of IPF, which leads to some heterogeneity in clinical characteristics. However, we think this cohort is representative of patients with IPF worldwide, as the practice of making working diagnoses of IPF in the absence of lung biopsy results is widespread. Furthermore, we have mainly investigated the humoral immune system, and did not evaluate T-cell function in detail. This would be an interesting topic for further studies. Last, with these results we cannot definitively differentiate between cause and effect. The association between increased inflammatory markers at diagnosis and development of AE-IPF and decreased survival could indicate that inflammation modulates the disease process in patients with IPF. Contrarily, this could also be a bystander effect of another factor that modulates disease progression, such as chronic or intermittent infection. A higher bacterial burden in the lungs of patients with IPF is associated with decreased survival, independent of the severity of the underlying disease.[34,35] Furthermore, in patients with an AE-IPF, the bacterial burden is increased compared to patients who are stable.[36] There are also clues that viral infections play a role in the development of AE-IPF.[6]

CONCLUSION

In conclusion, the prevalence of humoral immune deficiency is low in patients with IPF. Patients that did have immune defects were not more prone to develop AE-IPF. Contrarily, higher levels of lymphocytes in BAL fluid at the time of diagnosis were associated with the development of AE-IPF and shorter survival. Higher serum IgA and IgG at diagnosis, as well as higher blood leukocytes and neutrophils at diagnosis, might also be associated with shorter survival. This emphasizes the role of inflammation as a disease modifier in patients with IPF. The association between increased inflammatory markers and both AE-IPF and survival warrants further investigations. This local immune response in the lung may be a target for future therapies.

REFERENCES

1. Gauldie J, Jordana M, Cox G. Cytokines and pulmonary fibrosis. *Thorax*. 1993;48(9):931-935. doi:10.1136/thx.48.9.931
2. Raghu G, Anstrom KJ, King TE, Lasky JA, Martinez FJ. Prednisone, Azathioprine, and *N* -Acetylcysteine for Pulmonary Fibrosis. *N Engl J Med*. 2012;366(21):1968-1977. doi:10.1056/NEJMoa1113354
3. Lederer DJ, Martinez FJ. Idiopathic Pulmonary Fibrosis. *N Engl J Med*. 2018;378(19):1811-1823. doi:10.1056/NEJMra1705751 [doi]
4. Desai O, Winkler J, Minasyan M, Herzog EL. The role of immune and inflammatory cells in idiopathic pulmonary fibrosis. *Front Med*. 2018;5:43. doi:10.3389/fmed.2018.00043
5. Heukels P, Moor CC, von der Thüsen JH, Wijsenbeek MS, Kool M. Inflammation and immunity in IPF pathogenesis and treatment. *Respir Med*. 2019;147:79-91. doi:10.1016/j.rmed.2018.12.015
6. Invernizzi R, Molyneaux PL. The contribution of infection and the respiratory microbiome in acute exacerbations of idiopathic pulmonary fibrosis. *Eur Respir Rev*. 2019;28(152). doi:10.1183/16000617.0045-2019
7. Armanios M. Telomerase and idiopathic pulmonary fibrosis. *Mutat Res*. 2012;730(1-2):52-58. doi:10.1016/j.mrfmmm.2011.10.013 [doi]
8. Wagner CL, Hanumanthu VS, Conover Talbot C, et al. Short telomere syndromes cause a primary T cell immunodeficiency. *J Clin Invest*. 2018;128(12):5222-5234. doi:10.1172/JCI120216
9. Najarro K, Nguyen H, Chen G, et al. Telomere Length as an Indicator of the Robustness of B- and T-Cell Response to Influenza in Older Adults. *J Infect Dis*. 2015;212(8):1261-1269. doi:10.1093/infdis/jiv202
10. Collard HR, Ryerson CJ, Corte TJ, et al. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. *Am J Respir Crit Care Med*. 2016;194(3):265-275. doi:10.1164/rccm.201604-0801CI [doi]
11. Kishaba T. Acute exacerbation of idiopathic pulmonary fibrosis. *Med*. 2019;55(3). doi:10.3390/medicina55030070
12. Lynch DA, Sverzellati N, Travis WD, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. *Lancet Respir Med*. 2018;6(2):138-153. doi:10.1016/S2213-2600(17)30433-2
13. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med*. 2011;183(6):788-824. doi:10.1164/rccm.2009-040GL [doi]
14. Ley B, Ryerson CJ, Vittinghoff E, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. *Ann Intern Med*. 2012;156(10):684-695. doi:10.7326/0003-4819-156-10-201205150-00004
15. Elberse KEM, Tcherniaeva I, Berbers GAM, Schouls LM. Optimization and application of a multiplex bead-based assay to quantify serotype-specific IgG against Streptococcus pneumoniae polysaccharides: response to the booster vaccine after immunization with the pneumococcal 7-valent conjugate vaccine. *Clin Vaccine Immunol*. 2010;17(4):674-682. doi:10.1128/CVI.00408-09

16. Bonilla FA, Khan DA, Ballas ZK, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *J Allergy Clin Immunol*. 2015;136(5):1186-205.e1-78. doi:10.1016/j.jaci.2015.04.049 [doi]

17. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;30(10):e47.

18. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010 [doi]

19. Hoffman TW, van Kessel DA, Rijkers GT. Impact of Using Different Response Criteria for Pneumococcal Polysaccharide Vaccination for Assessment of Humoral Immune Status. *J Clin Immunol*. 2018;38(2):149-152. doi:10.1007/s10875-017-0470-y

20. Scott MKD, Quinn K, Li Q, et al. Increased monocyte count as a cellular biomarker for poor outcomes in fibrotic diseases: a retrospective, multicentre cohort study. *Lancet Respir Med*. 2019;7(6):497-508. doi:10.1016/S2213-2600(18)30508-3

21. Feghali-Bostwick CA, Tsai CG, Valentine VG, et al. Cellular and Humoral Autoreactivity in Idiopathic Pulmonary Fibrosis. *J Immunol*. 2007;179(4):2592-2599. doi:10.4049/jimmunol.179.4.2592

22. Asai Y, Chiba H, Nishikiori H, et al. Aberrant populations of circulating T follicular helper cells and regulatory B cells underlying idiopathic pulmonary fibrosis. *Respir Res*. 2019;20(1):244. doi:10.1186/s12931-019-1216-6

23. Ten Klooster L, van Moorsel CHM, 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(2):357-361. doi:10.1111/cei.12636

24. Heukels P, Van Hulst JAC, Van Nimwegen M, et al. Enhanced Bruton's tyrosine kinase in B-cells and autoreactive IgA in patients with idiopathic pulmonary fibrosis. *Respir Res*. 2019;20(1):232. doi:10.1186/s12931-019-1195-7

25. Todd NW, Scheraga RG, Galvin JR, et al. Lymphocyte aggregates persist and accumulate in the lungs of patients with idiopathic pulmonary fibrosis. *J Inflamm Res*. 2013;6(1):63-70. doi:10.2147/JIR.S40673

26. Ali MF, Egan AM, Shaughnessy GF, et al. Antifibrotics Modify B-Cell–induced Fibroblast Migration and Activation in Patients with Idiopathic Pulmonary Fibrosis. *https://doi.org/101165/rcmb2020-0387OC*. 2021;64(6):722-733. doi:10.1165/RCMB.2020-0387OC

27. Balestro E, Calabrese F, Turato G, et al. Immune Inflammation and Disease Progression in Idiopathic Pulmonary Fibrosis. Eickelberg O, ed. *PLoS One*. 2016;11(5):e0154516. doi:10.1371/journal.pone.0154516

28. Farrand E, Vittinghoff E, Ley B, Butte AJ, Collard HR. Corticosteroid use is not associated with improved outcomes in acute exacerbation of IPF. *Respirology*. 2020;25(6):629-635. doi:10.1111/resp.13753

29. Kolb M, Bondue B, Pesci A, et al. Acute exacerbations of progressive-fibrosing interstitial lung diseases. *Eur Respir Rev*. 2018;27(150). doi:10.1183/16000617.0071-2018

30. Hozumi H, Hasegawa H, Miyashita K, et al. Efficacy of corticosteroid and intravenous cyclophosphamide in acute exacerbation of idiopathic pulmonary fibrosis: A propensity score-matched analysis. *Respirology*. 2019;24(8):792-798. doi:10.1111/resp.13506

31. Donahoe M, Valentine VG, Chien N, et al. Autoantibody-targeted treatments for acute exacerbations of idiopathic pulmonary fibrosis. *PLoS One*. 2015;10(6). doi:10.1371/journal.pone.0127771

32. Ryerson CJ, Cottin V, Brown KK, Collard HR. Acute exacerbation of idiopathic pulmonary fibrosis: Shifting the paradigm. *Eur Respir J*. 2015;46(2):512-520. doi:10.1183/13993003.00419-2015

33. Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2071-2082. doi:10.1056/NEJMoa1402584 [doi]

34. Molyneaux PL, Cox MJ, Willis-Owen SAG, et al. The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2014;190(8):906-913. doi:10.1164/rccm.201403-0541OC

35. Invernizzi R, Barnett J, Rawal B, et al. Bacterial burden in the lower airways predicts disease progression in idiopathic pulmonary fibrosis and is independent of radiological disease extent. *Eur Respir J*. 2020;55(4):1901519. doi:10.1183/13993003.01519-2019

36. Molyneaux PL, Cox MJ, Wells AU, et al. Changes in the respiratory microbiome during acute exacerbations of idiopathic pulmonary fibrosis. *Respir Res*. 2017;18(1). doi:10.1186/s12931-017-0511-3

SUPPLEMENTARY DATA

Supplementary Table 1: patient characteristics at diagnosis in 409 patients with pulmonary fibrosis.

	Immunoglobulins (n=157)	**Pneumococcal vaccination response (n=260)**	**Bronchoalveolar lavage (n=237)**	**Total (n=409)**
Male (%)	113 (72)	195 (75)	186 (79)	313 (77)
Median age (IQR)	65.3 (59.2-73.3) #	68.1 (62.9-74.3)	67.7 (62.0-73.6)	68.2 (62.0-74.5)
Age <60 (%)	45 (29) #	43 (16.5)	47 (20)	78 (19)
Ever smoker (%)	122 (78)	213 (82)	188 (79)	331 (81)
Smoked >20 packyears (%)	74 (47)	117 (45)	111 (47)	190 (47)
First-degree family member with pulmonary fibrosis (%)	45 (29)	76 (29)	63 (27)	116 (28)
Consensus diagnosis IPF (%)	67 (43)	137(53)	100 (42)	190 (47)
CT-imaging				
UIP (%)	94 (60)	153 (59)	127 (54)	238 (58)
Probable UIP or indeterminate for UIP (%)	31 (20)	54 (25)	65 (27)	101 (25)
Inconsistent with UIP (%)	32 (20)	42 (16)	45 (19)	70 (17)
Histopathology (%)	46 (30)	65 (25)	64 (27)	99 (24)
UIP (%)	30 (19)	48 (19)	43 (18)	70 (17)
Probable UIP or indeterminate for UIP (%)	8 (5)	10 (4)	12 (5)	16 (4)
Inconsistent with UIP (%)	8 (5)	7 (3)	9 (4)	13 (3)
Lung function				
Median FVC % of predicted (IQR)	77.7 (62.9-94.0)	78.0 (64.1-90.0)	79.2 (65.9-92.1)	78.0 (64.0-91.0)
Median DLCO % of predicted (IQR)	41.0 (32.1-53.0)	41.1 (33.0-50.0)	44.5(35.0-53.9)	41.0 (32.0-51.0)
Leukocyte telomere length <1st percentile	23 (15)	32 (12)	31 (13)	58 (14)
Leukocyte telomere length <10th percentile	66 (42)	107 (41)	101 (43)	170 (42)

There were no significant differences in age, sex, smoking status, family history of pulmonary fibrosis, proportion of patients with a consensus diagnosis if IPF, lung function, radiology findings, histopathology findings and leukocyte telomere length between patients in whom response to pneumococcal vaccination or BAL fluid cell counts were available and patients in whom these were not available. Patients in whom immunoglobulin levels were available, were significantly younger compared to patients in whom these were not available (Median 65.3 (59.2-73.3) versus 69.9 (64.6-75.0) years (IQR); $p<0.001$; 29% versus 13% with age <60 years at diagnosis; $p<0.001$). The other baseline characteristics did not differ between the two groups. IQR = interquartile range. # = $p<0.05$ compared to the rest of the cohort.

Supplementary Table 2: blood immunoglobulin and IgG subclass levels at time of diagnosis in patients with pulmonary fibrosis.

Median IgM g/L (IQR)	0.80 (0.56-1.28)
IgM <0.3 g/L (%)	0 (0)
Median IgA g/L (IQR)	3.59 (2.66-5.00)
IgA < 0.7 g/L (%)	1 (0)
Median IgG g/L (IQR)	12.65 (10.25-16.68)
IgG < 7.0 g/L (%)	4 (2)
Median IgG1 g/L (IQR)	7.90 (6.98-9.63)
IgG1 < 4.9 g/L (%)	4 (6)
Median IgG2 g/L (IQR)	2.95 (2.38-4.30)
IgG2 < 1.5 g/L (%)	2 (3)
Median IgG3 g/L (IQR)	0.50 (0.36-0.65)
IgG3 < 0.2 g/L (%)	0 (0)
Median IgG4 g/L (IQR)	0.71 (0.32-1.36)
IgG4 < 0.08 g/L (%)	2 (3)
Any immunoglobulin below the lower limit of normal (%)	10 (6)

Lower limit of normal values were derived from the local hospital reference ranges. Serum immunoglobulin levels were available for 157 patients. IgG subclass levels were available for 64 patients. IgA and IgG levels were significantly higher in patients with leukocyte telomere length <10th percentile for age (IgA median 3.90 g/L (IQR 2.72-5.35) versus 3.26 g/L (IQR 2.56-4.44); *p*=0.02; (IgG median 13.00 g/L (IQR10.58-17.43) versus 11.90 g/L (IQR 10.13-14.78); *p*=0.005). IQR = interquartile range

Supplementary Table 3: blood leukocyte subset counts at time of diagnosis in 406 patients with pulmonary fibrosis.

Leukocyte subsets	
Mean leukocytes x 10^9/L (95%-CI)	8.17 (7.73-8.60)
Leukocytes < 2.50 x 10^9/L (%)	0 (0)
Leukocytes > 8.20 x 10^9/L (%)	227 (56)
Mean neutrophils x 10^9/L (95%-CI)	5.06 (4.70-5.42)
Neutrophils > 5.45 x 10^9/L (%)	186 (46)
Mean lymphocytes x 10^9/L (95%-CI)	2.08 (1.92-2.23)
Lymphocytes > 2.40 x 10^9/L (%)	114 (28)
Mean monocytes x 10^9/L (95%-CI)	0.71 (0.66-0.75)
Monocytes > 0.70 x 10^9/L (%)	185 (46)
Monocytes > 0.95 x 10^9/L (%)	66 (16)
Mean basophils x 10^9/L (95%-CI)	0.05 (0.05-0.06)
Basophils > 0.10 x 10^9/L (%)	20 (5)
Mean eosinophils x 10^9/L (95%-CI)	0.27 (0.24-0.30)
Eosinophils > 0.50 x 10^9/L (%)	28 (7)
Lymphocyte subsets	
Mean CD3+ % (95%-CI)	71 (69-72)

Supplementary Table 3: blood leukocyte subset counts at time of diagnosis in 406 patients with pulmonary fibrosis. (continued)

Mean CD4+ % (95%-CI)	41 (39-43)
Mean CD8+ % (95%-CI)	27 (25-29)
Mean CD56+CD16+ % (95%-CI)	18 (16-20)
Mean CD19+ % (95%-CI)	10 (9-11)
Median CD4-CD8-ratio (IQR)	1.50 (1.00-2.82)

Lymphocyte subsets were available in 126 patients. Lower limit of normal values were derived from the local hospital reference ranges. IQR = interquartile range; 95%-CI = 95%-confidence interval

Supplementary Table 4: leukocyte subset counts and percentages in bronchoalveolar lavage fluid from 237 patients with pulmonary fibrosis.

Leukocyte subsets	
Median leukocytes x 10^7/L (IQR)	22.4 (17.0-33.1)
Median macrophages x 10^7/L (IQR)	17.9 (12.1-27.3)
Median macrophage % (IQR)	31.1 (72.0-90.7)
Median neutrophils x 10^7/L (IQR)	1.31 (0.65-2.55)
Median neutrophil % (IQR)	5.0 (3.0-9.0)
Median lymphocytes x 10^7/L (IQR)	1.0 (0.4-2.3)
Median lymphocyte % (IQR)	4.6 (2.1-10.2)
Median basophils x 10^7/L (IQR)	0.0 (0.0-0.1)
Median basophil % (IQR)	0.1 (0.0-0.4)
Median eosinophils x 10^7/L (IQR)	0.6 (0.3-1.2)
Median eosinophil % (IQR)	2.4 (1.1-5.6)
Lymphocyte subsets	
Median CD3+ % (IQR)	93.0 (86.0-95.0)
Median CD19+ % (IQR)	1.0 (0.4-2.0)
Median CD4-CD8-ratio (IQR)	2.0 (1.1-3.8)

Lymphocyte subsets were available for 159 patients.

Supplementary Table 5: results of immune status investigations at diagnosis compared between patients with pulmonary fibrosis who developed an acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) and patients who did not develop an AE-IPF.

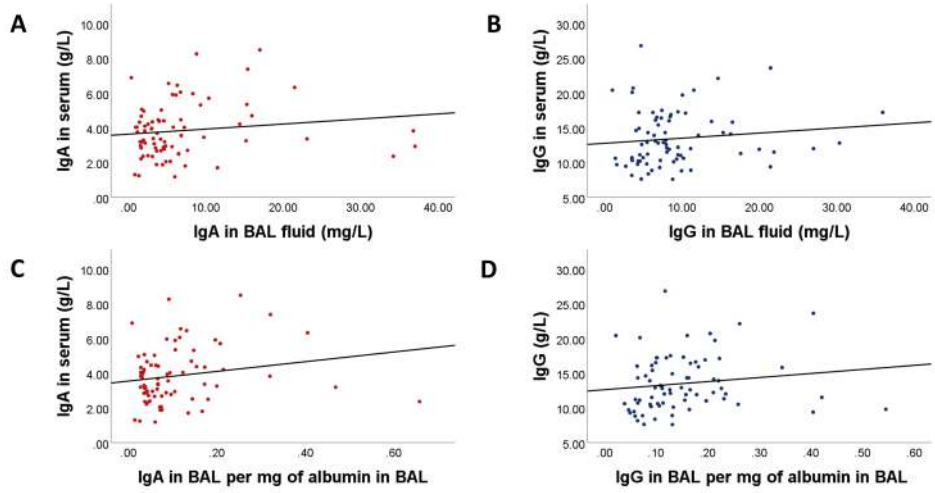
	AE-IPF (n=47)	**No AE-IPF (n=362)**	***p*-value**
Immunoglobulin levels available N (%)	22 (47)	135 (37)	
Median IgM g/L (IQR)	1.29 (0.72-2.41)	0.91 (0.63-1.37)	0.11
Median IgA g/L (IQR)	4.24 (3.79-8.15)	4.03 (2.66-5.02)	0.13
Median IgG g/L (IQR)	12.90 (12.55-17.80)	11.30 (9.46-16.00)	0.78
Median IgG1 g/L (IQR)	9.20 (7.65-13.35)	7.38 (6.20-8.70)	0.20
Median IgG2 g/L (IQR)	2.82 (2.25-3.95)	3.15 (2.30-4.30)	0.72
Median IgG3 g/L (IQR)	0.37 (0.20-0.75)	0.50 (0.40-0.80)	0.59
Median IgG4 g/L (IQR)	0.40 (0.27-1.12)	0.84 (0.30-1.15)	0.51
Any immunoglobulin level below lower limit of normal	0 (0)	10 (7)	0.36
Response to pneumococcal vaccination available N (%)	26 (55)	234 (65)	
Mildly impaired response	2 (8)	16 (7)	0.70
Moderately impaired response	8 (31)	66 (28)	0.82
Severely impaired response	0 (0)	3 (1)	1.00
Any impaired response	10 (38)	85 (36)	0.83
Median number of serotypes with post-vaccination antibody level >1.3 μg/ml	10 (7-13)	10 (8-12)	0.74
Median number of serotypes with >2-fold increase of pre-vaccination over post-vaccination antibody level	11 (9-12)	11 (10-12)	0.85
Leukocyte subsets available N (%)	47 (100)	359 (99)	
Mean leukocytes x 10^9/L (95%-CI)	7.33 (6.44-8.22)	8.39 (7.89-8.88)	0.53
Leukocytes > 8.20 x 10^9/L (%)	23 (49)	204 (57)	0.33
Mean neutrophils x 10^9/L (95%-CI)	4.44 (3.72-5.16)	5.22 (4.81-5.64)	0.97
Neutrophils > 5.45 x 10^9/L (%)	22 (47)	164 (47)	0.88
Mean lymphocytes x 10^9/L (95%-CI)	1.92 (1.64-2.21)	2.12 (1.94-2.30)	0.16
Lymphocytes > 2.40 x 10^9/L (%)	11 (23)	103 (29)	0.46
Mean monocytes x 10^9/L (95%-CI)	0.67 (0.58-0.76)	0.72 (0.67-0.77)	0.32
Monocytes > 0.70 x 10^9/L (%)	20 (43)	165 (46)	0.66
Monocytes > 0.95 x 10^9/L (%)	6 (13)	60 (17)	0.49
Mean basophils x 10^9/L (95%-CI)	0.04 (0.03-0.05)	0.05 (0.05-0.06)	0.16
Basophils > 0.10 x 10^9/L (%)	1 (2)	19 (5)	0.49
Mean eosinophils x 10^9/L (95%-CI)	0.25 (0.19-0.30)	0.28 (0.24-0.31)	0.59
Eosinophils > 0.50 x 10^9/L (%)	3 (6)	25 (7)	1.00
Lymphocyte subsets available N (%)	25 (53)	96 (27)	
Mean CD3+ % (95%-CI)	70 (66-73)	71 (69-73)	0.62
Mean CD4+ % (95%-CI)	43 (38-47)	41 (38-43)	0.43
Mean CD8+ % (95%-CI)	24 (20-29)	27 (25-30)	0.24
Mean CD56+CD16+ % (95%-CI)	18 (15-22)	18 (16-20)	0.74
Mean CD19+ % (95%-CI)	11 (9-13)	10 (9-11)	0.36
Median CD4-CD8-ratio (IQR)	1.40 (1.25-5.21)	1.10 (0.60-2.40)	0.19

Lower limit of normal values were derived from the local hospital reference ranges. Percentages were based on the number of patients in whom the relevant investigations were available. Interpretation of the response to pneumococcal vaccination was based on the 2015 AAAAI/ACAAI criteria. IQR = interquartile range; 95%-CI = 95%-confidence interval

Supplementary Table 6: leukocyte subsets, lymphocyte subsets, and immunoglobulins A and G in bronchoalveolar lavage fluid from patients with pulmonary fibrosis, around time of diagnosis.

	AE-IPF (n=47)	**No AE-IPF (n=362)**	***p*-value**
Leukocyte subsets available N (%)	31 (66)	202 (56)	
Median leukocytes x 10^7/L (IQR)	20.8 (15 3-30.1)	22.7 (15.3-33.7)	0.32
Median macrophages x 10^7/L (IQR)	14.8 (9.0-25.2)	18.1 (13.1-27.4)	0.10
Median macrophage % (IQR)	81.7 (66.7-90.7)	85.6 (74.5-92.2)	0.68
Median neutrophils x 10^7/L (IQR)	1.2 (0.4-1.7)	1.2 (0.5-2.6)	0.37
Median neutrophil % (IQR)	4.4 (2.9-6.8)	4.6 (2.4-10.5)	0.30
Neutrophil % >5 N (%)	15 (48)	110 (54)	0.57
Neutrophil % >10 N (%)	3 (10)	61 (30)	0.02
Neutrophil % >15 N (%)	3 (10)	40 (20)	0.22
Median lymphocytes x 10^7/L (IQR)	1.2 (0.7-3.2)	0.8 (0.3-1.9)	0.09
Median lymphocyte % (IQR)	7.6 (2.8-12.2)	3.6 (1.6-8.0)	0.05
Lymphocyte % >5 N (%)	19 (61)	94 (47)	0.15
Lymphocyte % >10 N (%)	12 (39)	42 (21)	0.03
Lymphocyte % >15 N (%)	7 (23)	20 (10)	0.05
Median basophils x 10^7/L (IQR)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.70
Median basophil % (IQR)	0.1 (0.0-0.4)	0.1 (0.0-0.3)	0.75
Median eosinophils x 10^7/L (IQR)	0.4 (0.2-0.9)	0.6 (0.2-1.2)	0.78
Median eosinophil % (IQR)	2.6 (1.1-3.9)	2.2 (1.0-5.5)	0.85
Eosinophil % >5 N (%)	7 (23)	69 (34)	0.20
Eosinophil % >10 N (%)	4 (13)	27 (13)	1.00
Eosinophil % >15 N (%)	3 (10)	11 (5)	0.40
Lymphocyte subsets available N (%)	26 (55)	113 (31)	
Median CD3+ % (IQR)	94.4 (91.5-96.0)	92.0 (84.0-95.0)	0.01
Median CD19+ % (IQR)	1.0 (0.5-2.0)	1.0 (0.4-2.0)	1.00
Median CD4-CD8-ratio (IQR)	2.3 (1.4-4.5)	1.8 (0.9-3.8)	0.03
Immunoglobulin levels available N (%)	10 (21)	66 (18)	0.69
Median IgA mg/L (IQR)	4.4 (3.4-12.2)	4.5 (2.0-6.8)	0.41
Median IgG mg/L (IQR)	7.9 (6.2-12.4)	7.5 (4.7-10.1)	0.41
Median IgA per mg/L of albumin mg/L (IQR)	0.08 (0.06-0.16)	0.07 (0.03-0.13)	0.37
Median IgG per mg/L of albumin mg/L (IQR)	0.13 (0.10-0.18)	0.12 (0.09-0.19)	0.55

Values are compared between patients who developed an AE-IPF and patients who did not develop an AE-IPF. Percentages were based on the number of patients in whom the relevant investigations were available. IQR = interquartile range; 95%-CI = 95%-confidence interval



Supplementary Figure 1: immunoglobulin levels in bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis. Immunoglobulin levels in BAL fluid were measured in 76 patients. (A) the median level of IgA in BAL fluid was 4.53 mg/L (IQR 2.16-7.29), the correlation between IgA in serum and bronchoalveolar lavage fluid was not significant (correlation coefficient 0.119 (p=0.126)). (B) the median level of IgG in BAL fluid was 7.59 mg/L (IQR 4.77-10.14), the correlation between IgG in serum and bronchoalveolar lavage fluid was not significant (correlation coefficient 0.120 (p=0.127)). (C) the correlation between IgA in serum and IgA in BAL fluid was not statistically significant when the latter was corrected for the concentration of albumin in BAL fluid (correlation coefficient 0.119 (p=0.130)). (D) the correlation between IgG in serum and IgG in BAL fluid was statistically significant when the latter was corrected for the concentration of albumin in BAL fluid (correlation coefficient 0.171 (p=0.031)). There was a significant correlation between BAL lymphocyte count and IgA in BAL fluid (correlation coefficient 0.206(p=0.01)), as well as IgG in BAL fluid (correlation coefficient 0.160 (p=0.04)). Correlation coefficients are Kendall's Tau. BAL = bronchoalveolar lavage

Chapter 5

Coronary artery calcification score is associated with survival in patients with idiopathic pulmonary fibrosis

T.W. Hoffman, H.W. van Es, D.H. Biesma, J.C. Grutters, and C.H.M. van Moorsel

ABSTRACT

Background:

Patients with idiopathic pulmonary fibrosis (IPF) frequently suffer from comorbidities, sometimes in the context of a short telomere syndrome (a form of accelerated aging). We took advantage of extra-pulmonary features available from CT-scans at the time of diagnosis, and investigated if these were related to clinical characteristics or patient outcomes.

Methods:

We retrospectively interpreted routinely obtained CT-scans that were performed without contrast-enhancement or cardiac triggering. Scans were scored for coronary artery calcification score, presence or absence of diaphragmatic hernia, and vertebral bone attenuation in 406 patients diagnosed with IPF between 2011 and 2017. Radiographic findings were correlated with clinical characteristics and outcomes.

Results:

The presence of any type of cardiovascular disease (n=178; 44%) was associated with decreased survival, also when corrected for age, sex, and lung function (p=0.02). Higher coronary artery calcification score associated with decreased survival, independent of a history of coronary artery disease (p=0.03 in a multivariate model correcting for age, sex, lung function, smoking status, and a history of coronary artery disease). Coronary artery calcification score did not associate with cardiac events after diagnosis. Complaints of gastro-oesophageal reflux and diaphragmatic hernia (present in 148 (37%) and 175 (43%) patients, respectively) did not associate with survival. Leukocyte telomere length did not correlate with radiographic findings.

Conclusions:

In this cohort of patients with IPF, a history of cardiovascular disease and increased coronary artery calcification score were independent predictors of decreased survival. There was no association between gastro-oesophageal reflux complaints or diaphragmatic hernia on CT scan and patient outcomes.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is characterized by a progressive fibrotic process in the lungs, which leads to increasing breathlessness and eventually death.[1] The diagnosis of IPF can be made using clinical characteristics and the radiological pattern seen on high-resolution computed tomography scan (HRCT) of the chest, sometimes in combination with the histologic pattern seen on lung biopsy specimens.[2] Median survival is approximately 4 years, and there are no curative treatments available, except for lung transplantation.[1]

Classical risk factors for IPF include old age, male sex, cigarette smoking, other fibrogenic exposures, and having a first-degree family member with pulmonary fibrosis.[1] There is significant overlap between these risk factors and risk factors for coronary artery disease (CAD), and the prevalence of CAD in patients with IPF ranges from 4-25% in most studies.[3] The presence of CAD seems has been associated with worse outcomes.[4] Even though CAD is usually diagnosed using left-heart catheterization, previous work suggests that the standard diagnostic HRCT-scan in patients with IPF might be used as a screening tool for CAD.[5] At present it is unknown whether coronary artery calcification, as seen on the HRCT-scan in patients with IPF, has prognostic implications.

Gastro-oesophageal reflux is also considered to be a risk factor for the development and progression of IPF, but this is rather controversial.[6] Gastro-oesophageal reflux and hiatal hernia are both very common in IPF, but the association between these factors and IPF could also be the result of reverse causation, as fibrotic lungs could lead to distortion of mediastinal structures.[6]

An additional risk factor for IPF development and disease evolution is leukocyte telomere length.[7] Short telomeres can lead to disease in various organ systems, but IPF is the most common form of a telomere syndrome.[8] Other potential disease manifestations include bone marrow failure, malignancies, pulmonary fibrosis and liver cirrhosis. But also neurological, gastrointestinal, cardiovascular and skeletal problems can be found.[9] It is not known at present if cardiovascular disease or osteopenia in patients with IPF is associated with leukocyte telomere length.

For this study we took advantage of the fact that all IPF patients receive a HRCT-scan of the chest during their diagnostic work-up. HRCT-scans were evaluated for coronary artery calcification, bone attenuation, and the presence of diaphragmatic hernia. We then investigated whether CT-findings are related to clinical characteristics and leukocyte telomere length.

PATIENTS AND METHODS

Patients were retrospectively included from the Biobank for interstitial lung diseases of St. Antonius Hospital. Participants of the Biobank have given informed consent for the use of their clinical data in scientific research, and have provided blood to be stored and used for later analyses (approved by the local medical ethics committee (MEC-U); study number R05-08A). This study included all patients in the Biobank with a working or consensus diagnosis of IPF from January 2011 to October 2017. Only patients in whom a DNA sample was available were included. The diagnosis of IPF was always made by a multidisciplinary team using clinical, radiological, and if necessary histopathological data. This was done in accordance with the Fleischner Society recommendations, as well as ATS/ERS/JRS/ALAT guidelines.[10,11]. A consensus diagnosis of IPF is given in the appropriate clinical context of IPF (age >60 years, absence of significant exposure, no evidence of collagen vascular disease), with a definite CT-pattern of usual interstitial pneumonia (UIP), or after integration of clinical, imaging and histologic features. A working diagnosis of IPF is given to patients with probable UIP on CT or progressive fibrosing interstitial pneumonia, in the absence of an alternative diagnosis, and when IPF was thought to be the most likely diagnosis.

Clinical characteristics, including demographics, presence of any form of cardiovascular disease, osteoporosis and gastro-oesophageal reflux were retrospectively collected from patient records. Patients were followed up until December 2018. During the course of follow up acute exacerbations of IPF were diagnosed based on the definition of a 2016 International Working Group Report.[12]

HRCT-scans of the chest were retrieved from the hospital radiology database when available. Bone attenuation was scored using a modification of a previously published method.[13] Coronary and aortic calcification was scored using visual grading according to a modification on a previously published scoring system.[14] The definitions used are provided in Supplementary Table 1. The four main coronary artery branches (left main coronary artery, left anterior descending artery, left circumflex artery, and right coronary artery) were each scored on a scale of 0-3, calcification of the ascending and descending aorta were each scored on a scale of 0-3, and calcification of the supra-aortic branches of the aortic arch was scored on a scale of 0-2. The cut-off level of the ascending aorta was after the left subclavian artery, and the cut-off level of the descending aorta was the 11th thoracic vertebra. Scores were summed into a single score for coronary artery calcification (0-12), aortic calcification (0-8), and an overall calcification score (0-20).

For measurement of bone attenuation, the most central section of thoracic vertebrae 4, 7, and 10 was identified with the help of coronal or sagittal images. On these locations on

the transversal images, a circular region of interest was placed in the central part of the vertebral body. Care was taken to avoid the cortex and the basivertebral venous plexus. The size of the region of interest varied, but a distance of one-third of the radius of the vertebral body was always maintained from the cortex. In case of obvious intravertebral abnormalities, such as a haemangioma or enostoses, the vertebral body superior to the vertebral body of interest was used (e.g. T3 instead of T4).

Scans made with various CT-machines and imaging protocols were included. Only CT-imaging without intravenous contrast and cardiac triggering was used, as the scoring system for coronary artery calcification that we used was developed using similarly obtained CT-imaging.[14] When the scan on which the diagnosis was based was not available or when this was a scan with intravenous contrast, any other chest CT-imaging within 6 months of the diagnosis was used. Scans were viewed in a commercially available software program (Sectra PACS; Sectra AB, Linköping, Sweden). For all scans coronal and sagittal reconstructions were made. Scans were read by one of the authors (TWH), who was blinded to patient history and telomere length. Data were collected on standardised forms. The use of the score form was trained under supervision of an experienced board-certified radiologist (HWvE). Collected data included presence of vertebral compression fractures, presence of aortic valve calcification, presence of mitral annular calcification, and presence of diaphragmatic hernia.

Blood samples were taken around time of diagnosis and leukocyte telomere length was determined using a previously described quantitative polymerase chain reaction (PCR) method.[15] Briefly, telomere length was estimated for each sample from the ratio of telomere repeat copy number to a single gene (human β-globin gene) copy number (T/S ratio). Measurements were performed on the Bio-Rad CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The samples were stored at -80 degrees Celsius until use. DNA was isolated from white blood cells using a magnetic beads-based method (chemagic DNA blood 10k kit; PerkinElmer.chemagen Technologie GmbH, Baesweiler, Germany). All sample measurements were performed in duplicate, with additional measurements if the duplicates differed more than 0.05; the mean value of the measured samples was used (in case of suspected faulty measurements, that value was excluded when calculating the mean). Furthermore, quality control samples were included on each PCR-run, with a <0.05 margin of variance to reference values allowed. The control cohort included 164 healthy adults. Age-adjusted normal values for the T/S-ratio were calculated by determining the best-fitting linear regression line through the data, and percentiles were derived from the regression line.

Study data were collected and managed using REDCap electronic data capture tools hosted at St. Antonius Hospital.[16] Data analyses were performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). For survival analyses, Kaplan-Meier curves were calculated and Cox-regression analyses were performed, with the latter correcting for age at diagnosis, sex, and lung function at diagnosis in multivariate analyses. Patients were censored when lost to follow up, when they underwent lung transplantation, or at the time of data collection.

RESULTS

The cohort included 406 patients in whom chest CT-scans around time of diagnosis were available. Baseline characteristics are shown in Table 1.

Table 1: Baseline characteristics in 406 patients with pulmonary fibrosis.

Clinical parameter	N (% of total)
Male (%)	310 (78)
Median age at diagnosis (IQR)	68 (62-75)
1st degree family member with pulmonary fibrosis (%)	113 (28)
Forced vital capacity, percentage of predicted, median (IQR)	78.0 (64.0-91.1)
Diffusion capacity of the lung for carbon monoxide, percentage of predicted, median (IQR)	41.0 (32.0-51.0)
Former or current smokers (%)	328 (81)
>5 packyears	299 (74)
>20 packyears	189 (47)
Diabetes mellitus	71 (17)
Hypercholesterolemia	164 (40)
Hypertension (%)	117 (29)
Pulmonary hypertension (%)	60 (15)
Any cardiovascular disease (%)	178 (44)
Coronary artery disease (%)	122 (30)
Acute coronary syndrome (%)	54 (13)
Peripheral arterial disease (%)	20 (5)
Heart failure (%)	19 (5)
Atrial fibrillation (%)	22 (5)
Cerebrovascular accident or transient ischemic attack (%)	35 (9)
Other cardiovascular disease (%)	53 (13)
Osteoporosis	15 (4)
Gastro-oesophageal reflux	148 (37)

IQR = interquartile range.

One-hundred-and-twenty-two patients had known CAD. There were 178 patients who had any type of cardiovascular comorbidity, excluding hypertension and pulmonary hypertension (44%). CAD specifically associated with male sex (n=108; p<0.001 compared to patients without CAD), and having smoked more than 5 or 20 packyears (n=105 and n=72; p<0.001 and p=0.001, respectively). CAD was associated with decreased survival in a univariate analysis (Figure 1; p=0.02; Log-rank test), but not in a multivariate analysis that corrected for age at diagnosis, lung function at diagnosis and smoking status (p=0.26; Cox-regression). The presence of any type of cardiovascular disease associated with decreased survival (p<0.001), also when corrected for age at diagnosis, sex, Forced Vital Capacity at diagnosis, and Diffusion Capacity of the Lung for Carbon Monoxide at diagnosis (p=0.02; Cox regression).

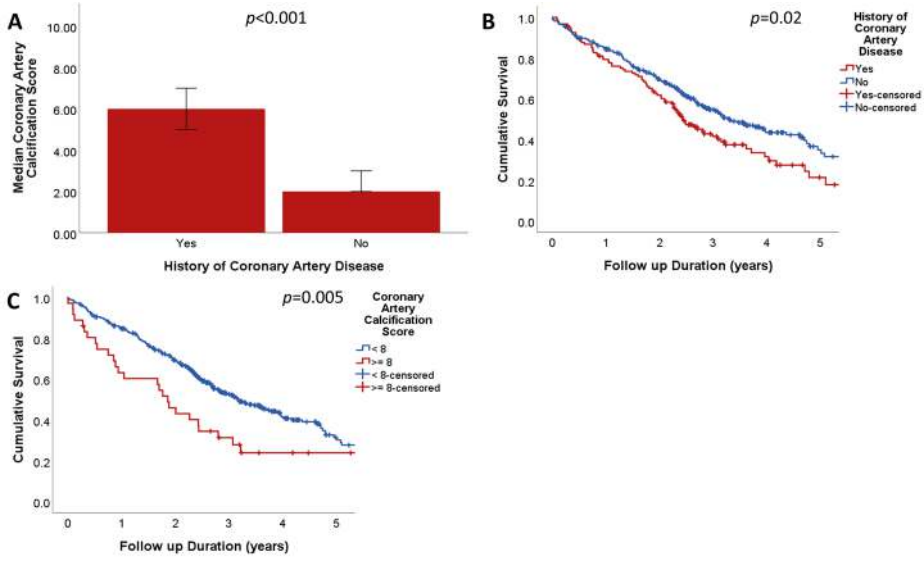


Figure 1: (A) median coronary artery calcification score for patients with and without a history of coronary artery disease. Whiskers represent 95% confidence intervals for the median. *P*-value calculated using Mann-Whitney U test. (B) survival stratified by presence (red) or absence (blue) of coronary artery disease. *P*-value calculated using Log-rank test. This association was no longer significant in a multivariate analysis that corrected for age at diagnosis, lung function at diagnosis and smoking status (p=0.26; Cox-regression). (C) survival stratified by coronary artery calcification score ≥8 (red) or <8 (blue). *P*-value calculated using Log-rank test. This association was still significant in a multivariate model that corrected for age at diagnosis, sex, lung function at diagnosis, smoking status, and a history of coronary artery disease (p=0.03; Cox-regression).

Coronary artery calcification score ranged from 0-12, and had a median value of 3 (interquartile range 1-5). Eighteen percent of patients had a coronary artery calcification score of 0. Coronary artery calcification score was significantly higher in patients with a history

of CAD (median 6 (interquartile range 4-8) versus median 2 (interquartile range 0.25-4); $p<0.001$). Higher coronary artery calcification score was associated with decreased survival ($p=0.04$ Log-rank test), also when corrected for age at diagnosis, sex, lung function at diagnosis, smoking status, and a history of CAD ($p=0.03$; Cox regression). In the same multivariate model, coronary artery calcification score ≥8 was significantly associated with shorter survival (n=36; $p=0.03$; hazard-ratio 1.708 (95% confidence interval 1.066-2.738) Cox-regression). Patients with coronary artery calcification score ≥8 were significantly older at diagnosis, had lower baseline diffusion capacity, were more likely to be current or former smokers and more often had a history of cardiovascular or coronary artery disease (Table 2). There was no relation between a history of CAD, any type of cardiovascular disease, or coronary artery calcification score and leukocyte telomere length.

Table 2: Baseline characteristics of patients with coronary artery calcification score ≥8 and <8.

Clinical parameter	**Coronary artery calcification score ≥8 (N=36)**	**Coronary artery calcification score <8 (N=370)**	***p*-value**
Male (%)	32 (89)	278 (75)	0.07
Median age at diagnosis (IQR)	74.6 (67.5-78.3)	67.7 (61.4-73.7)	<0.001
Forced vital capacity, percentage of predicted, median (IQR)	74.5 (63.5-85.0)	78.8 (64.1-92.0)	0.58
Diffusion capacity of the lung for carbon monoxide, percentage of predicted, median (IQR)	34.0 (26.8-42.3)	42.0 (32.2-53.0)	0.003
Former or current smokers (%)	34 (94)	294 (79)	0.03
>5 packyears	34 (94)	265 (72)	0.002
>20 packyears	28 (78)	161 (44)	<0.001
Diabetes mellitus	6 (17)	65 (18)	0.89
Hypercholesterolemia	27 (75)	137 (370)	<0.001
Hypertension (%)	17 (47)	100 (27)	0.01
Pulmonary hypertension (%)	6 (17)	54 (15)	0.74
Any cardiovascular disease	32 (89)	146 (39)	<0.001
Coronary artery disease (%)	31 (86)	91 (25)	<0.001
Acute coronary syndrome (%)	13 (34)	41 (11)	<0.001
Peripheral arterial disease (%)	6 (17)	14 (4)	0.001
Heart failure (%)	4 (11)	15 (4)	0.08
Atrial fibrillation (%)	3 (8)	19 (5)	0.43
Cerebrovascular accident or transient ischemic attack (%)	2 (6)	33 (9)	0.76
Other cardiovascular disease (%)	13 (36)	40 (11)	<0.001

IQR = interquartile range.

There were 20 patients (5%) who developed acute coronary syndrome, heart failure, atrial fibrillation, or stroke after the diagnosis of IPF. Thirteen of these patients (65%) had a prior history of any cardiovascular disease (p=0.05) and two (10%) had a coronary artery calcification score ≥8 (p=0.69). The median coronary artery calcification score in these patients was 4 (interquartile range 2-6) compared to a median of 3 (interquartile range 1-5) in other patients (p=0.08).

Fifteen patients (4%) had a history of osteoporosis. In these patients, average bone attenuation on CT-scans was significantly lower compared to patients without osteoporosis (mean 122, 95% CI 104-141 versus mean 157, 95% CI 152-161; p=0.003). Seventeen patients had one or more vertebral compression fractures on the CT-scan. This was significantly more common in patients with osteoporosis (n=5; p<0.001). Osteoporosis was not associated with survival. There was no association between osteoporosis and leukocyte telomere length, nor was there a correlation between bone attenuation and leukocyte telomere length (Supplementary Figure 1).

One-hundred-forty-eight patients (37%) had complaints of gastro-oesophageal reflux, and on CT-imaging 175 patients (43%) had a diaphragmatic hernia. Presence of diaphragmatic hernia on CT-imaging associated with gastro-oesophageal reflux complaints (Figure 2A; p=0.04). Gastro-oesophageal reflux was not related to smoking status (p=0.45), but diaphragmatic hernia was significantly less common in former or current smokers compared to never-smokers (42% versus 15% versus 58%; p<0.001).

An acute exacerbation of IPF was noted for 47 patients (12%), and was not associated with either the presence of gastro-oesophageal reflux complaints (n=19; p=0.63) or diaphragmatic hernia (n=25; p=0.64). Neither gastro-oesophageal reflux complaints, nor diaphragmatic hernia were associated with survival in a univariate model (Figure 2B and C; Log-rank test; p=0.54 and p=0.41, respectively). The presence of a diaphragmatic hernia was also not associated with lung function at diagnosis, or the observed pulmonary fibrosis pattern on CT-imaging (data not shown).

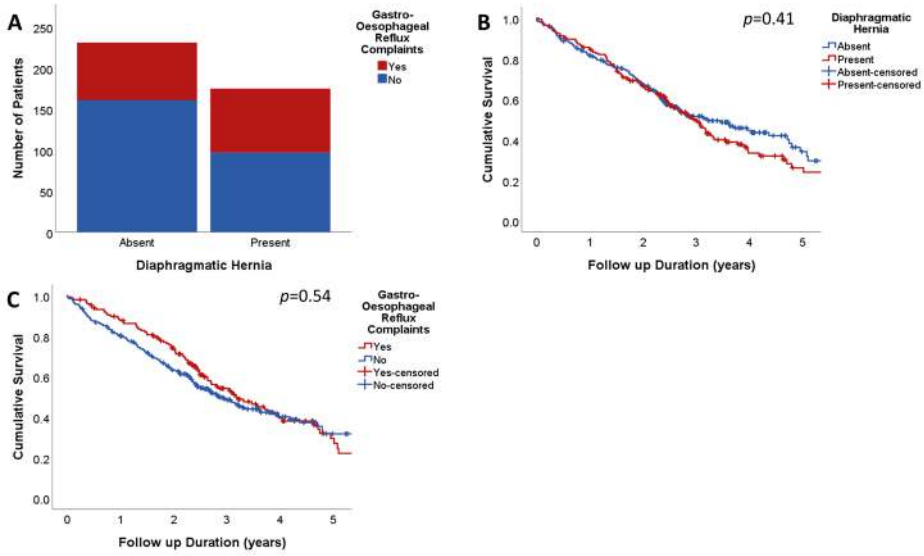


Figure 2: (A) number of patients with diaphragmatic hernia on CT-imaging, stratified by presence or absence of gastro-oesophageal reflux. Presence of diaphragmatic hernia on CT-imaging associated with gastro-oesophageal reflux complaints (p=0.04). (B) survival stratified by presence (red) or absence (blue) of diaphragmatic hernia. Survival did not significantly differ between the two groups (Log-rank test). (C) survival stratified by presence (red) or absence (blue) of gastro-oesophageal reflux complaints. Survival did not significantly differ between the two groups (Log-rank test).

DISCUSSION

In this study we investigated the value of unrequested information from CT-imaging of the chest in 406 patients with IPF. This included coronary artery calcification, bone attenuation of the thoracic vertebrae, and the presence of diaphragmatic hernia. We found that coronary artery calcification score was an independent predictor for survival, with higher scores being associated with lower survival. Notably, the predictive value of coronary artery calcification score was independent of a clinical history of CAD. None of the CT-findings associated with leukocyte telomere length.

Significantly, we found that higher coronary artery calcification score, as read from standard HRCT-imaging, was associated with decreased survival, independent of a history of CAD. In a previous study Nathan and colleagues found a prevalence of 29% and 66% for significant and any degree of CAD, respectively, in patients with IPF who underwent screening for lung transplantation.[4] Patients with significant CAD in that study had a significantly worse survival, but not in a multivariate model that corrected for sex, lung function, and age. In another study by the same authors, they showed that coronary calcification score had a sensitivity and specificity of 81% and 85%, respectively, for significant

CAD as determined by left heart catheterization.[5] It must be noted that the authors used a different calcification score than the one used in the present study. However, it seems that coronary artery calcification score as determined from standard CT-imaging is a useful, non-invasive, prognostic factor. The use of this prognostic factor could be implemented in practice quite easily, although it should still be determined if different types of (automated) coronary artery calcification scoring systems could be used. It remains to be investigated if serial calculation of coronary artery calcification has prognostic implications as well, as HRCT-scans are typically done repeatedly in the course of follow up of patients with IPF. In addition, whether patients with significant CAD would benefit from a cardiology referral and possible treatment of CAD remains to be determined. In this study, coronary artery calcification score did not associate with cardiac events during follow up, but this might be related to a relatively small percentage of patients (5%) who actually developed such an event.

The roles of gastro-oesophageal reflux and diaphragmatic hernia in IPF pathogenesis and disease progression have been the subject of debate.[6] Rates of gastro-oesophageal reflux complaints (37%) and diaphragmatic hernia (43%) in the present cohort were comparable to those in earlier cohorts.[17–20] The present study comprised a much larger patient population of over 400 patients compared to around or below 100 patients in previous studies. In contrast to previous studies, we did not find an association between gastro-oesophageal reflux and survival.[19,20] Taken together, our findings are not indicative of a role of gastro-oesophageal reflux or diaphragmatic hernia in disease progression for IPF patients. This seems to fit within the context of several recent studies. Notably, the WRAP-IPF trial randomised IPF patients with proven gastro-oesophageal reflux to reflux surgery or conservative treatment and found no difference in lung function decline or survival between both groups.[21] A recent meta-analysis did find a significant association between the presence of gastro-oesophageal reflux and IPF, but noted that this association was no longer significant when correcting for smoking.[22] A meta-analysis of pharmacologic treatment of gastro-oesophageal reflux in patients with IPF found a reduction of IPF-related mortality in the treated patients, but no reduction in all-cause mortality.[23] Furthermore, a recent Australian registry-based study did not find an effect of proton-pump inhibitor use or gastro-oesophageal reflux symptoms on long-term outcomes in 587 IPF patients.[24]

This study has several limitations. First, this was a retrospective study and the availability of data was limited by what was recorded in patient files. This might lead to an underestimation of the prevalence of some clinical characteristics, such as osteoporosis. However, our cohort was large and all patients underwent a relatively standardized and thorough evaluation. This means that the results are representative of the information that is available in clinical practice. Second, we have not corrected our survival analyses for the use

of antifibrotic therapies, which are now an integral part of the management of patients with IPF. This was deemed likely to introduce bias, as the choice for an antifibrotic agent was time-dependent, pirfenidone having become available on the Dutch market earlier than nintedanib. With regard to the absence or presence of gastro-oesophageal reflux we have only included clinical complaints, and not invasive measurement of oesophageal pH. It has been shown in previous studies that gastro-oesophageal reflux can be clinically occult,[25] and therefore we might have missed gastro-oesophageal reflux in patients without symptoms.

CONCLUSION

An increased coronary artery calcification score was an independent predictor of decreased survival in a cohort of 406 patients with IPF. We found no association between coronary artery calcification score or vertebral bone attenuation and leukocyte telomere length. Furthermore, in contrast to earlier studies, we did not find an association between the presence of gastro-oesophageal reflux complaints or diaphragmatic hernia and patient outcomes.

REFERENCES

1. Lederer DJ, Martinez FJ. Idiopathic Pulmonary Fibrosis. *N Engl J Med*. 2018;378(19):1811-1823. doi:10.1056/NEJMra1705751 [doi]
2. Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2018;198(5):e44-e68. doi:10.1164/rccm.201807-1255ST [doi]
3. Oldham JM, Collard HR. Comorbid conditions in idiopathic pulmonary fibrosis: Recognition and management. *Front Med*. 2017;4(AUG):123. doi:10.3389/FMED.2017.00123
4. Nathan SD, Basavaraj A, Reichner C, et al. Prevalence and impact of coronary artery disease in idiopathic pulmonary fibrosis. *Respir Med*. 2010;104(7):1035-1041. doi:10.1016/j.rmed.2010.02.008 [doi]
5. Nathan SD, Weir N, Shlobin OA, et al. The value of computed tomography scanning for the detection of coronary artery disease in patients with idiopathic pulmonary fibrosis. *Respirology*. 2011;16(3):481-486. doi:10.1111/j.1440-1843.2010.01919.x [doi]
6. Johannson KA, Strambu I, Ravaglia C, et al. Antacid therapy in idiopathic pulmonary fibrosis: more questions than answers? *Lancet Respir Med*. 2017;5(7):591-598. doi:10.1016/S2213-2600(17)30219-9
7. Duckworth A, Gibbons MA, Allen RJ, et al. Telomere length and risk of idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease: a mendelian randomisation study. *Lancet Respir Med*. 2021;9(3):285-294. doi:10.1016/S2213-2600(20)30364-7
8. Armanios M. Telomerase and idiopathic pulmonary fibrosis. *Mutat Res*. 2012;730(1-2):52-58. doi:10.1016/j.mrfmmm.2011.10.013 [doi]
9. Niewisch MR, Savage SA. An update on the biology and management of dyskeratosis congenita and related telomere biology disorders. *Expert Rev Hematol*. September 2019:1-16. doi:10.1080/17474086.2019.1662720 [doi]
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(6):788-824. doi:10.1164/rccm.2009-040GL [doi]
11. Lynch DA, Sverzellati N, Travis WD, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. *Lancet Respir Med*. 2018;6(2):138-153. doi:10.1016/S2213-2600(17)30433-2
12. Collard HR, Ryerson CJ, Corte TJ, et al. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. *Am J Respir Crit Care Med*. 2016;194(3):265-275. doi:10.1164/rccm.201604-0801CI [doi]
13. Romme EA, Murchison JT, Phang KF, et al. Bone attenuation on routine chest CT correlates with bone mineral density on DXA in patients with COPD. *J Bone Miner Res*. 2012;27(11):2338-2343. doi:10.1002/jbmr.1678 [doi]
14. Jacobs PC, Gondrie MJ, van der Graaf Y, et al. Coronary artery calcium can predict all-cause mortality and cardiovascular events on low-dose CT screening for lung cancer. *AJRAmerican J Roentgenol*. 2012;198(3):505-511. doi:10.2214/AJR.10.5577 [doi]
15. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;30(10):e47.

16. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010 [doi]

17. Noth I, Zangan SM, Soares R V, et al. Prevalence of hiatal hernia by blinded multidetector CT in patients with idiopathic pulmonary fibrosis. *Eur Respir J*. 2012;39(2):344-351. doi:10.1183/09031936.00099910 [doi]

18. Savarino E, Carbone R, Marabotto E, et al. Gastro-oesophageal reflux and gastric aspiration in idiopathic pulmonary fibrosis patients. *Eur Respir J*. 2013;42(5):1322-1331. doi:10.1183/09031936.00101212 [doi]

19. Mackintosh JA, Desai SR, Adamali H, et al. In patients with idiopathic pulmonary fibrosis the presence of hiatus hernia is associated with disease progression and mortality. *Eur Respir J*. 2019;53(5):1802412. doi:10.1183/13993003.02412-2018

20. Tossier C, Dupin C, Plantier L, et al. Hiatal hernia on thoracic computed tomography in pulmonary fibrosis. *Eur Respir J*. 2016;48(3):833-842. doi:10.1183/13993003.01796-2015 [doi]

21. Raghu G, Pellegrini CA, Yow E, et al. Laparoscopic anti-reflux surgery for the treatment of idiopathic pulmonary fibrosis (WRAP-IPF): a multicentre, randomised, controlled phase 2 trial. *Lancet Respir Med*. 2018;6(9):707-714. doi:https://doi.org/10.1016/S2213-2600(18)30301-1

22. Bedard Methot D, Leblanc E, Lacasse Y. Meta-analysis of Gastroesophageal Reflux Disease and Idiopathic Pulmonary Fibrosis. *Chest*. 2019;155(1):33-43. doi:10.1016/j.chest.2018.07.038

23. Fidler L, Sitzer N, Shapera S, Shah PS. Treatment of Gastroesophageal Reflux in Patients With Idiopathic Pulmonary Fibrosis: A Systematic Review and Meta-Analysis. *Chest*. 2018;153(6):1405-1415. doi:10.1016/j.chest.2018.03.008

24. Jo HE, Corte TJ, Glaspole I, et al. Gastroesophageal reflux and antacid therapy in IPF: analysis from the Australia IPF Registry. *BMC Pulm Med*. 2019;19(1):84-019-0846-2. doi:10.1186/s12890-019-0846-2 [doi]

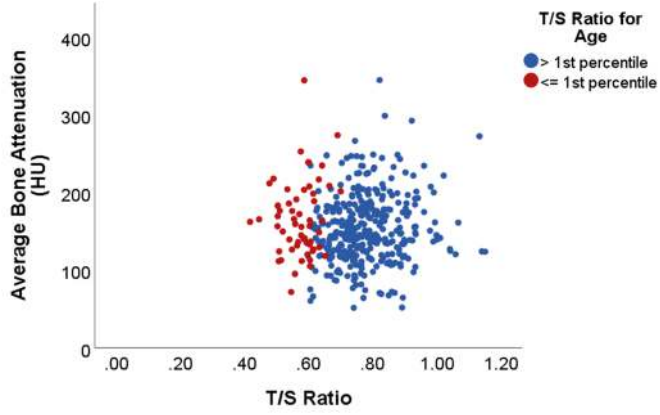
25. Raghu G, Freudenberger TD, Yang S, et al. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. *Eur Respir J*. 2006;27(1):136-142. doi:10.1183/09031936.06.00037005

SUPPLEMENTARY DATA

Supplementary Table 1: Visual grading system for calcification of coronary arteries and aorta.

	Grade 0	Grade 1	Grade 2	Grade 3
Coronary artery calcification	Absent	1-2 foci	>2 foci or 1 calcification extending over 10mm	Calcified arteries covering a large segment of a coronary branch
Supra-aortic artery calcification	Absent	Calcifications in 1 supra-aortic artery	Calcifications in >1 supra-aortic artery	-
Aortic wall calcification	Absent	≤3 foci	4-5 foci or 1 calcification extending over 15mm	>5 foci or 1 calcification extending over 25mm

Coronary arteries scored are the left main coronary artery, left anterior descending artery, left circumflex artery and right coronary artery. The ascending and descending aorta are scored separately. Adapted from Jacobs *et al.*[14]



Supplementary Figure 1: Average vertebral bone attenuation in Hounsfield Units (HU) in 406 patients with idiopathic pulmonary fibrosis. As measured for thoracic vertebrae 4, 7 and 10, subcategorized according to telomere length below (red) or above (blue) the first percentile for age. There was no significant difference in vertebral bone attenuation between patients with telomere length below or above the first percentile for age (median 161 (interquartile range 131-199) versus 147 (124-179); *p*=0.08).

Chapter 6

Pulmonary phenotypes associated with genetic variation in telomere-related genes

T.W. Hoffman, C.H.M. van Moorsel, R. Borie, and B. Crestani

ABSTRACT

Genomic mutations in telomere-related genes have been recognized as a cause of familial forms of idiopathic pulmonary fibrosis (IPF). However, it has become increasingly clear that telomere syndromes and telomere shortening are associated with various types of pulmonary disease. Additionally, it was found that also single nucleotide polymorphisms (SNPs) in telomere-related genes are risk factors for the development of pulmonary disease. This review focuses on recent updates on pulmonary phenotypes associated with genetic variation in telomere-related genes.

Genomic mutations in seven telomere-related genes cause pulmonary disease. Pulmonary phenotypes associated with these mutations range from many forms of pulmonary fibrosis to emphysema and pulmonary vascular disease. Telomere-related mutations account for up to 10% of sporadic IPF, 25% of familial IPF, 10% of connective-tissue disease-associated interstitial lung disease, and 1% of COPD. Mixed disease forms have also been found. Furthermore, SNPs in *TERT*, *TERC*, *OBFC1*, and *RTEL1*, as well as short telomere length, have been associated with several pulmonary diseases. Treatment of pulmonary disease caused by telomere-related gene variation is currently based on disease diagnosis and not on the underlying cause.

Pulmonary phenotypes found in carriers of telomere-related gene mutations and SNPs are primarily pulmonary fibrosis, sometimes emphysema and rarely pulmonary vascular disease. Genotype–phenotype relations are weak, suggesting that environmental factors and genetic background of patients determine disease phenotypes to a large degree. A disease model is presented wherever genomic variation in telomere-related genes cause specific pulmonary disease phenotypes whenever triggered by environmental exposure, comorbidity, or unknown factors.

INTRODUCTION

Telomeres are repetitive hexanucleotide structures located at the end of chromosomes. Telomeres protect chromosome ends from being recognized as DNA breaks by the cell's DNA damage repair machinery.[1] Because chromosome ends cannot be fully replicated, telomeres shorten with every cell division.[2] This is counteracted by activation of the telomerase enzyme in embryonic and stem cells, which can add nucleotides.[3] However, in most cells the telomerase enzyme does not completely keep up with telomere loss, and thus telomeres become increasingly short with age.[4] When telomere length falls below a critical threshold, cellular senescence or apoptosis ensues.[5]

Genomic mutations in genes encoding the telomerase enzyme, the shelterin protein complex (which binds to telomeres, and is required for their protection and proper functioning of the telomerase enzyme), and other telomere-related genes can lead to increased telomere shortening.[6] This can become clinically manifest in various tissues, and is termed a telomere syndrome. Telomere syndromes are systemic diseases in principle, but disease presentation can be very variable.[6] Manifestations of a telomere syndrome can include nail dystrophy, skin hyperpigmentation, oral leucoplakia (the characteristic clinical triad of dyskeratosis congenita (DKC)), premature hair greying, liver cirrhosis (or milder liver abnormalities), bone marrow failure (or milder haematological abnormalities), acute leukaemia, and pulmonary disease, among others.[6] Patients with different disease phenotypes can have the same underlying mutation and vice versa.[7]

In 2007 it was discovered that mutations in telomerase genes were the cause of the familial form of Idiopathic pulmonary fibrosis (IPF) in several families.[8,9] IPF is a very severe lung disease, and is the most frequent form of the idiopathic interstitial pneumonias.[10] The diagnosis of IPF is based on radiological and histological features, which should show a characteristic usual interstitial pneumonia (UIP) pattern. Although IPF is rare, with an estimated prevalence of 1-28/100000 persons in western countries,[11] it is by far the most common manifestation of a telomere syndrome.[6,12] After these initial discoveries, disease-causing genomic mutations in various other telomere-related genes have been found in IPF patients.[13–18]

In early studies of telomere genes in patients with pulmonary disease, even when focussed on patients with the familial form of IPF, it was already seen that some family members of the index patients had pulmonary disease incompatible with the diagnosis of IPF.[12] In the past years, it has become increasingly clear that telomere syndromes are associated with various types of pulmonary disease. Additionally, it has been found that not only telomere gene mutations, but also short telomere length in the absence of a mutation, as well as

single nucleotide polymorphisms (SNPs) in telomere-related genes may be associated with developing pulmonary disease.[19,20] Almost 10 years after the landmark descriptions of the first mutations in telomere-related gene in patients with familial IPF, this review addresses recent updates in pulmonary phenotypes associated with genetic variation in telomere-related genes. The review will focus on diseases associated with short telomeres, and the genetic variation discussed will be of a genomic nature, rather than somatic.

INTERSTITIAL LUNG DISEASES ASSOCIATED WITH TELOMERE-RELATED GENE MUTATIONS

An overview of the pulmonary diseases associated with mutations in telomere-related genes is provided in Table 1. Interstitial lung disease (ILD) is the best-known pulmonary manifestation of mutations in telomere-related genes, with the most common diagnosis being IPF.[12] The frequency of mutations in telomere-related genes in familial IPF is shown in Figure 1. The prevalence of ILD in carriers of telomere gene mutations increases with age. In an American cohort of *TERT* mutation carriers, none of the patients younger than 40 years showed ILD, but the prevalence was greater than 60% in those older than 60.[21] Patients with *TERC* mutations may be relatively younger at ILD diagnosis than patients with mutations in other genes. In a recent series of 114 patients with ILD associated with telomerase complex mutation, the ILD was diagnosed at a mean age of 51 years (n=7) for *TERC* mutation carriers, 58 years (n=75) for *TERT* mutation carriers, 60 years (n=14) for *RTEL1* mutation carriers and 65 years (n=19) for *PARN* mutation carriers (p=0.03).[22] In case of *TERT* mutations, male mutation carriers are more often affected compared to female carriers, and present with disease at an earlier age.[21] Smoking and pneumotoxic exposure are frequently observed in patients with ILD (40-96%).[21–23] Also in patients who were initially diagnosed with silicosis or drug-induced pulmonary fibrosis, telomere gene mutations have been found after family members had been diagnosed with pulmonary fibrosis.[8,23]

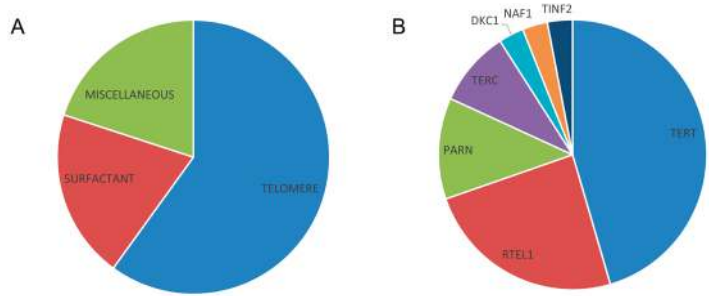


Figure 1: Frequency of mutations in telomere-related genes in patients with familial pulmonary fibrosis. (A) frequency of telomere-related gene mutations compared to mutations in surfactant or other genes. (B) relative frequencies of mutations in specific telomere-related genes.

Table 1: pulmonary diseases associated with mutations in telomere-related genes.

Pulmonary phenotype	Radiological pattern*	Histopathological pattern*	Phenotypic characteristics	Implicated telomere-related genes	% of cases with telomere gene mutation	Phenotype associated with telomere gene mutation
Interstitial lung disease						
IPF[8,9,24–27,13–18,22,23]	UIP	UIP	-	TERT, TERC, DKC1, TINF2, RTEL1, PARN, NAF1	5-10% (sporadic); 20-25% (familial)	Extrapulmonary signs of telomeropathy in some patients
NSIP[17,22,23,28,29]	NSIP	NSIP	-	TERT, (TERC,) PARN, DKC1, RTEL1	?	
DIP[22]	DIP	DIP	Usually smokers	TERT	?	
PPFE [22,30,31]	PPFE	PPFE/UIP	Pneumothorax common	TERT, TERC, RTEL1	50%	F>M
Unclassifiable PF[8,17,22,23,32]	PF	PF	-	TERT, TERC, RTEL1, PARN, DKC1	?	
HP[22,23,25]	HP	HP	Exposure to inhalation antigens; mostly non-smokers	TERT, TERC, RTEL1, PARN	?	
CTD-ILD[15,17,22,23,28,33]	NSIP/UIP/other	NSIP/UIP/other	Co-existing connective tissue disease	TERT, TERC, RTEL1, PARN	10%	Younger age at diagnosis
Drug-induced PF[23]	NSIP/UIP/other	NSIP/UIP/other	Exposure to pneumotoxic drug	TERT, (TERC)	?	
Other						
CPFE[18,34,35]	UIP/NSIP + emphysema	UIP/NSIP	Usually smoking	TERT, TERC, NAF1	?	
COPD[18,34,36]	Emphysema	Emphysema	Usually smoking	NAF1, TERT, TERC	1%	Mostly smoking females; pneumothorax relatively common
Pulmonary arteriovenous malformations[37]	PAVM	PAVM		TERT, RTEL1, PARN, DKC1, TINF2	?	Young patients with dyskeratosis congenita
Hepatopulmonary syndrome[38]	Intrapulmonary vascular dilatation	Intrapulmonary vascular dilatation, arteriovenous communications	Liver disease	DKC1, TERT, RTEL1	?	Extrapulmonary features of telomere syndrome (premature hair greying, bone marrow failure, mucocutaneous abnormalities); presentation at early age

Interstitial lung disease (idiopathic interstitial pneumonia) classification according to the 2013 ATS/ERS criteria.[10] * most common, but not exclusive, radiological or histopathological pattern. UIP= usual interstitial pneumonia; NSIP= non-specific

interstitial pneumonia; DIP= desquamative insterstitial pneumonia; PPFE= pleuroparenchymal fibroelastosis; PF= pulmonary fibrosis; HP= hypersensitivity pneumonitis; CTD-ILD= connective tissue disease-associated interstitial lung disease; CPFE=combined pulmonary fibrosis and emphysema; COPD= chronic obstructive pulmonary disease; PAVM= pulmonary arteriovenous malformation

A typical UIP pattern on chest CT was reported in 46% to 74% of patients with a telomere-related gene mutation.[21–23,33] Atypical features are found in 13% to 20% of patients including upper-lung predominance of fibrosis, a pleuro-parenchymal fibroelastosis pattern, or centrilobular fibrosis.[21–23] IPF is the most frequent diagnosis given to patients with telomere gene mutations (45% to 86%).[21–23] Unclassifiable fibrosis (19-30%), pleuroparenchymal fibroelastosis (up to 10%), chronic hypersensitivity pneumonitis (HP) (7-11%), and connective tissue disease (CTD)-associated ILD (2%) can also be found.[21–23,28,30,31] The

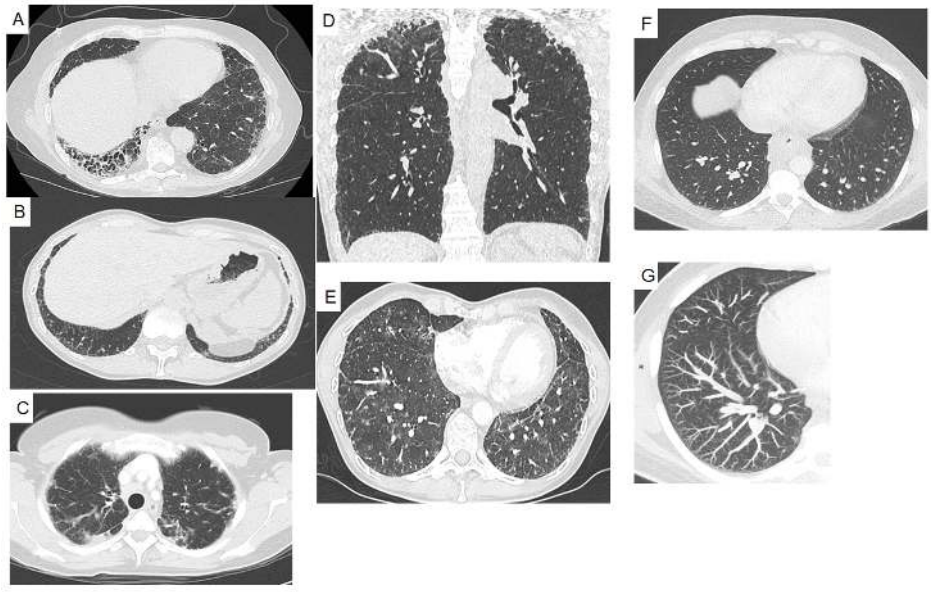


Figure 2: Computed-tomography of the chest in patients with pulmonary disease due to telomere-related gene mutations. (A) usual interstitial pneumonia (UIP). (B) possible UIP. (C and D) inconsistent with UIP suggestive of pleuro-parenchymal fibroelastosis. (E) ground glass opacities superimposed with fibrosis revealed to be secondary to pneumocystis infection. (F) normal chest CT scan in a patient with hypoxemia and liver disease. (G) reconstruction with maximum intensity projection revealed intrapulmonary vascular dilatation.

variation in fibrotic patterns that can be observed in patients with telomere-related gene mutations is illustrated in Figure 2. The frequency of telomere gene mutations in patients with a given ILD phenotype has not been studied extensively for most phenotypes. In IPF, up to 10% of patients have a mutation in a telomere-related gene.[28] The proportion in CTD-ILD could be similar,[33] and the proportion of patients with telomere gene mutations in

PPFE was 50% in a small group.[31] A family history of pulmonary fibrosis is associated with an increased chance of finding a mutation in all subtypes of ILD reported here.

The decline of lung function (forced vital capacity (FVC)) in patients with telomere-related gene mutations seems unexpectedly high when compared to placebo arms of IPF clinical trials (130-210 mL/year).[39] Newton and colleagues recently reported a 300 mL/year decline of FVC whatever the gene (*TERC*, *TERT*, *RTEL1* or *PARN*), or the clinical diagnosis (IPF or not) involved.[22] Pulmonary fibrosis associated with telomere-related gene mutations is lethal, and most patients die of respiratory causes. The mean survival for symptomatic patients is 2.8 to 5.2 years after diagnosis. However, disease evolution can be longer, particularly in asymptomatic carriers of mutations.[40] Transplant-free survival could be lower for carriers of *TERT* or *TERC* mutations than for patients with familial ILD without *TERT* or *TERC* mutation.[23]

COPD AND EMPHYSEMA

Chronic obstructive pulmonary disease (COPD) is much more common than interstitial lung disease, and genetic causes of this disease were long limited to alpha-1 antitrypsin deficiency. After telomere dysfunction was implicated as a cause of IPF, mouse models interestingly showed that mice with short telomeres developed emphysema and not fibrosis in response to cigarette smoke. Thereafter, a family with a *TERC* mutation was identified, where carriers presented with either early-onset pulmonary fibrosis or emphysema.[34] Subsequently, mutations in *TERT* and *NAF1* were found in patients with emphysema.[18,36] COPD in patients with telomere-related gene mutations seems to be more severe compared to other COPD patients. Within families with telomere-related gene mutations, especially females who smoke, seem to be susceptible to develop emphysema.[41] The frequency of *TERT* mutations in a cohort of 292 patients with severe COPD was 1%.[36] Emphysema has been observed in families with mutations in other telomere genes as well (*RTEL1*, *PARN*), but extensive clinical data were not available in those cases.[17]

In all, up to 40% of patients with IPF show a combined pulmonary fibrosis and emphysema (CPFE) pattern.[42] CPFE is usually characterized by pulmonary fibrosis in the lower lobes and emphysema in the upper lobes. Whether it is a distinct clinical syndrome or a combination of different lung phenotypes is still under debate.[10,43] There are several reports of patients with telomere gene mutations and CPFE.[18,25,34,35] However, concurrent emphysema in patients with ILD and mutations in telomere-related genes has not been studied extensively. As CPFE is not included in the current and past classifications of ILD,[10] previous studies might often have reported a patient's diagnosis to be IPF, when that patient also

had emphysema. In one study that did separately report emphysema on chest imaging, the prevalence was 20% in a cohort of *TERT* mutation carriers with pulmonary fibrosis.[21] This is lower than the 40% prevalence in all IPF patients mentioned above, but the latter percentage comes from a study that specifically scored chest imaging for emphysema, whereas the former used available clinical data. Furthermore, it has not been studied whether mutations in telomere-related genes are associated with more emphysema in IPF patients when correcting for smoking behavior.

PULMONARY VASCULAR DISEASE

Mutations in telomere-related genes have been associated with several types of liver disease, most characteristically cryptogenic liver cirrhosis.[6] Recently, Gorgy and colleagues studied the prevalence of hepatopulmonary syndrome (HPS) in patients with telomere gene mutations who presented with dyspnoea.[38] In most cases the dyspnoea was caused by pulmonary fibrosis or emphysema, but in 21% of patients the cause was HPS. Histology of liver tissue showed nodular regenerative hyperplasia in most patients, without cirrhosis. Interestingly, HPS is a rare complication of non-cirrhotic portal hypertension, which is rare in itself. Therefore, the association of both features with telomere gene mutations is remarkable. The precise pathophysiological mechanisms underlying this phenotype remain to be determined. Possibly, dysfunction of hepatic endothelial cells leads to liver disease which in turn causes a vascular response in a lung that is more susceptible to vascular changes because of lung endothelial cell dysfunction.[38]

In addition to these findings, some patients with telomere syndromes who do not have HPS do develop pulmonary arteriovenous malformations (PAVMs).[37] The precise pathophysiologic mechanism underlying the forming of PAVMs in this case needs to be elucidated, but could involve transforming growth factor β-signalling.[37] Importantly, both these vascular diseases have only been observed in the setting of patients with telomere syndrome manifestations in other organs as well, although in some patients with HPS shortness of breath was the first presenting sign.

INFECTION AND IMMUNOLOGY

Respiratory tract infections are the most common infectious manifestation of immunodeficiency.[44] Patients with DKC have been found to have immunodeficiencies, including lymphopenia, low B-cell numbers, hypogammaglobulinemia, and decreased T-cell function.[45,46] These patients therefore frequently have respiratory tract infections. However,

in patients with telomere gene mutations who have pulmonary disease but not DKC, leukopenia is rare.[21,22] Furthermore, most pulmonary fibrosis patients with telomere gene mutations have immunoglobulin levels within the normal range (Hoffman/van Moorsel, unpublished observation). However, immune dysfunction is observed in some telomere gene mutation carriers with pulmonary disease, as some patients have concomitant myelodysplastic syndrome (MDS).[21–23] Overall, the infection risk in patients with pulmonary disease and mutations in telomere-related genes seems to be comparable to pulmonary disease patients without telomere-related gene mutations. DKC patients are particularly at risk for pneumocystis infection, and we recently reported the case of a patient with a *TERC* mutation and primary immunodeficiency revealed by pneumocystosis, which could have been misdiagnosed as an acute exacerbation of ILD (Figure 2).[47] Furthermore, we previously reported the case of an IPF patient with a *TINF2* mutation who first presented with pneumonia and was concurrently diagnosed with common variable immunodeficiency (CVID). This patient did not have a history of recurrent infections, but did fulfill the diagnostic criteria for CVID.[26] Following lung transplantation, patients with lung fibrosis and telomere gene mutations did not appear to present a higher risk of opportunistic infection, but recurrent respiratory tract infections are common.[48–50]

SINGLE NUCLEOTIDE POLYMORPHISMS IN TELOMERE GENES ASSOCIATED WITH PULMONARY DISEASE

Apart from rare genetic mutations that cause shortening of telomeres and can directly lead to development of disease, common variants with smaller effect sizes can also contribute to disease development. Table 2 provides an overview of common variants in telomere genes that are associated with telomere shortening and pulmonary disease. Genome-wide association studies (GWAS) have found several common genetic variants in telomere genes to be associated with IPF.[20,51] These include variants in *TERT* and near *TERC* (rs6793295), as well as *OBFC1* (rs11191865), a gene that is also implicated in telomere biology. One variant in the *TERT* gene (rs2736100) was subsequently found to be associated with other idiopathic interstitial pneumonias as well.[52] Interestingly, this same variant has also been associated with lung cancer.[53] However, in lung cancer it is not the major allele (A), but the minor allele (C) that increases disease risk. The minor allele has been associated with longer telomeres, whereas the major allele is associated with shorter telomeres.[54] Other variants in *TERT* are associated with development of lung cancer as well, and are also associated with longer telomeres.[55–57] Furthermore, variants in the *OBFC1* and *RTEL1* genes have also been associated with lung cancer.[57] This lung-cancer associated variant in the *OBFC1* gene has also been associated with longer telomeres.[54]

Table 2: common genetic variants in telomere-related genes associated with pulmonary disease.

Gene	Risk allele	Pulmonary disease associated with variant	Variant associated with shorter telomeres?	Independent replication?	References
TERT	rs2736100 major allele (A)	IPF	Yes	Yes	[20,51,52]
		IIP other than IPF	Yes	No	[52]
(near) TERC	rs1881984 minor allele (G)	IPF	Unknown	No*	[51]
OBFC1	rs11191865 major allele (A)	IPF	No	No*	[51,58]
RTEL1	rs4809324 (T)	COPD	Unknown	No	[59]
	rs6010621 (G)	HAPE	Unknown	No	[60]

The Fingerlin et al study included patients with diagnoses other than IPF as well, but IPF patients comprised the vast majority of the fibrotic IIP cohort. *the Fingerlin study included a validation cohort. For Fingerlin et al. only sentinel variants were included. Telomere length was measured in blood in healthy controls for all variants. IPF= idiopathic pulmonary fibrosis; COPD= chronic obstructive pulmonary disease; HAPE= high-altitude pulmonary edema

Claar and colleagues found that a common variant near the *TERC* gene was significantly associated with asthma, as well as pulmonary fibrosis using a so-called PheWAS approach.[61] The variant they selected (rs6793295) was found to be associated with IPF by Fingerlin and colleagues, as well as in a confirmation study in a Mexican population by Peljto and colleagues.[51,62] Unfortunately, because extensive data on other SNPs was not available in their study, Claar and colleagues could only examine the effect of this variant. Future studies will have to determine whether this variant is indeed associated with asthma. Asthma GWAS have thus far not identified any common variants in telomere genes.[63] An association between COPD and a variant in *RTEL1* (rs4809324) was found by one candidate-gene study,[59] but COPD GWAS have not identified any telomere-related gene variants.[64] This association will also need to be confirmed in future studies.

Interestingly, a recent candidate-gene study by Rong and colleagues found a common variant in the *RTEL1* gene (rs6010621) to be associated with the development of high-altitude pulmonary oedema (HAPE).[60] HAPE is a condition of acute pulmonary oedema caused by pulmonary hypertension in response to ascension to high altitude.[65] Other studies have found an association between the risk of developing HAPE and common variants in the *ACYP2* gene.[66,67] The *ACYP2* gene is found in a locus that has been linked to telomere length.[54] However, it is not clear if *ACYP2* plays a role in telomere biology. The link between telomere biology and HAPE is still elusive, and these results require confirmation in future studies.

RELATION BETWEEN TELOMERE LENGTH AND PULMONARY DISEASE

Telomere length is often used as a proxy for telomere dysfunction and the likelihood that a given telomere gene mutation causes disease or that a person develops clinical manifestations of a telomere syndrome. This is at least partially correct, as most known mutations that cause telomere syndromes lead to markedly shortened telomeres (50-90% of cases).[21,23,68] Furthermore, the phenomenon of genetic anticipation can be observed in families with telomere gene mutations. This is defined as an earlier onset of disease with each next generation.[6] Telomeres shorten from generation to generation in patients with *TERT*, *TERC*, *PARN* or *RTEL1* mutations because of transmission of the short telomeres independent of transmission of the mutation.[21–23] This epigenetic-like inheritance in human disorders of telomerase also explains observations of affected patients who do not carry the familial mutation.[69,70]

In patients with genomic mutations in telomere-related genes, the disease phenotype is clearly caused by telomere dysfunction due to shortening of telomeres. However, in other patients a link between short telomere length and disease development may not be as straightforward. Telomere length is not only dependent on inherited telomere length and functioning of telomere maintenance proteins, but also on environmental factors.[71] For example, exposure to cigarette smoke can shorten lung telomeres in mice and cell lines, and is associated with shorter blood telomeres in humans.[72–74] In general, if more pulmonary tissue needs to be renewed during life, telomeres will become shorter. The same principle applies to blood cells, which is also relevant since telomere length is usually measured in blood. In this light it is important to note that a correlation between lung and blood telomere length has not unequivocally been shown.[19,32,75] This all makes that associations between telomere length and various diseases cannot easily be interpreted as causative relations. Even so, in the past years a large number of studies have investigated telomere length in relation to a multitude of phenotypes.

With regard to the lung, several studies have focused on ILD. Patients with ILD have shorter telomeres than controls, and patients with IPF have shorter telomeres than patients with non-IPF ILD. Within familial pulmonary fibrosis, patients with TERT mutations have the shortest telomeres. Compared to other ILD, patients with sarcoidosis have relatively long telomeres, albeit still shorter than controls.[68] Patients with COPD have shorter telomeres than non-smoking and smoking controls, and non-COPD smokers have shorter telomeres than non-smokers.[76] Asthma patients were also found to have shorter telomere length than controls, though this was limited to patients with life-course persistent asthma in one study.[77,78] Telomere length has not been studied in patients with HAPE. In lung transplant

patients, short donor telomere length seems to be associated with the development of chronic lung allograft disease (CLAD),[79] although other studies did not find this association.[80] Short recipient telomere length was associated with the development of CLAD in one large study,[81] but not in other studies.[79,80]

At first, it was thought that lung cancer patients have shorter telomeres than controls. Larger and more recent studies, on the other hand, indicate that lung cancer patients have longer telomeres compared to controls.[82–84] This also fits with the observed associations between lung cancer and SNPs in telomere genes that are associated with longer telomeres.[53,57] These findings suggest a disease model where short telomeres can lead to degenerative diseases, whereas long telomeres lead to malignant disease.[85]

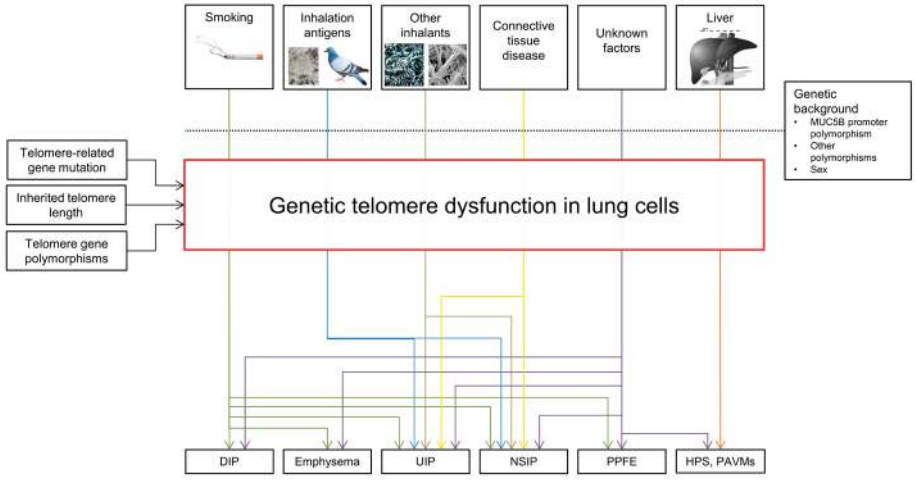


Figure 3: Disease model of pulmonary disease caused by variance in telomere-related genes. Environmental triggers or comorbidity, in combination with telomere dysfunction in lung cells leads to development of pulmonary disease. The specific phenotype is dependent on the trigger involved, and on the genetic background of an individual. HP: hypersensitivity pneumonitis; HPS: hepatopulmonary syndrome; NSIP: non-specific interstitial pneumonia; PAVMs: pulmonary arteriovenous malformations; PPFE: pleuroparenchymal fibroelastosis; UIP: usual interstitial pneumonia

DISEASE MODEL FOR PULMONARY DISEASE CAUSED BY GENETIC VARIATION IN TELOMERE-RELATED GENES

The above findings show that telomeres play an important role in the development of various types of pulmonary disease. In case of genetic mutations and variance in common genetic variants, a causative role for telomere biology in the development of pulmonary disease can be inferred. However, not all associations between telomere length and pulmonary disease indicate a causative relation. The current state of knowledge points to a

pathogenetic model where telomere shortening, which is influenced by common genetic variants, rare genetic mutations, and inherited telomere length, can lead to pulmonary disease dependent on environmental factors, comorbid conditions, and the general genetic background of an individual (Figure 3). This model applies to at least IIP, pulmonary emphysema and vascular malformations. For other diseases, such as asthma and HAPE, pathophysiological relations with telomere dysfunction have yet to be confirmed.

The best illustration of this model is IPF, as telomere dysfunction in disease pathogenesis has been studied most extensively there. In both the familial and sporadic form of IPF genomic mutations in telomere-related genes have been identified and related to increased telomere shortening. IPF GWAS have also identified a common promoter polymorphism in the *MUC5B* gene (a non-telomere-related gene) as an important risk factor for developing disease.[86] Moreover, the risk MUC5B allele is also significantly more common in IPF patients with telomere gene mutations,[23,28,87] illustrating the role of the patients' genetic background in development of the pulmonary phenotype associated with telomere mutations. Environmental factors, smoking and fibrogenic exposures are important risk factors for IPF, and a majority of patients (up to 80%) are current or former smokers.[88] In patients with *TERT* or *TERC* mutations, this percentage was 55.2%. Additionally, fibrogenic exposure was reported in 42.1% of patients and either smoking or fibrogenic exposure in 73.6%.[23] In another study, 96% of pulmonary fibrosis patients with a *TERT* mutation reported a history of smoking or exposure to other fibrinogens (ever smoker 63%, fibrogenic exposure 71%).[21] Patients with *RTEL1* mutations who had smoked or had other fibrinogenic exposure (or both) presented at an earlier age compared to patients with no known exposures.[15]

For other pulmonary diseases the contributing factors are less clear. Most forms of pulmonary disease related to exposure, as well as a pulmonary disease related to autoimmune disease, can cause various pulmonary phenotypes in combination with telomere dysfunction. In these cases, the genetic background of an individual likely determines the precise phenotype. However, genetic factors that are strongly associated with non-IPF diagnoses have not yet been identified.

SPECIFIC TREATMENT FOR PULMONARY PHENOTYPES OF TELOMERE GENE MUTATIONS

When a group of diseases is caused by the same underlying mechanism, this gives the opportunity for specific treatments that affect that mechanism. Potentially, telomere-targeted treatment can be used for various pulmonary diseases caused by telomere gene mutations. These drugs are not yet available, but they would provide a unique opportunity

in IPF patients.[89] There is currently no curative treatment for IPF (except lung transplantation), and patients with telomere-related disease have a worse prognosis. The safety and efficacy of antifibrotic drugs has not specifically been evaluated in patients with genetic forms of pulmonary fibrosis. Telomere-related forms of other fibrotic ILD all have a similarly bad prognosis as IPF, indicating that the efficacy of standard treatment is low.[22] Treatment efficacy in COPD patients with mutations in telomere-related genes has not been studied.

Danazol, a synthetic sex hormone with androgenic properties, showed promise for pulmonary fibrosis associated with telomere disease, with stabilization of diffusing capacity of the lung for CO (DLCO), FVC and CT scan findings during a 2-year treatment period.[90] Moreover, treatment with danazol was associated with telomere elongation and hematological response in 79% of the cases (19/24). However, this drug is poorly tolerated in many patients, and has potential liver adverse effects and increased risk of venous thrombosis. Other drugs targeting telomere homeostasis are being evaluated. Original *in vitro* studies also demonstrated that *TERC, PARN or DKC1* deficiency could be reversed by posttranscriptional manipulations suggesting a potential therapeutic strategy.[91,92] In the future, specific telomere-targeting drugs will likely become available. These would be a unique therapeutic opportunity in patients with IPF, as current therapies do not treat the cause of the disease (i.e. telomere dysfunction (in part of the patients)), but the fibrotic process in itself.[89] These telomere-targeting drugs might be of use in patients with other types of pulmonary fibrosis and emphysema caused by telomere disease as well. On the other side of the spectrum, several promising telomere-targeting drugs for the treatment of various malignancies are currently under investigation, and might become of use in lung cancer patients.[89]

Given the young age of most patients with IPF caused by telomere gene mutations, lung transplantation is often discussed. This may explain the high frequency of telomere-related gene mutations in a cohort of sporadic IPF patients who underwent lung transplantation reported by Petrovski and colleagues.[28] Three retrospective series reported the outcome of lung transplantation in 26 patients with *TERT* and *TERC* mutations.[48–50] Almost all patients required adjustment of immunosuppression because of hematological toxicity. Thrombocytopenia and a need for platelet transfusion were frequent, and myelodysplastic syndrome and/or bone marrow failure occurred in some patients. Acute kidney failure requiring dialysis support seemed unexpectedly frequent in two series (0-50%). Prevalence of infections and acute/chronic rejections did not differ from historical data, but there was no control group.[48–50]

GENETIC SCREENING FOR TELOMERE-RELATED GENE MUTATIONS

As pulmonary fibrosis is the most common pulmonary manifestation of a telomere-related gene mutation, it has been discussed recently if and when pulmonary fibrosis patients should undergo genetic testing for mutations in telomere-related genes. In line with other authors,[93,94] we would now recommend screening for telomere-related gene mutations in pulmonary fibrosis in the following situations: any case of familial pulmonary fibrosis; any case of pulmonary fibrosis in the context of personal or familial features suggestive of a telomere syndrome; or before lung transplantation. As the frequency of telomere-related gene mutations is lower in non-fibrotic pulmonary disease, and the role of telomere dysfunction in these diseases needs to be better studied, we only recommend genetic screening in these patients in case of personal or family features suggestive of a telomere syndrome.

CONCLUSION

Genomic mutations in seven telomere-related genes cause pulmonary disease. Pulmonary phenotypes associated with these mutations range from different forms of pulmonary fibrosis (most commonly IPF) to emphysema and pulmonary vascular disease. Telomere-related mutations account for up to 10% of sporadic IPF, 25% of familial IPF, 10% of connective-tissue disease-associated interstitial lung disease, and 1% of COPD. The frequency of telomere-related gene mutations in other pulmonary phenotypes has not yet been studied. There regularly is overlap between different forms of pulmonary disease in the same patient, and different types of pulmonary disease can be found within families that carry telomere-related gene mutations. Furthermore, SNPs in *TERT*, *TERC*, *OBFC1* and *RTEL1*, have been associated with pulmonary disease, and some of these variants have been associated with short telomere length. Short telomere length in itself has also been associated with many types of pulmonary disease, but causal relations have not been established for asthma, CLAD, and sarcoidosis. Genotype-phenotype relations are weak, suggesting that environmental factors and genetic background of patients determine disease phenotypes to a large degree. In patients with pulmonary fibrosis and a telomere-related gene mutation, the prognosis seems to be worse compared to other patients regardless of the precise diagnosis. All these findings point to a disease model where genomic variation in telomere-related genes can cause pulmonary disease when triggered by environmental exposure, comorbidity, or unknown factors. The pulmonary phenotype then depends on the nature of the trigger, as well as genetic background of the individual. At present, treatment of pulmonary disease caused by telomere-related gene

variation is based on disease diagnosis and not on the underlying cause, but telomere-targeting therapies are currently under development.

REFERENCES

1. Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet*. 2008;42:301-334. doi:10.1146/annurev.genet.41.110306.130350 [doi]
2. Levy MZ, Allsopp RC, Futcher AB, Greider CW, Harley CB. Telomere end-replication problem and cell aging. *J Mol Biol*. 1992;225(4):951-960. doi:0022-2836(92)90096-3
3. Schmidt JC, Cech TR. Human telomerase: biogenesis, trafficking, recruitment, and activation. *Genes Dev*. 2015;29(11):1095-1105. doi:10.1101/gad.263863.115 [doi]
4. Daniali L, Benetos A, Susser E, et al. Telomeres shorten at equivalent rates in somatic tissues of adults. *Nat Commun*. 2013;4:1597. doi:10.1038/ncomms2602 [doi]
5. Shay JW, Wright WE. Senescence and immortalization: role of telomeres and telomerase. *Carcinogenesis*. 2005;26(5):867-874. doi:10.1093/carcin/bgh296
6. Armanios M, Blackburn EH. The telomere syndromes. *Nat Rev*. 2012;13(10):693-704. doi:10.1038/nrg3246 [doi]
7. Holohan B, Wright WE, Shay JW. Cell biology of disease: Telomeropathies: An emerging spectrum disorder. *J Cell Biol*. 2014;205(3):289-299. doi:10.1083/jcb.201401012 [doi]
8. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med*. 2007;356(13):1317-1326. doi:10.1056/NEJMoa066157
9. Tsakiri KD, Cronkhite JT, Kuan PJ, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci U S A*. 2007;104(18):7552-7557. doi:10.1073/pnas.0701009104
10. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med*. 2013;188(6):733-748. doi:10.1164/rccm.201308-1483ST [doi]
11. Nalysnyk L, Cid-Ruzafa J, Rotella P, Esser D. Incidence and prevalence of idiopathic pulmonary fibrosis: review of the literature. *Eur Respir Rev*. 2012;21(126):355-361. doi:10.1183/09059180.00002512 [doi]
12. Armanios M. Telomerase and idiopathic pulmonary fibrosis. *Mutat Res*. 2012;730(1-2):52-58. doi:10.1016/j.mrfmmm.2011.10.013 [doi]
13. Alder JK, Stanley SE, Wagner CL, Hamilton M, Hanumanthu VS, Armanios M. Exome sequencing identifies mutant TINF2 in a family with pulmonary fibrosis. *Chest*. December 2014. doi:10.1378/chest.14-1947 [doi]
14. Kropski JA, Mitchell DB, Markin C, et al. A novel dyskerin (DKC1) mutation is associated with Familial Interstitial Pneumonia. *Chest*. February 2014. doi:10.1378/chest.13-2224 [doi]
15. Kannengiesser C, Borie R, Menard C, et al. Heterozygous RTEL1 mutations are associated with familial pulmonary fibrosis. *Eur Respir J*. May 2015;46(2):474-485. doi:10.1183/09031936.00040115
16. Cogan JD, Kropski JA, Zhao M, et al. Rare Variants in RTEL1 are Associated with Familial Interstitial Pneumonia. *Am J Respir Crit Care Med*. January 2015. doi:10.1164/rccm.201408-1510OC [doi]
17. Stuart BD, Choi J, Zaidi S, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. *Nat Genet*. 2015;47(5):512-517. doi:10.1038/ng.3278 [doi]

18. Stanley SE, Gable DL, Wagner CL, et al. Loss-of-function mutations in the RNA biogenesis factor NAF1 predispose to pulmonary fibrosis-emphysema. *Sci Transl Med*. 2016;8(351):351ra107. doi:10.1126/scitranslmed.aaf7837 [doi]

19. Alder JK, Chen JJ, Lancaster L, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proc Natl Acad Sci U S A*. 2008;105(35):13051-13056. doi:10.1073/pnas.0804280105 [doi]

20. Mushiroda T, Wattanapokayakit S, Takahashi A, et al. A genome-wide association study identifies an association of a common variant in TERT with susceptibility to idiopathic pulmonary fibrosis. *J Med Genet*. 2008;45(10):654-656. doi:10.1136/jmg.2008.057356 [doi]

21. Diaz de Leon A, Cronkhite JT, Katzenstein AL, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. *PLoS One*. 2010;5(5):e10680. doi:10.1371/journal.pone.0010680 [doi]

22. Newton CA, Batra K, Torrealba J, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J*. 2016;48(6):1710-1720. doi:10.1183/13993003.00308-2016 [doi]

23. Borie R, Tabeze L, Thabut G, et al. Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. *Eur Respir J*. 2016;48(6):1721-1731. doi:10.1183/13993003.02115-2015 [doi]

24. Dai J, Cai H, Li H, et al. Association between telomere length and survival in patients with idiopathic pulmonary fibrosis. *Respirology*. 2015;20(6):947-952. doi:10.1111/resp.12566 [doi]

25. George G, Rosas IO, Cui Y, et al. Short telomeres, telomeropathy, and subclinical extrapulmonary organ damage in patients with interstitial lung disease. *Chest*. 2015;147(6):1549-1557. doi:10.1378/chest.14-0631

26. Hoffman TW, van der Vis JJ, van Oosterhout MF, et al. TINF2 Gene Mutation in a Patient with Pulmonary Fibrosis. *Case Rep Pulmonol*. 2016;2016:1310862. doi:10.1155/2016/1310862 [doi]

27. Kropski JA, Reiss S, Markin C, et al. Rare Genetic Variants in PARN Are Associated with Pulmonary Fibrosis in Families. *Am J Respir Crit Care Med*. 2017;196(11):1481-1484. doi:10.1164/rccm.201703-0635LE [doi]

28. Petrovski S, Todd JL, Durheim MT, et al. An Exome Sequencing Study to Assess the Role of Rare Genetic Variation in Pulmonary Fibrosis. *Am J Respir Crit Care Med*. January 2017. doi:10.1164/rccm.201610-2088OC [doi]

29. Hisata S, Sakaguchi H, Kanegane H, et al. A Novel Missense Mutation of DKC1 In Dyskeratosis Congenita With Pulmonary Fibrosis. *Sarcoidosis, Vasc Diffus lung Dis Off J WASOG*. 2013;30(3):221-225.

30. Newton CA, Batra K, Torrealba J, Meyer K, Raghu G, Garcia CK. Pleuroparenchymal fibroelastosis associated with telomerase reverse transcriptase mutations. *Eur Respir J*. 2017;49(5):17006962017. doi:10.1183/13993003.00696-2017

31. Nunes H, Jeny F, Bouvry D, et al. Pleuroparenchymal fibroelastosis associated with telomerase reverse transcriptase mutations. *Eur Respir J*. 2017;49(5):1602022. doi:10.1183/13993003.02022-2016.

32. Kropski JA, Pritchett JM, Zoz DF, et al. Extensive phenotyping of individuals at risk for familial interstitial pneumonia reveals clues to the pathogenesis of interstitial lung disease. *Am J Respir Crit Care Med*. 2015;191(4):417-426. doi:10.1164/rccm.201406-1162OC [doi]

33. Juge PA, Borie R, Kannengiesser C, et al. Shared genetic predisposition in rheumatoid arthritis-interstitial lung disease and familial pulmonary fibrosis. *Eur Respir J*. 2017;49(5):1602314. doi:10.1183/13993003.02314-2016

34. Alder JK, Guo N, Kembou F, et al. Telomere length is a determinant of emphysema susceptibility. *Am J Respir Crit Care Med*. 2011;184(8):904-912. doi:10.1164/rccm.201103-0520OC [doi]

35. Nunes H, Monnet I, Kannengiesser C, et al. Is telomeropathy the explanation for combined pulmonary fibrosis and emphysema syndrome?: report of a family with TERT mutation. *Am J Respir Crit Care Med*. 2014;189(6):753-754. doi:10.1164/rccm.201309-1724LE [doi]

36. Stanley SE, Chen JJ, Podlevsky JD, et al. Telomerase mutations in smokers with severe emphysema. *J Clin Invest*. 2015;125(2):563-570. doi:10.1172/JCI78554 [doi]

37. Khincha PP, Bertuch AA, Agarwal S, et al. Pulmonary arteriovenous malformations: an uncharacterised phenotype of dyskeratosis congenita and related telomere biology disorders. *Eur Respir J*. 2017;49(1):1601640. doi:10.1183/13993003.01640-2016.

38. Gorgy AI, Jonassaint NL, Stanley SE, et al. Hepatopulmonary syndrome is a frequent cause of dyspnea in the short telomere disorders. *Chest*. 2015;148(4):1019-1026. doi:10.1378/chest.15-0825

39. Karimi-Shah BA, Chowdhury BA. Forced vital capacity in idiopathic pulmonary fibrosis--FDA review of pirfenidone and nintedanib. *N Engl J Med*. 2015;372(13):1189-1191. doi:10.1056/NEJMp1500526 [doi]

40. El-Chemaly S, Ziegler SG, Calado RT, et al. Natural history of pulmonary fibrosis in two subjects with the same telomerase mutation. *Chest*. 2011;139(5):1203-1209. doi:10.1378/chest.10-2048

41. Stanley SE, Merck SJ, Armanios M. Telomerase and the Genetics of Emphysema Susceptibility. Implications for Pathogenesis Paradigms and Patient Care. *Ann Am Thorac Soc*. 2016;13(Supplement_5):S447-S451. doi:10.1513/AnnalsATS.201609-718AW [doi]

42. Jacob J, Bartholmai BJ, Rajagopalan S, et al. Likelihood of pulmonary hypertension in patients with idiopathic pulmonary fibrosis and emphysema. *Respirology*. December 2017. doi:10.1111/resp.13231 [doi]

43. Cottin V. Combined pulmonary fibrosis and emphysema: bad and ugly all the same? *Eur Respir J*. 2017;50(1):1700846. doi:10.1183/13993003.00846-2017

44. Solder B, Weiss M, Jager A, Belohradsky BH. Dyskeratosis congenita: multisystemic disorder with special consideration of immunologic aspects. A review of the literature. *Clin Pediatr (Phila)*. 1998;37(9):521-530. doi:10.1177/000992289803700901 [doi]

45. Jyonouchi S, Forbes L, Ruchelli E, Sullivan KE. Dyskeratosis congenita: a combined immunodeficiency with broad clinical spectrum--a single-center pediatric experience. *Pediatr Allergy Immunol*. 2011;22(3):313-319. doi:10.1111/j.1399-3038.2010.01136.x [doi]

46. Bousfiha A, Jeddane L, Picard C, et al. The 2017 IUIS Phenotypic Classification for Primary Immunodeficiencies. *J Clin Immunol*. 2018;38(1):129-143. doi:10.1007/s10875-017-0465-8 [doi]

47. Borie R, Kannengiesser C, Sicre de Fontbrune F, et al. Pneumocystosis revealing immunodeficiency secondary to TERC mutation. *Eur Respir J*. 2017;50(5):1701443. doi:10.1183/13993003.01443-2017

48. Silhan LL, Shah PD, Chambers DC, et al. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. *Eur Respir J*. 2014;44(1):178-187. doi:10.1183/09031936.00060014 [doi]

49. Borie R, Kannengiesser C, Hirschi S, et al. Severe hematologic complications after lung transplantation in patients with telomerase complex mutations. *J Heart Lung Transplant*. 2015;34(4):538-546. doi:10.1016/j.healun.2014.11.010 [doi]

50. Tokman S, Singer JP, Devine MS, et al. Clinical outcomes of lung transplant recipients with telomerase mutations. *J Heart Lung Transplant*. 2015;34(10):1318-1324. doi:10.1016/j.healun.2015.05.002 [doi]

51. Fingerlin TE, Murphy E, Zhang W, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. *Nat Genet*. 2013;45(6):613-620. doi:10.1038/ng.2609 [doi]

52. Wei R, Li C, Zhang M, et al. Association between MUC5B and TERT polymorphisms and different interstitial lung disease phenotypes. *Transl Res*. 2014;163(5):494-502. doi:10.1016/j.trsl.2013.12.006 [doi]

53. Nie W, Zang Y, Chen J, Xiu Q. TERT rs2736100 polymorphism contributes to lung cancer risk: a meta-analysis including 49,869 cases and 73,464 controls. *Tumour Biol*. 2014;35(6):5569-5574. doi:10.1007/s13277-014-1734-2 [doi]

54. Codd V, Nelson CP, Albrecht E, et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet*. 2013;45(4):422-427, 427e1-2. doi:10.1038/ng.2528 [doi]

55. Bojesen SE, Pooley KA, Johnatty SE, et al. Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. *Nat Genet*. 2013;45(4):371-384, 384e1-2. doi:10.1038/ng.2566 [doi]

56. Cao JL, Yuan P, Abuduwufuer A, Lv W, Yang YH, Hu J. Association between the TERT Genetic Polymorphism rs2853676 and Cancer Risk: Meta-Analysis of 76,108 Cases and 134,215 Controls. *PLoS One*. 2015;10(6):e0128829. doi:10.1371/journal.pone.0128829 [doi]

57. McKay JD, Hung RJ, Han Y, et al. Large-scale association analysis identifies new lung cancer susceptibility loci and heterogeneity in genetic susceptibility across histological subtypes. *Nat Genet*. 2017;49(7):1126-1132. doi:10.1038/ng.3892 [doi]

58. Prescott J, Kraft P, Chasman DI, et al. Genome-wide association study of relative telomere length. *PLoS One*. 2011;6(5):e19635. doi:10.1371/journal.pone.0019635 [doi]

59. Ding Y, Xu H, Yao J, et al. Association between RTEL1 gene polymorphisms and COPD susceptibility in a Chinese Han population. *Int J Chron Obstruct Pulmon Dis*. 2017;12:931-936. doi:10.2147/COPD.S131246 [doi]

60. Rong H, He X, Zhu L, et al. Association between regulator of telomere elongation helicase1 (RTEL1) gene and HAPE risk: A case-control study. *Medicine (Baltimore)*. 2017;96(39):e8222. doi:10.1097/MD.0000000000008222 [doi]

61. Claar DD, Larkin EK, Bastarache L, et al. A Phenome-Wide Association Study Identifies a Novel Asthma Risk Locus Near TERC. *Am J Respir Crit Care Med*. 2016;193(1):98-100. doi:10.1164/rccm.201507-1267LE [doi]

62. Peljto AL, Selman M, Kim DS, et al. The MUC5B promoter polymorphism is associated with idiopathic pulmonary fibrosis in a Mexican cohort but is rare among Asian ancestries. *Chest*. 2015;147(2):460-464. doi:10.1378/chest.14-0867

63. Ramasamy A, Kuokkanen M, Vedantam S, et al. Genome-wide association studies of asthma in population-based cohorts confirm known and suggested loci and identify an additional association near HLA. *PLoS One*. 2012;7(9):e44008. doi:10.1371/journal.pone.0044008 [doi]

64. Hobbs BD, de Jong K, Lamontagne M, et al. Genetic loci associated with chronic obstructive pulmonary disease overlap with loci for lung function and pulmonary fibrosis. *Nat Genet*. 2017;49(3):426-432. doi:10.1038/ng.3752 [doi]

65. Bhagi S, Srivastava S, Singh SB. High-altitude pulmonary edema: review. *J Occup Health*. 2014;56(4):235-243. doi:10.1539/joh.13-0256-ra

66. He Y, Zhang X, Li X, et al. Telomere length-related gene ACYP2 polymorphism is associated with the risk of HAPE in Chinese Han population. *J Gene Med*. 2016;18(9):244-249. doi:10.1002/jgm.2896 [doi]

67. Zhu L, Liu L, He X, et al. Association between genetic polymorphism of telomere-associated gene ACYP2 and the risk of HAPE among the Chinese Han population: A Case-control study. *Medicine (Baltimore)*. 2017;96(13):e6504. doi:10.1097/MD.0000000000006504 [doi]

68. Snetselaar R, van Moorsel CH, Kazemier KM, et al. Telomere length in interstitial lung diseases. *Chest*. 2015;148(4):1011-1018. doi:10.1378/chest.14-3078 [doi]

69. Alder JK, Cogan JD, Brown AF, et al. Ancestral mutation in telomerase causes defects in repeat addition processivity and manifests as familial pulmonary fibrosis. *PLoS Genet*. 2011;7(3):e1001352. doi:10.1371/journal.pgen.1001352 [doi]

70. Collopy LC, Walne AJ, Cardoso S, et al. Triallelic and epigenetic-like inheritance in human disorders of telomerase. *Blood*. 2015;126(2):176-184. doi:10.1182/blood-2015-03-633388 [doi]

71. Bar C, Blasco MA. Telomeres and telomerase as therapeutic targets to prevent and treat age-related diseases. *F1000Research*. 2016;5:10.12688/f1000research.7020.1. eCollection 2016. doi:10.12688/f1000research.7020.1 [doi]

72. Huang J, Okuka M, McLean M, Keefe DL, Liu L. Telomere susceptibility to cigarette smoke-induced oxidative damage and chromosomal instability of mouse embryos in vitro. *Free Radic Biol Med*. 2010;48(12):1663-1676. doi:10.1016/j.freeradbiomed.2010.03.026 [doi]

73. Huang J, Okuka M, Lu W, et al. Telomere shortening and DNA damage of embryonic stem cells induced by cigarette smoke. *Reprod Toxicol*. 2013;35:89-95. doi:10.1016/j.reprotox.2012.07.003 [doi]

74. Astuti Y, Wardhana A, Watkins J, Wulaningsih W, Network PR. Cigarette smoking and telomere length: A systematic review of 84 studies and meta-analysis. *Environ Res*. 2017;158:480-489. doi:10.1016/j.envres.2017.06.038

75. Snetselaar R, van Batenburg AA, van Oosterhout MFM, et al. Short telomere length in IPF lung associates with fibrotic lesions and predicts survival. *PLoS One*. 2017;12(12):e0189467. doi:10.1371/journal.pone.0189467 [doi]

76. Rode L, Bojesen SE, Weischer M, Vestbo J, Nordestgaard BG. Short telomere length, lung function and chronic obstructive pulmonary disease in 46,396 individuals. *Thorax*. 2013;68(5):429-435. doi:10.1136/thoraxjnl-2012-202544 [doi]

77. Belsky DW, Shalev I, Sears MR, et al. Is chronic asthma associated with shorter leukocyte telomere length at midlife? *Am J Respir Crit Care Med*. 2014;190(4):384-391. doi:10.1164/rccm.201402-0370OC [doi]

78. Albrecht E, Sillanpaa E, Karrasch S, et al. Telomere length in circulating leukocytes is associated with lung function and disease. *Eur Respir J*. 2014;43(4):983-992. doi:10.1183/09031936.00046213 [doi]

79. Faust HE, Golden JA, Rajalingam R, et al. Short Lung Transplant Donor Telomere Length is Associated with Decreased CLAD-Free Survival. *Thorax*. 2017;72(11):1052. doi:10.1136/THORAXJNL-2016-209897

80. Courtwright AM, Fried S, Villalba JA, et al. Association of Donor and Recipient Telomere Length with Clinical Outcomes following Lung Transplantation. *PLoS One*. 2016;11(9):e0162409. doi:10.1371/journal.pone.0162409 [doi]

81. Newton CA, Kozlitina J, Lines JR, Kaza V, Torres F, Garcia CK. Telomere length in patients with pulmonary fibrosis associated with chronic lung allograft dysfunction and post-lung transplantation survival. *J Heart Lung Transplant*. 2017;36(8):845-853. doi:10.1016/j.healun.2017.02.005

82. Seow WJ, Cawthon RM, Purdue MP, et al. Telomere length in white blood cell DNA and lung cancer: a pooled analysis of three prospective cohorts. *Cancer Res*. 2014;74(15):4090-4098. doi:10.1158/0008-5472.CAN-14-0459 [doi]

83. Machiela MJ, Hsiung CA, Shu XO, et al. Genetic variants associated with longer telomere length are associated with increased lung cancer risk among never-smoking women in Asia: a report from the female lung cancer consortium in Asia. *Int J cancer*. 2015;137(2):311-319. doi:10.1002/ijc.29393 [doi]

84. de-Torres JP, Sanchez-Salcedo P, Bastarrika G, et al. Telomere length, COPD and emphysema as risk factors for lung cancer. *Eur Respir J*. 2017;49(1):1601521. doi:10.1183/13993003.01521-2016

85. Stanley SE, Armanios M. The short and long telomere syndromes: paired paradigms for molecular medicine. *Curr Opin Genet Dev*. 2015;33:1-9. doi:10.1016/j.gde.2015.06.004 [doi]

86. Seibold MA, Wise AL, Speer MC, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1503-1512. doi:10.1056/NEJMoa1013660 [doi]

87. 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*. 2016;21(4):712-717. doi:10.1111/resp.12728 [doi]

88. Oh CK, Murray LA, Molfino NA. Smoking and idiopathic pulmonary fibrosis. *Pulm Med*. 2012;2012:808260. doi:10.1155/2012/808260 [doi]

89. Martinez P, Blasco MA. Telomere-driven diseases and telomere-targeting therapies. *J Cell Biol*. 2017;216(4):875-887. doi:10.1083/jcb.201610111 [doi]

90. Townsley DM, Dumitriu B, Liu D, et al. Danazol Treatment for Telomere Diseases. *N Engl J Med*. 2016;374(20):1922-1931. doi:10.1056/NEJMoa1515319 [doi]

91. Shukla S, Schmidt JC, Goldfarb KC, Cech TR, Parker R. Inhibition of telomerase RNA decay rescues telomerase deficiency caused by dyskerin or PARN defects. *Nat Struct Mol Biol*. 2016;23(4):286-292. doi:10.1038/nsmb.3184 [doi]

92. Boyraz B, Moon DH, Segal M, et al. Posttranscriptional manipulation of TERC reverses molecular hallmarks of telomere disease. *J Clin Invest*. 2016;126(9):3377-3382. doi:10.1172/JCI87547 [doi]

93. Kropski JA, Young LR, Cogan JD, et al. Genetic Evaluation and Testing of Patients and Families with Idiopathic Pulmonary Fibrosis. *Am J Respir Crit Care Med*. 2017;195(11):1423-1428. doi:10.1164/rccm.201609-1820PP [doi]

94. Borie R, Kannengiesser C, Debray MP, Crestani B. The Genetic Diagnosis of Interstitial Lung Disease: A Need for an International Consensus. *Am J Respir Crit Care Med*. 2017;195(11):1538-1539. doi:10.1164/rccm.201611-2209LE [doi]

Chapter 7

Pulmonary fibrosis linked to variants in the *ACD* gene, encoding the telomere protein TPP1

T.W. Hoffman, J.J van der Vis, J.J. van der Smagt, M.P.G. Massink, J.C. Grutters, and C.H.M. van Moorsel

ABSTRACT

Background:

Disease-causing mutations in telomere-related genes are identified in 10-25% of patients with sporadic and familial idiopathic pulmonary fibrosis (IPF). Mutations in the *ACD* gene, encoding the telomere protein TPP1, have been identified in patients with other manifestations of a telomere syndrome, but not in patients with IPF.

Methods:

Sixty patients with pulmonary fibrosis underwent genetic screening through the use of whole exome sequencing, filtered for variants in genes related to telomere syndromes or pulmonary fibrosis.

Results:

Potentially disease-causing variants were identified in three patients. The first patient was a 33-year-old female who also had bone marrow failure, in whom a pathogenic mutation in *ACD* was identified. The second patient was a 62-year-old male who had a family history of pulmonary fibrosis, in whom a likely pathogenic mutation in *ACD* was identified. The third patient was a 57-year-old male who also had liver cirrhosis, in whom a variant of unknown significance in *ACD* was identified. All patients had leukocyte telomere length below the first percentile for age.

Conclusions:

Pulmonary fibrosis is associated with mutations in the *ACD* gene, encoding the telomere protein TPP1. Potentially disease-causing variants were found in 5% of a cohort of 60 unrelated patients with pulmonary fibrosis who were referred for genetic screening.

INTRODUCTION

Telomeres are a repetitive DNA sequence at the ends of chromosomes. Telomeres shorten with every cell division, and thus become increasingly short with age. Stable telomeres are necessary for cellular survival, and critically short or dysfunctional telomeres lead to cellular senescence or apoptosis. Mutations in genes encoding telomere-associated proteins can lead to increased telomere shortening or telomere dysfunction.[1] These mutations can cause various disease manifestations, which are termed telomere syndromes.[1]

Disease-causing mutations in genes encoding telomere-associated proteins, most commonly *TERT*, *TERC*, *RTEL1* and *PARN*, have been identified in up to 11% of patients with sporadic idiopathic pulmonary fibrosis, and in up to 25% of patients with familial pulmonary fibrosis (≥2 first-degree relatives with pulmonary fibrosis).[2–4] Here we studied the *ACD* gene, which encodes the telomere protein TPP1, in patients with pulmonary fibrosis.

PATIENTS AND METHODS

Between October 2017 and December 2018 60 unrelated patients with pulmonary fibrosis from St. Antonius Hospital in Nieuwegein, a referral center for patients with interstitial lung diseases in The Netherlands, underwent genetic screening. Screening was offered in case of a family history of pulmonary fibrosis, clinical suspicion of a telomere syndrome, or relatively young age at presentation (44, 9, and 7 patients, respectively). Clinical suspicion of a telomere syndrome was based on haematological abnormalities such as bone marrow failure or anaemia in 6 patients and liver cirrhosis in 3 patients. The cohort included 18 females, and the median age at the time of genetic screening was 69 years (range 33 - 84).

DNA was isolated from blood samples. Exome sequencing and processing of the data were performed at the University Medical Center Utrecht. After enrichment of the exome with the Agilent SureSelect CREV2 kit (Agilent Technologies, Santa Clara, California, USA), whole exome sequencing was performed on an Illumina Novaseq 6000 sequencer. The Illumina sequencing data was processed with the in-house pipeline, IAP v2.6.1, including GATK v3.4-46, according to the best practices guidelines.[5] Results were filtered for exonic variants with a population frequency below 0.5% in 36 genes related to telomere syndromes or pulmonary fibrosis (*ABCA3*, *ACD, AP3B1, CSF2RA, CSF2RB, CTC1, DKC1, FAM111B, HPS1, HPS4, ITGA4, LIG4, MARS, NAF1, NKX2-1, NOP10, PARN, POT1, RNF168, RTEL1, SAMD9L, SFTPA1, SFTPA2, SFTPB, SFTPC, SFTPD, STN1, TEN1, TERC, TERF1, TERF2, TERT, TINF2, TMEM173, USB1,* and *WRAP53)*. Leukocyte telomere length was measured using previously described methods.[6] Briefly, telomere length was estimated for each

sample from the ratio of telomere repeat copy number to a single gene (human β-globin gene) copy number (T/S ratio). Measurements were performed on the Bio-Rad CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Reference values were derived from a cohort of 164 healthy controls. Pulmonary fibrosis diagnoses were made after multidisciplinary discussion, in accordance with the 2018 ATS/ERS/JTS/ALAT guideline.[7] All patients gave written informed consent in research project R05-08A.

RESULTS

In 19 patients potentially disease-causing variants in one or more telomere genes were found, including four patients with pathogenic mutations in *TERT* and four patients with pathogenic mutations in *RTEL1*. Potentially disease-causing variants in *ACD* were identified in three patients (Figure 1). The first patient was a 33-year-old female referred to our centre because of dyspnoea on exertion. She had been diagnosed with bone marrow failure at age 25 and had also experienced several transient episodes of gastro-intestinal mucositis, which had not led to a definitive diagnosis, and had improved without specific treatment. She reported having grey hair since she was 21 years old. The family history was only available for her mother and sister, and was not suggestive of a telomere syndrome (Figure 1A). She had a smoking history of eight packyears, but no other fibrogenic exposures. On physical examination, reticular hyperpigmentation of the skin on the neck was noted, but no oral leucoplakia or nail dystrophy. No hepatic abnormalities were seen on abdominal ultrasound. A high-resolution CT-scan (HRCT) of the thorax showed pulmonary fibrosis with honeycombing, but inconsistent with a Usual Interstitial Pneumonia (UIP) pattern. (Figure 1B) The patient was given a working diagnosis of pulmonary fibrosis in the context of a telomere syndrome. Genetic analysis revealed a heterozygous deletion in *ACD*: c.508-510del; p.(Lys170del) (NM_001082486.1), and no abnormalities in the other tested genes.

The second patient was a 62-year-old male who presented with dyspnoea on exertion. His past medical history revealed no clues suggestive of telomere disease, and he reported no early greying of his hair. His family history was notable for pulmonary and autoimmune diseases in several family members, including pulmonary fibrosis in an uncle (Figure 1A). The patient did not report any significant fibrogenic exposures and had a smoking history of two packyears when he was in his twenties. No skin abnormalities were noted on physical examination, and laboratory investigations revealed normal platelet, erythrocyte and leukocyte counts and no macrocytosis. Pulmonary fibrosis with a probable UIP pattern (Figure 1C) was seen on HRCT, and the patient was given a working diagnosis of idiopathic pulmonary fibrosis (IPF). Genetic screening revealed a heterozygous variant in *ACD*: c.508A>G; p.(Lys170Glu), and no abnormalities in the other tested genes.

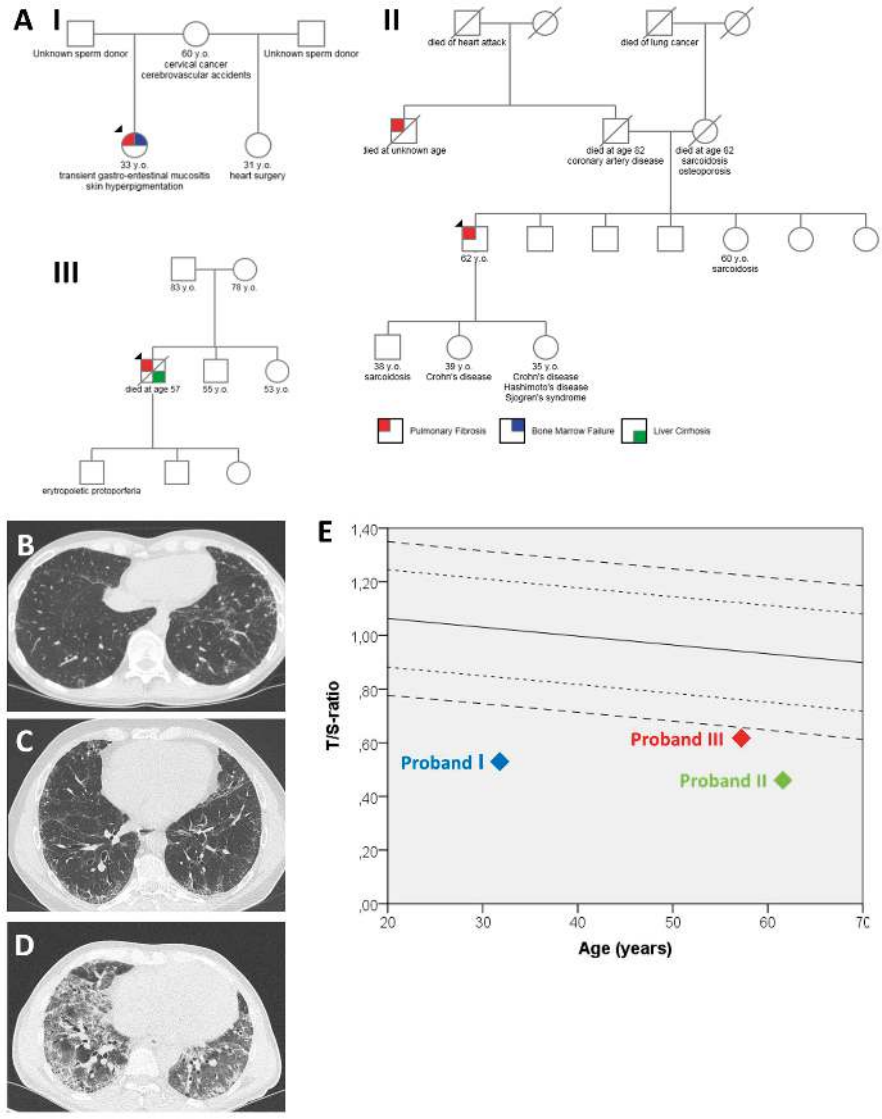


Figure 1: (A) Pedigrees for patients 1-3. Partially filled squares indicate specific diagnoses: red represents pulmonary fibrosis, blue represents bone marrow failure, and green represents liver cirrhosis. (B-D) Chest high-resolution CT-scans for patients 1-3, respectively, showing pulmonary fibrosis in the basal fields. In patient 1, there was bilateral subpleural reticulation and honeycombing, as well as minimal traction bronchiectasis on the left. The pattern was inconsistent with a UIP pattern because of central extension of the fibrosis as well as airtrapping. The scan in patient 2 showed subpleural reticulation, predominantly in the basal fields, but without honeycombing, consistent with a possible UIP pattern. In patient 3 there was subpleural reticulation with traction bronchiectasis predominantly in the lower fields, as well as honeycombing, consistent with a UIP pattern. Groundglass opacities were noted, but this was not the dominant pattern. (E) Leukocyte telomere length in patients 1-3 compared to a cohort of 164 healthy controls. The uninterrupted line represents the 50th percentile for age. The dashed lines represent the 99th, 90th, 10th and 1st percentiles for age.

The third patient was a 57-year-old male who presented with dyspnoea on exertion and chronic cough. His past medical history was notable for cryptogenic liver cirrhosis since age 24, and he reported grey hair since age 30. His family history revealed no additional clues for a telomere syndrome (Figure 1A). The patient had never smoked, but he worked as an electrician and reported exposure to rockwool and dust at work. On physical examination, vitiligo of his fingers, toes and right lower leg was seen, but no other skin abnormalities were noted. Laboratory investigations revealed mild thrombocytopenia, with normal erythrocyte and leukocyte counts and no macrocytosis. Pulmonary fibrosis with honeycombing, consistent with a UIP pattern (Figure 1D), was seen on HRCT. The patient was given a diagnosis of IPF. Genetic screening revealed a heterozygous variant in *ACD*: c.215C>A; p.(Ala72Glu), and no abnormalities in the other tested genes.

Leukocyte telomere length was below the first percentile for age in all three patients (Figure 1E). Blood samples were not available for the family members of patients 1 and 2. However, genetic analysis could be performed in the parents of patient 3, and the patient's 82-year-old father was found to have the same *ACD*-variant. The father had no signs or symptoms suggestive of telomere disease, but did not undergo additional investigations such as lung function testing or CT-scanning.

DISCUSSION

This study shows that pulmonary fibrosis patients can have heterozygous mutations in *ACD*. In 5% of patients with pulmonary fibrosis referred for genetic screening, (potentially) disease-causing variants were present. *ACD* encodes the shelterin component TPP1, which is responsible for telomerase recruitment to telomeres.[8] Disease-causing mutations in *ACD* have been previously identified in patients with aplastic anemia and Hoyeraal-Hreidarsson syndrome, the most severe form of dyskeratosis congenita.[9,10]

The variants that were identified in patients 1 and 2 are located in the OB-fold domain of TPP1, which is the telomerase-interacting domain. The variant identified in patient 3 is located in the N-terminal domain, the function of which is currently not known. The variant in patient 1 is classified as a pathogenic mutation, as the same variant has previously been functionally characterized in patients with a aplastic anemia and Hoyeraal-Hreidarsson syndrome.[9–11] This showed that it disrupts TPP1 binding to telomerase, and the mutation itself is sufficient for causing telomere dysfunction in a human cell line.[12] The phenotype of patient 1 is of interest because it fits well within the concept of a telomere syndrome but is not a classic case of dyskeratosis congenita despite the young age at presentation and involvement of multiple organs. Because of the extremely short telomeres in patients

2 and 3, as well as liver cirrhosis and early greying consistent with a telomere syndrome phenotype in patient 3, a telomere gene mutation would be a likely explanation for their disease. The *ACD*-variants from patient 2 and 3 were absent in the Exome Sequencing Project, 1000 Genomes Project and the Exome Aggregation Consortium. Also, PolyPhen-2 analysis (at http://genetics.bwh.harvard.edu/pph2/index.shtml) predicted both variants to be probably damaging. Of note, it has been suggested that the qPCR technique is less reliable for measuring telomere length than other techniques.[13]

The variant in patient 2 is classified as likely pathogenic,[11] because it concerns the same amino acid, Lys170, as the pathogenic mutation in patient 1. The variant in patient 3 is classified as a variant of unknown significance.[11] The fact that the father of patient 3 was healthy, despite carrying the same *ACD*-variant, would suggest that the variant is not disease-causing. Alternatively, genetic anticipation, where symptoms of a telomere syndrome become more severe and have an earlier onset in each successive generation, occurs in families with telomere syndrome. This allows for incomplete penetrance of telomere syndrome features in the father.[1] Unfortunately, there was no blood sample available for telomere length measurement.

CONCLUSION

In conclusion, this study shows that pulmonary fibrosis is associated with mutations in the *ACD* gene, encoding the telomere protein TPP1. Potentially disease-causing variants were found in 5% of the patients in this cohort, including two mutations classified as pathogenic (3% of patients).

REFERENCES

1. Armanios M, Blackburn EH. The telomere syndromes. *Nat Rev*. 2012;13(10):693-704. doi:10.1038/nrg3246 [doi]
2. Petrovski S, Todd JL, Durheim MT, et al. An Exome Sequencing Study to Assess the Role of Rare Genetic Variation in Pulmonary Fibrosis. *Am J Respir Crit Care Med*. January 2017. doi:10.1164/rccm.201610-2088OC [doi]
3. Borie R, Tabeze L, Thabut G, et al. Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. *Eur Respir J*. 2016;48(6):1721-1731. doi:10.1183/13993003.02115-2015 [doi]
4. Kannengiesser C, Borie R, Menard C, et al. Heterozygous RTEL1 mutations are associated with familial pulmonary fibrosis. *Eur Respir J*. 2015;46(2):474-485. doi: 10.1183/09031936.00040115
5. Van der Auwera GA, Carneiro MO, Hartl C, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinforma*. 2013;43:11.10.1-33. doi:10.1002/0471250953.bi1110s43 [doi]
6. Snetselaar R, van Moorsel CH, Kazemier KM, et al. Telomere length in interstitial lung diseases. *Chest*. 2015;148(4):1011-1018. doi:10.1378/chest.14-3078 [doi]
7. Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2018;198(5):e44-e68. doi:10.1164/rccm.201807-1255ST [doi]
8. Rajavel M, Mullins MR, Taylor DJ. Multiple facets of TPP1 in telomere maintenance. *Biochim Biophys Acta*. 2014;1844(9):1550-1559. doi:10.1016/j.bbapap.2014.04.014 [doi]
9. Guo Y, Kartawinata M, Li J, et al. Inherited bone marrow failure associated with germline mutation of ACD, the gene encoding telomere protein TPP1. *Blood*. 2014;124(18):2767-2774. doi:10.1182/blood-2014-08-596445 [doi]
10. Kocak H, Ballew BJ, Bisht K, et al. Hoyeraal-Hreidarsson syndrome caused by a germline mutation in the TEL patch of the telomere protein TPP1. *Genes Dev*. 2014;28(19):2090-2102. doi:10.1101/gad.248567.114 [doi]
11. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30 [doi]
12. Bisht K, Smith EM, Tesmer VM, Nandakumar J. Structural and functional consequences of a disease mutation in the telomere protein TPP1. *Proc Natl Acad Sci U S A*. 2016;113(46):13021-13026. doi:1605685113
13. Khincha PP, Dagnall CL, Hicks B, et al. Correlation of Leukocyte Telomere Length Measurement Methods in Patients with Dyskeratosis Congenita and in Their Unaffected Relatives. *Int J Mol Sci*. 2017;18(8):1765. doi:10.3390/ijms18081765

Chapter 8

TINF2 gene mutation in a patient with idiopathic pulmonary fibrosis

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ABSTRACT

Background:

Pulmonary fibrosis is a frequent manifestation of a telomere syndrome. Telomere-related gene mutations are found in up to 25% and 3% of patients with familial disease and sporadic disease, respectively. The telomere gene *TINF2* encodes an eponymous protein that is part of the shelterin complex, a complex involved in telomere protection and maintenance. A *TINF2* gene mutation was recently reported in a family with pulmonary fibrosis.

Methods:

A sequence analysis of *TINF2* was performed in a cohort of 158 pulmonary fibrosis patients.

Results:

We identified a heterozygous Ser245Tyr mutation in the *TINF2* gene of previously healthy female patient that presented with progressive cough due to pulmonary fibrosis as well as an underlying panhypogammaglobulinemia at age 52. Retrospective multidisciplinary evaluation classified her as a case of possible idiopathic pulmonary fibrosis. Telomere length-measurement indicated normal telomere length in the peripheral blood compartment.

Conclusions:

This is the first report of a TINF2 mutation in a sporadic patient with pulmonary fibrosis, which strengthens the association between *TINF2* mutations and this disease. Furthermore, this case underlines the potential importance of telomere dysfunction and not telomere length alone in telomere syndromes and draws attention to hypogammaglobulinemia as a manifestation of telomere syndromes.

INTRODUCTION

Idiopathic Pulmonary Fibrosis (IPF) has been described as a condition of "accelerated aging" of the lungs and the prevalence of IPF rises dramatically with age.[1] IPF is characterized by progressive fibrosis, leading to respiratory failure and eventually death. Median survival after diagnosis of IPF is 3-4 years,[2] although recently two new treatments have come available that slow disease progression and might increase survival.[3] The pathogenetic processes leading to IPF are still not completely understood, but in a subset of patients, telomere dysfunction plays a key role.[4]

Telomere dysfunction is the hallmark of the telomere syndromes, conditions that are clinically characterized by premature aging and are exemplified by dyskeratosis congenita (DC).[5] Telomeres are repetitive TTAGGG sequences at chromosome ends that serve as a solution to the end-replication problem and end-protection problem that arise in cells with linear DNA.[6] Every cell division results in shorter telomeres, and with increasing age telomeres become critically short and induce cellular senescence or apoptosis.[7]

Telomeres are highly regulated structures that are maintained by various regulatory proteins. Mutations have been found in genes encoding parts of the telomerase and the shelterin complex, as well as other genes involved in telomere biology.[8] The telomerase complex is active in embryonic cells and stem cells and can increase telomere length by adding the repetitive telomere sequence to telomere ends.[9] The shelterin complex specifically locates to telomeres and is involved in their protection and maintenance.[10] In IPF patients, mutations have been found in *TERT*, *TERC*, *DKC1*, *RTEL1* and *PARN* genes.[11–15]

Mutations in telomere genes can lead to critically short telomeres in both high-turnover and low-turnover tissues, resulting in various disease phenotypes.[5] In the lung, a slow-turnover tissue, exogenous damage in combination with short telomeres due to telomere gene mutations, is suggested to trigger pulmonary fibrosis.[5] Mutations in telomere genes have been found in up to 25% of familial and 1-3% of sporadic IPF patients.[5,13–15] In fact, although DC was the first telomere syndrome to be identified, it is now understood that IPF is by far the most common manifestation of a telomere syndrome.[16] What makes the lung especially susceptible to telomere dysfunction has not been definitively proven, but constant exposure to irritating or infectious substances from the outside world, as well as continuous mechanical stress caused by ventilation have been implicated as factors contributing to IPF pathogenesis.[1]

In addition to the previously mentioned telomere genes, a mutation has recently been reported in the TRF1-Interacting Nuclear Factor 2 gene (*TINF2*) in the setting of familiar

pulmonary fibrosis.[17] The *TINF2* gene encodes an eponymous protein, which is part of the shelterin complex. *TINF2* mutations have often been found to underlie telomere syndrome manifestations other than pulmonary fibrosis.[8] Therefore, we performed a sequence analysis of *TINF2* in our cohort of 158 pulmonary fibrosis patients, which revealed a heterozygous Ser245Tyr mutation in one patient (Figure 1). All patients provided formal written consent.

CASE PRESENTATION

The patient was a 52-year-old female that presented with progressive cough and dyspnea for 8 weeks. Her medical history was unremarkable; she did not suffer from chronic disease. She had never smoked and had no known allergies or exposure to toxics. She had no family history suggestive for telomere syndromes or lung disease and she had two healthy daughters. She was admitted to a general hospital, where she was diagnosed with IPF based on high-resolution computed tomography scan and open lung biopsy findings. Simultaneously, laboratory analysis revealed that she had severe panhypogammaglobulinemia (IgM 0.32 g/L; IgA <0.1 g/L and IgG 2.2 g/L), and she was given the diagnosis common variable immunodeficiency (CVID). There were no other clinical features suggestive of telomere disease or autoimmune disease. The patient had no history of recurrent infections. Bronchoalveolar lavage fluid showed no lymphocytosis or other abnormalities, and anti-nuclear antibody and anti-neutrophil cytoplasmic antibody tests were negative. She was started on oral corticosteroid treatment for her pulmonary fibrosis (as was common practice at the time) and immunoglobulin replacement therapy, both of which she would receive during her entire course of disease.

Her situation was relatively stable for 48 months, and she showed no signs of infections. When she became progressively dyspnoeic, she was screened for lung transplantation. Because there were no contra-indications for lung transplantation, she was placed on the waiting list 65 months after diagnosis. Unfortunately, a donor was not available in time and she passed away 71 months after diagnosis at the age of 58. As this all occurred a decade ago, we have retrospectively reviewed the case based on current guidelines [18] in our multidisciplinary interstitial lung diseases-team. Based on pathological and radiological findings, and in the absence of features suggestive of other diagnoses, our patient can be classified as a case of possible IPF (Figure 1). Because she failed to respond to corticosteroid treatment, there was multidisciplinary consensus on the clinical diagnosis of IPF. We retrospectively measured the T/S ratio in DNA extracted from peripheral blood monocytes obtained 65 months after diagnosis and found a T/S ratio of 1.03, indicating normal telomere length in this cell compartment.

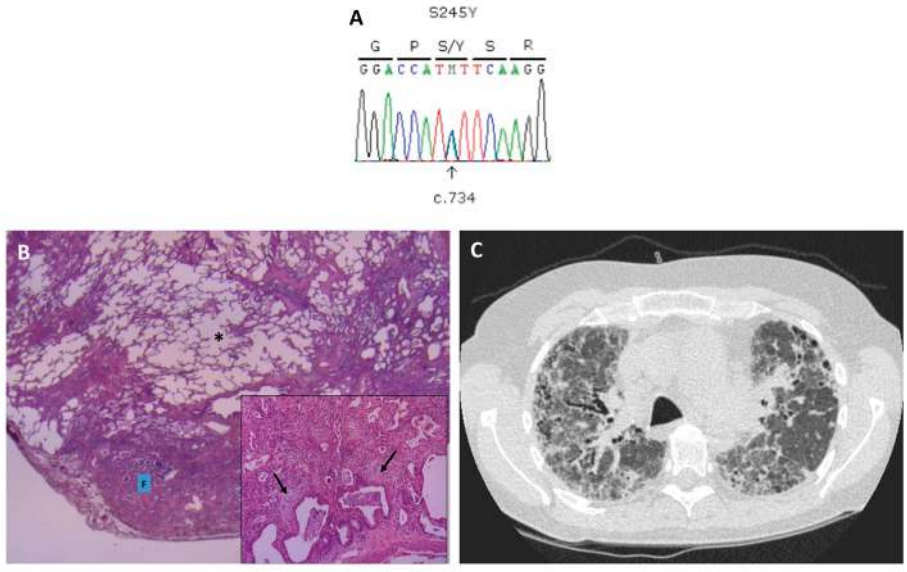

Figure 1: (A) DNA sequence of a segment of *TINF2* exon 6 demonstrates a cytosine to adenine change at position c.734 that leads to the amino acid substitution of serine to tyrosine at codon 245. The M denotes that both a cytosine and an adenine nucleotide at cDNA position 734 are present, indicating a heterozygous mutation. (B) Lung biopsy specimen of our patient taken at the time of diagnosis (H&E 12,5x). The biopsy shows temporal and spatial heterogeneous fibrosis consistent with a usual interstitial pneumonia (UIP) pattern: marked subpleural fibrosis with honeycombing (F) and central sparing (*), and the presence of fibroblast foci (inset 200x, arrows). No features suggestive of an alternative diagnosis were seen. Specifically, histologically there was no granulomatous disease or lymphocytic interstitial pneumonia pattern present suggestive of granulomatous-lymphocytic interstitial lung disease (GLILD) and there was no interstitial elastosis suggestive of pleuroparenchymal fibroelastosis (PPFE). (C) HRCT scan image of the lungs of our patient when she was referred for lung transplantation. The scan shows thickening of the inter- and intralobular septae, subpleural and peribronchovascular. Honeycombing is seen on the left. This is inconsistent with a UIP pattern, due to the peribronchovascular extension of the fibrosis. No radiological features suggestive of alternative diagnoses were seen. Specifically, there were no pulmonary micronodules that are typical of GLILD, and there was no pleuroparenchymal thickening in the upper lung zones, which is typical of PPFE. With these findings combined, the patient can be classified as a case of possible IPF, in accordance with current guidelines.[18]

DISCUSSION

This case is interesting for multiple reasons. First, this case strengthens the association between *TINF2* mutations and pulmonary fibrosis. Previously, *TINF2* mutations were found in three DC patients that developed pulmonary fibrosis[19–21] and in a family with pulmonary fibrosis, infertility and short telomeres[17] (Figure 2). In the literature, five persons with the same heterozygous Ser245Tyr mutation as our patient have been described. These mutation carriers include a 50-year-old male with aplastic anemia,[22] a 7-year-old girl with abnormal skin pigmentation and bone marrow failure and her asymptomatic 35-year-old

mother[23] and a 14-year-old boy with aplastic anemia and his asymptomatic father.[24] None of these patients had pulmonary fibrosis.

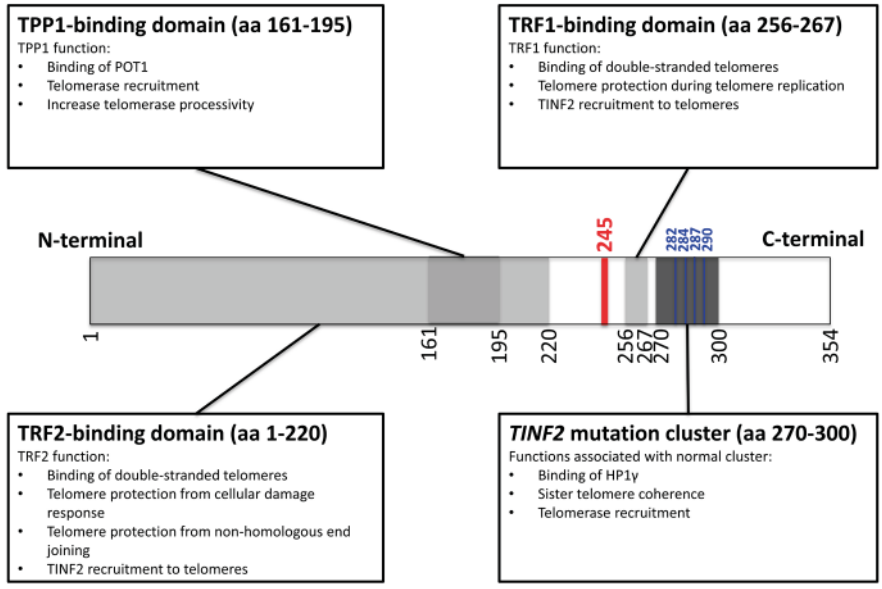

Figure 2: *TINF2* gene overview visualizing the sequence of protein domains and describing the interactions of the TINF2 protein in the shelterin complex. TINF2 mediates the formation of the shelterin complex by binding to the TRF1, TRF2 and TPP1 proteins. Numbers along the lower side of the *TINF2* gene denote encoded amino acid positions. The known binding domains of the TINF2 interaction partners TRF1, TRF2 and TPP1 are indicated in light grey.[25,26] TINF2 interacting protein functions are annotated in boxes. TRF1 protein function is based on references.[27,28] TRF2 protein function is based on reference.[27] TPP1 protein function is based on references.[27,29] TINF2 mutation cluster function is based on references.[30,31] Numbers along the upper side of the *TINF2* gene indicate amino acid positions of *TINF2* mutations in patients with pulmonary fibrosis. The Ser245Tyr mutation location is shown in red. The *TINF2* DC mutation cluster is indicated in dark grey.[32] Blue lines indicate *TINF2* mutations found in patients with pulmonary fibrosis at amino acids 282,[20] 284,[17] 287,[21] and 290.[19] aa= amino acid

Secondly, the nature of the Ser245Tyr mutation is of interest. The TINF2 protein has multiple roles in telomere maintenance, by way of the binding of TINF2 to several other telomere maintenance proteins (Figure 2), thereby forming the shelterin complex. One end of TINF2 binds to the TRF1 protein, while the other end binds to the TRF2 protein.[25] The area adjacent to the TRF2-binding site binds to the TPP1 protein.[26] All three aforementioned proteins are critical for the protection of telomeres against cellular DNA damage repair mechanisms. The majority of the *TINF2* mutations found in telomere syndrome patients, however, lie in a cluster outside the binding regions of TINF2 to TRF1, TRF2 and TPP1, and do not seem to influence the interaction of TINF2 with these proteins.[30,32] The precise pathogenetic consequences of mutations in this cluster have not been elucidated,

but it has been found that the mutations impair the binding of heterochromatin protein 1 gamma (HP1γ) to TINF2.[31] The TINF2-HP1γ interaction is involved in sister telomere cohesion during cell division, which is thought to be required for adequate telomerase functioning.[31] Impairment of this interaction leads to telomere shortening.[31] A recent study suggests that telomere shortening due to mutations in the *TINF2* mutation cluster is caused by impaired telomerase recruitment to telomeres rather than impaired telomerase functionality.[30]

The Ser245Tyr mutation lies outside of this mutation cluster, and also outside of the binding regions of TINF2 to TRF1, TRF2 and TPP1. The function of the 245th amino acid of TINF2 is not known at the present, as are the biochemical consequences of the serine to tyrosine substitution at this location. All reported patients with the Ser245Tyr mutation described, including our patient, have normal telomere length. This led the authors of one study to state that this mutation is non-pathogenic.[23] However, the mutation has been associated with a telomere syndrome phenotype in three patients (four when our patient is included), and therefore others consider the Ser245Tyr mutation to be pathogenic, but associated with a milder telomere syndrome phenotype compared to other *TINF2* mutations.[24]

In support of the latter position, Sorting Intolerant from Tolerant (SIFT) analysis[33] predicted this mutation to be deleterious (performed at http://sift.jcvi.org/ using default settings; SIFT value 0.00 (version 1.03, reference sequence: NP_001092744.1)). Polyphen-2 prediction[34] reported that this mutation is possibly damaging (performed at http://genetics.bwh.harvard.edu/pph2/ using default settings; value 0.907 (version 2.2, reference sequence: NP_001092744.1)). Furthermore, we screened 100 self-reported healthy hospital employees, 125 lung-transplantation donors, and 63 self-reported healthy other controls, none of whom were found to carry the *TINF2* Ser245Tyr mutation. In addition the minor allele frequency for the mutation in the 1000genome project[35] is 0.001 (at http://browser.1000genomes.org, data from Ensembl release 68), indicating very low population frequency.

That our patient, as well as other reported Ser245Tyr mutation carriers, had normal telomere length in the blood compartment does not fit within the model of critically short telomeres that lead to telomere syndrome manifestations. However, we believe that this view of telomere syndrome pathogenesis is incomplete. It has been shown in mice that conditional deletion of *Trf1* or *Trf2* in type 2 alveolar epithelial cells leads to a significant telomere damage response, cellular senescence and a pulmonary fibrosis phenotype, while maintaining normal telomere length.[7,36] In addition, in patients with severe telomere syndrome phenotypes, some show very short telomeres in the blood cell compartment, while others show a normal telomere length in the presence of a severe telomere damage

response.[37] This points to a model where telomere dysfunction, and not short telomere length per se, is the ultimate cause of a telomere syndrome phenotype.

In this model telomere shortening decreases the concentration of telomere maintenance proteins at telomeres, and thereby causes telomere protection to fall below a certain threshold, leading to a telomere damage response.[38] Mechanisms that influence telomere protective mechanisms without influencing telomere length, such as loss of TRF1 or TRF2, can lead to a telomere damage response as well. The telomere damage response then leads to telomere dysfunction and subsequent cellular senescence or apoptosis. Short telomeres are no prerequisite for telomere dysfunction, and the Ser245Tyr mutation in our patient might influence telomere protection in a manner that is not presently known.

Thirdly, this case draws attention to late-onset hypogammaglobulinemia as a manifestation of telomere syndromes. It is highly likely that our patient developed hypogammaglobulinemia later in life, as she had no history of recurrent infections. Immunodeficiency is commonly seen in DC and can even precede other bone marrow manifestations.[39] The working diagnosis of CVID could not be confirmed in accordance with current diagnostic criteria (available at http://esid.org/Working-Parties/Registry/Diagnosis-criteria) due to a lack of data on our patients' vaccination responses and isohemagglutinin titer levels. To our knowledge, the association of IPF and CVID has never been reported. CVID is known to have infectious pulmonary complications, such as bronchiectasis, and also non-infectious pulmonary complications, such as granulomatous-lymphocytic interstitial lung disease (GLILD).[40] In our patient, radiological and pathological studies found no features suggestive of GLILD or interstitial lung disease other than IPF. This report raises the question whether some cases of CVID are in fact manifestations of a telomere syndrome. Interestingly, a subset of CVID patients has significantly shorter telomeres in comparison with others and displays telomere-dependent B-cell senescence.[41]

CONCLUSION

In summary, we identified a heterozygous Ser245Tyr mutation in the *TINF2* gene of a sporadic pulmonary fibrosis patient. This case strengthens the association between *TINF2* mutations and pulmonary fibrosis. Furthermore, this case illustrates the importance of telomere dysfunction and not telomere length alone in telomere syndromes and draws attention to hypogammaglobulinemia as a manifestation of telomere syndromes.

REFERENCES

1. Selman M, Pardo A. Revealing the Pathogenic and Aging-related Mechanisms of the Enigmatic Idiopathic Pulmonary Fibrosis. An Integral Model. *Am J Respir Crit Care Med*. 2014;189(10):1161-1172. doi:10.1164/rccm.201312-2221PP [doi]
2. Strand MJ, Sprunger D, Cosgrove GP, et al. Pulmonary function and survival in idiopathic versus secondary usual interstitial pneumonia. *Chest*. April 2014. doi:10.1378/chest.13-2388 [doi]
3. Hunninghake GM. A New Hope for Idiopathic Pulmonary Fibrosis. *N Engl J Med*. May 2014. doi:10.1056/NEJMe1403448 [doi]
4. Renzoni E, Srihari V, Sestini P. Pathogenesis of idiopathic pulmonary fibrosis: review of recent findings. *F1000Prime Rep*. 2014;6:69-69. eCollection 2014. doi:10.12703/P6-69 [doi]
5. Armanios M, Blackburn EH. The telomere syndromes. *Nat Rev*. 2012;13(10):693-704. doi:10.1038/nrg3246 [doi]
6. de Lange T. How telomeres solve the end-protection problem. *Science*. 2009;326(5955):948-952. doi:10.1126/science.1170633 [doi]
7. Alder JK, Barkauskas CE, Limjunyawong N, et al. Telomere dysfunction causes alveolar stem cell failure. *Proc Natl Acad Sci U S A*. 2015;112(16):5099-5104. doi:10.1073/pnas.1504780112 [doi]
8. Holohan B, Wright WE, Shay JW. Cell biology of disease: Telomeropathies: An emerging spectrum disorder. *J Cell Biol*. 2014;205(3):289-299. doi:10.1083/jcb.201401012 [doi]
9. Aubert G. Telomere dynamics and aging. *Prog Mol Biol Transl Sci*. 2014;125:89-111. doi:10.1016/B978-0-12-397898-1.00004-9 [doi]
10. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev*. 2005;19(18):2100-2110. doi:19/18/2100
11. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med*. 2007;356(13):1317-1326. doi:356/13/1317
12. Kropski JA, Mitchell DB, Markin C, et al. A novel dyskerin (DKC1) mutation is associated with Familial Interstitial Pneumonia. *Chest*. February 2014. doi:10.1378/chest.13-2224 [doi]
13. Cogan JD, Kropski JA, Zhao M, et al. Rare Variants in RTEL1 are Associated with Familial Interstitial Pneumonia. *Am J Respir Crit Care Med*. January 2015. doi:10.1164/rccm.201408-1510OC [doi]
14. Stuart BD, Choi J, Zaidi S, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. *Nat Genet*. 2015;47(5):512-517. doi:10.1038/ng.3278 [doi]
15. Kannengiesser C, Borie R, Menard C, et al. Heterozygous RTEL1 mutations are associated with familial pulmonary fibrosis. *Eur Respir J*. 2015;46(2):474-485. doi:10.1183/09031936.00040115
16. Armanios M. Telomerase and idiopathic pulmonary fibrosis. *Mutat Res*. 2012;730(1-2):52-58. doi:10.1016/j.mrfmmm.2011.10.013 [doi]
17. Alder JK, Stanley SE, Wagner CL, Hamilton M, Hanumanthu VS, Armanios M. Exome sequencing identifies mutant TINF2 in a family with pulmonary fibrosis. *Chest*. December 2014. doi:10.1378/chest.14-1947 [doi]
18. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med*. 2011;183(6):788-824. doi:10.1164/rccm.2009-040GL [doi]

19. Fukuhara A, Tanino Y, Ishii T, et al. Pulmonary fibrosis in dyskeratosis congenita with TINF2 gene mutation. *Eur Respir J*. 2013;42(6):1757-1759. doi:10.1183/09031936.00149113 [doi]

20. Panichareon B, Seedapan T, Thongnoppakhun W, Limwongse C, Pithukpakorn M, Limjindaporn T. Novel Mutation of the TINF2 Gene in a Patient with Dyskeratosis Congenita. *Case Rep Dermatol*. 2015;7(2):212-219. doi:10.1159/000439042 [doi]

21. Zlateska B, Ciccolini A, Dror Y. Treatment of dyskeratosis congenita-associated pulmonary fibrosis with danazol. *Pediatr Pulmonol*. June 2015. doi:10.1002/ppul.23235 [doi]

22. Walne AJ, Vulliamy T, Beswick R, Kirwan M, Dokal I. TINF2 mutations result in very short telomeres: analysis of a large cohort of patients with dyskeratosis congenita and related bone marrow failure syndromes. *Blood*. 2008;112(9):3594-3600. doi:10.1182/blood-2008-05-153445 [doi]

23. Vulliamy T, Beswick R, Kirwan MJ, Hossain U, Walne AJ, Dokal I. Telomere length measurement can distinguish pathogenic from non-pathogenic variants in the shelterin component, TIN2. *Clin Genet*. 2012;81(1):76-81. doi:10.1111/j.1399-0004.2010.01605.x [doi]

24. Miano M, Lanciotti M, Giardino S, Dufour C. Ser245Tyr TINF2 mutation in a long-term survivor after a second myeloablative SCT following late graft failure for Aplastic Anaemia. *Blood Cells Mol Dis*. 2015;55(2):187-188. doi:10.1016/j.bcmd.2015.01.002

25. Chen Y, Yang Y, van Overbeek M, et al. A shared docking motif in TRF1 and TRF2 used for differential recruitment of telomeric proteins. *Science*. 2008;319(5866):1092-1096. doi:10.1126/science.1151804 [doi]

26. Frescas D, de Lange T. Binding of TPP1 protein to TIN2 protein is required for POT1a,b protein-mediated telomere protection. *J Biol Chem*. 2014;289(35):24180-24187. doi:10.1074/jbc.M114.592592 [doi]

27. Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet*. 2008;42:301-334. doi:10.1146/annurev.genet.41.110306.130350 [doi]

28. Zimmermann M, Kibe T, Kabir S, de Lange T. TRF1 negotiates TTAGGG repeat-associated replication problems by recruiting the BLM helicase and the TPP1/POT1 repressor of ATR signaling. *Genes Dev*. 2014;28(22):2477-2491. doi:10.1101/gad.251611.114 [doi]

29. Dalby AB, Hofr C, Cech TR. Contributions of the TEL-patch amino acid cluster on TPP1 to telomeric DNA synthesis by human telomerase. *J Mol Biol*. 2015;427(6 Pt B):1291-1303. doi:10.1016/j.jmb.2015.01.008 [doi]

30. Frank AK, Tran DC, Qu RW, Stohr BA, Segal DJ, Xu L. The Shelterin TIN2 Subunit Mediates Recruitment of Telomerase to Telomeres. *PLoS Genet*. 2015;11(7):e1005410. doi:10.1371/journal.pgen.1005410 [doi]

31. Canudas S, Houghtaling BR, Bhanot M, et al. A role for heterochromatin protein 1gamma at human telomeres. *Genes Dev*. 2011;25(17):1807-1819. doi:10.1101/gad.17325211 [doi]

32. Frescas D, de Lange T. A TIN2 dyskeratosis congenita mutation causes telomerase-independent telomere shortening in mice. *Genes Dev*. 2014;28(2):153-166. doi:10.1101/gad.233395.113 [doi]

33. 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(7):1073-1081. doi:10.1038/nprot.2009.86 [doi]

34. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7(4):248-249. doi:10.1038/nmeth0410-248 [doi]

35. Consortium 1000 Genomes Project, Abecasis GR, Auton A, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;491(7422):56-65. doi:10.1038/nature11632 [doi]

36. Povedano JM, Martinez P, Flores JM, Mulero F, Blasco MA. Mice with Pulmonary Fibrosis Driven by Telomere Dysfunction. *Cell Rep*. 2015;12(2):286-299. doi:10.1016/j.celrep.2015.06.028 [doi]

37. Touzot F, Gaillard L, Vasquez N, et al. Heterogeneous telomere defects in patients with severe forms of dyskeratosis congenita. *J Allergy Clin Immunol*. 2012;129(2):473-482, 482.e1-3. doi:10.1016/j.jaci.2011.09.043 [doi]

38. Denchi EL, de Lange T. Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. *Nature*. 2007;448(7157):1068-1071. doi:10.1038/nature06065

39. Jyonouchi S, Forbes L, Ruchelli E, Sullivan KE. Dyskeratosis congenita: a combined immunodeficiency with broad clinical spectrum--a single-center pediatric experience. *Pediatr Allergy Immunol*. 2011;22(3):313-319. doi:10.1111/j.1399-3038.2010.01136.x [doi]

40. Park JH, Levinson AI. Granulomatous-lymphocytic interstitial lung disease (GLILD) in common variable immunodeficiency (CVID). *Clin Immunol*. 2010;134(2):97-103. doi:10.1016/j.clim.2009.10.002; 10.1016/j.clim.2009.10.002

41. Visentini M, Cagliuso M, Conti V, et al. Telomere-dependent replicative senescence of B and T cells from patients with type 1a common variable immunodeficiency. *Eur J Immunol*. 2011;41(3):854-862. doi:10.1002/eji.201040862 [doi]

Chapter 9

No effect of danazol treatment in patients with advanced idiopathic pulmonary fibrosis

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ABSTRACT

Background:

Telomere dysfunction underlies the development of idiopathic pulmonary fibrosis (IPF) in at least part of the patients, and recent work suggests that patients with telomere syndromes might benefit from treatment with androgens, such as danazol.

Methods:

This was a prospective observational cohort study. Fifty patients with IPF received off-label treatment with danazol after they showed progressive disease under treatment with pirfenidone or nintedanib. The primary outcome was the difference in yearly decline in forced vital capacity (FVC) prior to (pre) and after (post) start of treatment with danazol.

Results:

There was no significant difference in FVC-decline between one-year pre and one-year post start of danazol treatment (mean decline pre 395 mL (95% confidence interval (CI) 290 – 500) compared to post 461 mL (95% CI 236 – 686); p=0.59; paired T-test). Eleven patients (22%) were still on danazol after 1 year, and thirty-seven patients (74%) had stopped danazol, mainly because of side effects (57%) or death (32%). In patients who were still using danazol after one year, FVC-decline significantly slowed down under danazol treatment (mean pre 512 mL (95% CI 308 – 716) versus post 198 mL (95% CI 16 – 380); p=0.04). Median survival after start of treatment with danazol was 15.1 months (95% CI 10.9 – 19.4).

Conclusion:

Danazol as a treatment of last resort in patients with IPF did not lead to slowing of lung function decline and was associated with significant side effects. It remains to be determined if earlier treatment or treatment of specific patient subgroups is beneficial.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a severe lung disease with a median survival of three years after diagnosis.[1] Progressive scarring of the lung leads to increasing breathlessness and eventually death. Treatment options include the anti-fibrotic drugs pirfenidone and nintedanib. Both drugs have been shown to slow disease progression, but do not cure the disease.[2,3] So, even under anti-fibrotic treatment patients progress towards advanced fibrosis, which highlights the need for other medication.

Telomere dysfunction plays an important role in the pathogenesis of IPF.[4] Telomeres are repetitive nucleotide sequences at the ends of chromosomes. Telomeres shorten with every cell division, and thus telomeres become increasingly short with age. Critically short telomeres will eventually lead to cellular senescence or apoptosis.[5] Abnormally short telomeres lead to conditions of accelerated aging, called short telomere syndromes. These conditions include dyskeratosis congenita and some forms of bone marrow failure, as well as IPF.[6] Telomere dysfunction in these patients is the result of a genetic mutation in a gene encoding a component of the telomere maintenance proteins.[6]

IPF is the most common manifestation of a short telomere syndrome,[6] and mutations in telomere genes have been found in up to 35% of familial IPF cases and up to 11% of sporadic IPF cases.[7,8] In addition, it has been found that a substantial proportion of IPF patients in whom no mutation was found has decreased telomere length compared to healthy controls and to patients with other chronic lung diseases.[9–11] In one study, over half of patients with IPF who had no known telomerase mutation did have short lung telomere length, in the same range as patients with a telomerase gene mutation.[12]

Clinical studies in patients with aplastic anaemia or dyskeratosis congenita showed that patients with telomere-related gene mutations may benefit from androgen therapy.[13–19] The most extensive study to date reported on the use of danazol in patients with telomere syndromes.[20] Danazol is a synthetic hormone with light androgenic effects. The study included 27 patients, of whom 10 had overt pulmonary fibrosis, and 2 had a primary diagnosis of IPF. Most of the other patients had a primary diagnosis of aplastic anaemia with secondary pulmonary fibrosis. Patients were treated with oral danazol for two years. The main outcome parameter was peripheral blood telomere length, and this significantly increased during danazol treatment. Secondary outcome parameters included follow up of chest computed tomography (CT) scans, which showed that the pulmonary fibrosis was radiologically stable in all but one patient. Also, the mean lung function was stable during the study period, whereas there was a mean lung function decline prior to the study period.[20,21]

Although these findings are promising, the efficacy of danazol for the treatment of patients with IPF is not yet well-known. This study reports on the efficacy of danazol in patients with IPF, to whom danazol was offered as an off-label treatment when there was progressive lung function decline despite treatment with pirfenidone or nintedanib.

PATIENTS AND METHODS

Study subjects

Patients with IPF who had disease progression under the current standard-of-care, based on lung function (decline in forced vital capacity >5% of predicted) and radiographic testing (worsening of fibrosis on high-resolution CT scan), and who started treatment with danazol at St. Antonius Hospital were included. Subjects were included starting August 2018 up to July 2022. Per local protocol, patients were not eligible for treatment with danazol in case of severe cardiac, hepatic, or renal disease, when they used statins metabolized by CYP3A4 (statins were switched if needed), when they had an androgen-dependent tumour (such as prostate cancer), increased thromboembolic risk without adequate prophylactic measures taken (e.g. anticoagulation use), or when they were pregnant or of childbearing potential and unwilling to take adequate contraceptive measures during danazol treatment. Patients who had concomitant (severe) disease that was deemed likely to result in death within 30 days, or precluded the ability to participate in the study protocol, were also excluded.

All patients underwent clinical screening prior to starting treatment with danazol. This screening included the drawing of blood for laboratory work-up (liver function tests, hormone profile, standard renal, electrolyte, and other general labs, full blood count with differential count, lipid spectrum, prostate specific antigen (in men)), as well as ultrasound investigation of the liver and reproductive organs and a thrombophilia screening questionnaire. Repeat laboratory testing was done at two, four, and eight weeks, as well as at three, six, nine, and twelve months. In the course of regular clinical care, follow-up of lung function was scheduled every three months, a chest X-ray was scheduled every three or six months and a CT-scan was scheduled after twelve months.

The starting dose of danazol was 400mg twice daily. Per local protocol, in case of suspected mild to moderate adverse effects, the dose was reduced in steps of 200 mg (i.e. to 300 mg twice daily, then to 200 mg twice daily, then to 100 mg twice daily). Step-wise dose reduction was continued until side effects are acceptable. If a patient was unable to tolerate a dose of 100 mg twice daily, danazol was discontinued.

Study design

This was a prospective observational cohort study. Danazol was prescribed off-label to patients with IPF after shared decision making. The study was registered locally under protocol number NL 62034.100.17, and was approved by the local medical ethics committee (MEC-U; study number R17.047). The study was conducted in accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent

All subjects included in the study were followed up for at least one year, regardless of withdrawal from treatment, or until the subject's death or lung transplant. Patients still on treatment after one year of follow up were followed further, while for patients that withdrew from treatment, follow up ceased one year after start of treatment.

Laboratory investigations

Blood samples were taken around time of diagnosis. The samples were stored at -80 degrees Celsius until use. DNA was isolated from white blood cells using a magnetic beads-based method (chemagic DNA blood 10k kit; PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany). Telomere length was determined using a previously described quantitative polymerase chain reaction (PCR) method.[33] Briefly, telomere length was estimated for each sample from the ratio of telomere repeat copy number to a single gene (human β-globin gene) copy number (T/S ratio). Measurements were performed on the Bio-Rad CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) in duplicate, with additional measurements if the duplicates differed more than 0.05; the mean value of the measured samples was used. Furthermore, quality control samples were included on each PCR-run, with a <0.05 margin of variance to reference values allowed. The control cohort included 164 healthy adults (71 male) with an age ranging between 20-70 years. Age-adjusted normal values for the T/S-ratio were calculated by determining the best-fitting linear regression line through the data, and percentiles were derived from the regression line.

After enrichment of the exome with the Agilent SureSelect CREV2 kit (Agilent Technologies, Santa Clara, California, USA), whole exome sequencing was performed on an Illumina Novaseq 6000 sequencer. The Illumina sequencing data was processed with the in-house pipeline, IAP v2.6.1, including GATK v3.4-46, according to the best practices guidelines.[34] Results were filtered for exonic variants with a population frequency below 0.5% in 36 genes related to telomere syndromes or pulmonary fibrosis (*ABCA3*, *ACD*, *AP3B1*, *CSF2RA*, *CSF2RB*, *CTC1*, *DKC1*, *FAM111B*, *HPS1*, *HPS4*, *ITGA4*, *LIG4*, *MARS*, *NAF1*, *NKX2-1*, *NOP10*, *PARN*, *POT1*, *RNF168*, *RTEL1*, *SAMD9L*, *SFTPA1*, *SFTPA2*, *SFTPB*, *SFTPC*, *SFTPD*, *STN1*, *TEN1*, *TERC*, *TERF1*, *TERF2*, *TERT*, *TINF2*, *TMEM173*, *USB1*, and *WRAP53*).

Statistical analysis

The main study parameter is difference in yearly decline in lung function between the period prior to danazol treatment and the first year after start of danazol treatment. Secondary outcome parameters are overall survival, radiological qualification of pulmonary fibrosis at baseline compared to during danazol treatment, and adverse events during danazol treatment.

For the primary analysis, yearly FVC-decline was compared between the period prior to therapy and the first year of the study period (i.e. the first year that a patient was receiving danazol) using a paired T-test. In case of missing data, FVC-decline after one year was imputed using linear extrapolation of available lung function measurements after start of danazol treatment. When no pulmonary function testing after the start of danazol was available, FVC-decline after one year was imputed using linear extrapolation of the FVC-decline prior to start of danazol.

Secondary analyses included restricting included patients to those in whom lung function measurements were available at 12 months and restricting included patients to those who were still using danazol after one year. Secondary end points were compared between the period prior to therapy and the treatment period using paired T-tests, Chi-squared test, or Fisher's exact test, where appropriate. For survival analyses, Log-rank and Cox-regression analyses were used, and patients were censored when follow up ended, when they underwent lung transplantation, or when they were lost to follow up. Data analyses were performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA).

RESULTS

The total cohort comprised 50 patients. Baseline characteristics at the time of diagnosis and when starting danazol treatment are provided in Table 1. Forty-four (88%) of the patients were male, sixteen (32%) had a first-degree family member with pulmonary fibrosis, ten (20%) had a family history suggestive of a telomere syndrome, and four (8%) had extrapulmonary disease suggestive of a telomere syndrome. All patients had received treatment with either pirfenidone or nintedanib prior to starting treatment with danazol, and 36 patients were still using pirfenidone or nintedanib when they started treatment with danazol. Patients started treatment with danazol a median of 2.44 years (interquartile range (IQR) 1.69-3.32) after the diagnosis of IPF.

Leukocyte telomere length at diagnosis was available for 43 patients (86%), and was below the 10th percentile for age in 22 (51%). Whole exome sequencing, with results filtered for genes related to telomere syndromes or pulmonary fibrosis, was performed in 36 patients (72%) and revealed a likely pathogenic variant in six patients (17%) and a variant of unknown significance in nine patients (25%).

Table 1: Baseline characteristics of 50 patients with idiopathic pulmonary fibrosis who started treatment with danazol.

Baseline characteristics	
Male sex (%)	44 (88)
Median age at diagnosis, years (IQR)	67.6 (61.5 – 71.1)
Median age at start of danazol, years (IQR)	70.0 (66.2 – 73.8)
First-degree family member with pulmonary fibrosis (%)	16 (32)
Smoking status	
Current (%)	3 (6)
Former (%)	39 (78)
Never (%)	8 (16)
Median FVC % of predicted at diagnosis (IQR)	79 (68 – 87)
Median FVC % of predicted at start of danazol (IQR)	64 (56 – 80)
Median DLCOc % of predicted at diagnosis (IQR)	45 (37 – 51)
Median DLCOc % of predicted at start of danazol (IQR)	32 (25 – 37)
Treatment with antifibrotic agent prior to start of danazol(%)	50 (100)
Nintedanib (%)	44 (88)
Still using nintedanib (%)	28 (56)
Pirfenidone (%)	29 (58)
Still using pirfenidone (%)	9 (18)
Leukocyte telomere length (%)	43 (86)
<10th percentile for age (%)	22 (51)
<1st percentile for age (%)	8 (19)
Whole exome sequencing (%)	36 (72)
Variant of unknown significance in telomere- or pulmonary fibrosis-related gene (%)	10 (28)
Likely pathogenic variant in telomere- or pulmonary fibrosis-related gene (%)	6 (17)
Any clinical suggestion of telomere syndrome (%)	18 (36)
Family history suggestive of telomere syndrome (%)	10 (20)
Extrapulmonary disease suggestive of telomere syndrome (%)	4 (8)
Haematological laboratory abnormalities	
Anaemia (%)	6 (12)
Macrocytosis (%)	9 (18)
Thrombocytopenia (%)	3 (6)
Leukopenia (%)	0 (0)

Percentages for subgroups calculated based upon the total number of patients for whom data was available. With whole exome sequencing, one patient was found to have two variants of unknown significance. DLCOc = diffusion capacity of the lung for carbon monoxide, corrected for haemoglobin level; FVC = forced vital capacity; IQR = interquartile range. Extrapulmonary disease suggestive of a telomere syndrome included liver cirrhosis in two patients, myelodysplastic syndrome in one patient, and otherwise unexplained cytopenia in two patients (one patient had cytopenia and liver cirrhosis). Anaemia, macrocytosis, thrombocytopenia, and leukopenia were defined based on local laboratory lower-limit-of-normal levels. Any clinical suggestion of a telomere syndrome was defined as either a clinical history, family history, or laboratory abnormality suggestive of a telomere syndrome.[22]

Danazol

Patients received danazol for a median of 123 days (IQR 48 – 314 days). Thirty-seven patients (74%) stopped danazol prior to having received one year of treatment, mainly because of side effects (57% of patients who stopped) or death (32% of patients who stopped) (Figure 1; Table 2). Presumed side effects of danazol treatment were observed in 29 patients (58%) and included elevated liver enzymes, malaise, decreased renal function, myalgia, and other (Table 3). Dose adjustments were made in fourteen patients (28%) and danazol was temporarily discontinued because of side effects in eleven patients (22%). There was no significant difference in terms of side effects between patients who had leukocyte telomere length below or above the 10th percentile for age (Supplementary Table 1), or patients with or without any clinical suggestion of a telomere syndrome (Supplementary Table 2).

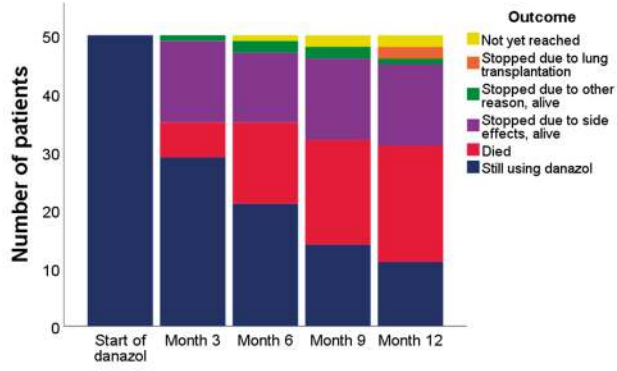


Figure 1: Outcomes of 50 patients with idiopathic pulmonary fibrosis treated with danazol, per 3 months. When a patient who had stopped using danazol died or did not yet reach a certain time of follow up, this was reported as the outcome.

Table 2: Duration of danazol use of 50 patients with idiopathic pulmonary fibrosis and reasons for stopping.

Danazol use	**N = 50**
Median duration of danazol use, days (IQR)	123 (48 – 314)
Stopped using danazol <1 year after start, N (%)	37 (74)
Side effects (%)	21 (57)
Deceased (%)	12 (32)
Progression of disease (%)	3 (8)
Lung transplant (%)	1 (3)
Still using danazol >1 year after start, N (%)	11 (22)
Started danazol <1 year ago, N (%)	2 (4)

Percentages for subgroups calculated based upon the total number of patients for whom data was available. IQR = interquartile range

Table 3: Presumed side effects of danazol in 50 patients with idiopathic pulmonary fibrosis

Side effects	N= 50
Temporarily discontinued because of side effects (%)	11 (22)
Permanently discontinued because of side effects (%)	23 (46)
Dose adjustment made (%)	14 (28)
Any side effects (%)	29 (58)
Elevated liver enzymes	10
Malaise	6
Decreased renal function	4
Myalgia	4
Oedema	3
Tiredness	2
Shivering	2
Stomach pain	2
Reduced appetite	2
Weight gain	2
Headache	2
Acne	1

Course of lung function

For all patients, the lung function declined significantly in the year prior to starting danazol, with a mean FVC-decline of 395 mL (95% confidence interval (CI) 290 – 500; $p<0.001$). In the year after starting danazol, the lung function also declined significantly, with a mean FVC-decline in the year after starting danazol of 461 mL (95% CI 236 – 686; $p=0.0001$; Figure 2A). Lung function measurements after the start of danazol were available in 31 patients, and lung function decline was imputed from lung function decline prior to danazol in the remainder. There was no significant difference between FVC-decline in the year prior to starting danazol and in the year after starting danazol ($p=0.59$; paired T-test). FVC-decline in the year after starting danazol was not significantly different between patients who had leukocyte telomere length below or above the 10th percentile for age (Supplementary Table 1), or patients with or without any clinical suggestion of a telomere syndrome (Supplementary Table 2). When restricting the analysis to patients in whom lung function measurements where available at 12 months (N=13), there was no significant difference between FVC-decline in the year prior to starting danazol and in the year after starting danazol (mean pre 399 mL (95% CI 203 – 594) compared to post 265 mL (95% CI 74 – 457); $p=0.32$). For patients who were still using danazol after one year (N=11), the rate of FVC-decline was significantly higher in the year prior to starting danazol then under danazol (mean pre 512 mL (95% CI 308 – 716) versus post 198 mL (95% CI 16 – 380); $p = 0.04$; Figure 2B).

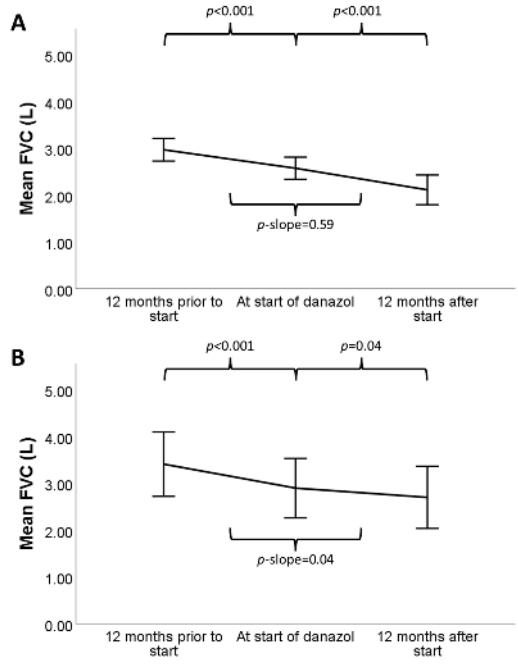


Figure 2: Forced vital capacity (FVC) in patients with IPF prior to, at start and 12-months after treatment with danazol. (A) Mean FVC in 50 patients for the primary analysis at 12 months prior to starting danazol, at start of danazol, and 12 months after starting danazol. (B) Mean FVC in 11 patients who were still using danazol after one year. Whiskers represent 95% confidence intervals. *P*-values represent differences between FVC at two time points, and *p*-slope represents the difference between rates of decline (paired T-test).

Radiographic imaging during follow up was available for 33 patients, including CT-scans in eighteen patients (3-12 months after start Danazol). CT-scans for twelve patients (67%) showed radiographic progression of fibrosis. In addition, radiographic progression of fibrosis was observed on chest X-ray in four patients for whom no CT-scan was available.

Survival

One year after starting treatment with danazol, 20 patients had died (eight of these patients had stopped danazol prior to dying), two patients underwent lung transplantation (one patient had stopped danazol prior to the transplantation), and 23 patients were still followed up (twelve of these patients had stopped using danazol), of which five patients had not yet completed the follow up period of one year (three of these patients had stopped using danazol). Patients who were still alive and using danazol after 12 months did not have significantly different baseline characteristics from the other patients (Table 4). Median survival after start of treatment with danazol was 15.1 months (95% CI 10.9 – 19.4); Figure 3).

Table 4: Patient characteristics of 11 patients still alive and using danazol after 12 months compared to the rest of the cohort.

	Still using danazol at 12 months (N=11)	**Not using danazol at 12 months (N=36)**	***p*-value**
Male sex (%)	9 (82)	32 (89)	0.61
Median age at start of danazol, years (IQR)	69.7 (65.8 – 73.2)	70.3 (66.6 – 75.7)	0.43
First-degree family member with pulmonary fibrosis (%)	4 (36)	10 (28)	0.71
Median time after IPF diagnosis before start Danazol, years (IQR)	3.6 (2.2 – 4.8)	2.3 (1.5 – 3.0)	0.08
Median FVC % of predicted at start (IQR)	68 (61 – 86)	66 (57 – 80)	0.43
Mean FVC-decline prior to start, mL (95% CI)	512 (308 – 716)	376 (242 – 509)	0.24
Median DLCOc % of predicted at start (IQR)	37 (28 – 43)	32 (22 – 37)	0.12
Still using antifibrotic agent	9 (82)	25 (69)	0.70
Still using nintedanib	7 (78)	18 (72)	1.00
Still using pirfenidone	2 (22)	7 (28)	1.00
Leukocyte telomere length (%)	10 (91)	32 (89)	1.00
<10th percentile for age (%)	7 (70)	15 (47)	0.28
<1st percentile for age (%)	2 (20)	6 (19)	1.00
Whole exome sequencing (%)	10 (91)	24 (67)	0.15
Variant of unknown significance in telomere- or pulmonary fibrosis-related gene (%)	2 (20)	8 (33)	0.68
Likely pathogenic variant in telomere- or pulmonary fibrosis-related gene (%)	1 (10)	4 (17)	1.00
Family history suggestive of telomere syndrome (%)	1 (9)	8 (22)	0.66
Extrapulmonary disease suggestive of telomere syndrome (%)	1 (9)	2 (6)	0.56
Any clinical suggestion of telomere syndrome (%)	3 (27)	13 (36)	0.73

Patients not using danazol because they underwent lung transplantation or because they did not yet complete the first year of follow up were excluded from this analysis. Percentages for subgroups calculated based upon the total number of patients for whom data was available. IQR = interquartile range

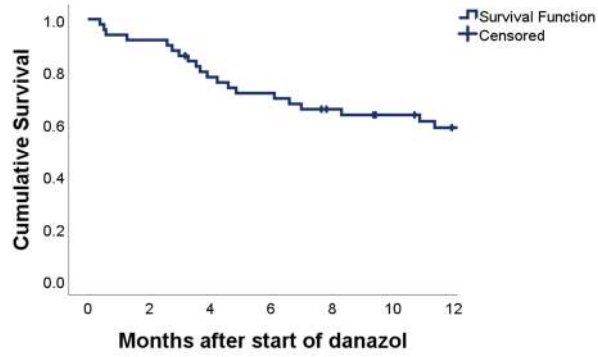


Figure 3: survival in patients with IPF who were treated with danazol. Subjects were censored when lost to follow-up or when they underwent lung transplantation.

DISCUSSION

In this prospective observational drug study, the use of danazol as a treatment of last resort for patients with IPF did not lead to slowing of lung function decline. Secondary outcomes, including radiographic progression and survival, also did not indicate a beneficial effect of danazol. Notably, the patients included in our study represent a subgroup of patients with an advanced stage of IPF. Prior to the start of danazol, patients showed a greater mean decline in FVC than observed in both the treatment and placebo arms of pirfenidone and nintedanib trials in patients with IPF.[2,3] Median time after diagnosis and before starting danazol treatment was 2.44 years. After start of danazol, survival in the study cohort was low. It could be speculated that the patients included already had a too advanced stage of IPF, where the therapeutic potential for any treatment might have been limited. Therapeutic potential for telomere-targeting treatments may be higher in earlier stages of the disease. Notably, patients who remained on danazol for ≥1 year appeared to have a better DLCOc percentage of predicted at the start of danazol and showed a significant decrease in the rate of FVC-decline after 1 year of danazol therapy. This suggests that earlier start with danazol may have a positive effect.

Previously, danazol was shown to have a beneficial effect in patients with proven short telomere syndromes.[20] Alternative to the advanced stage of IPF, the poor treatment effect might be related to the fact that not all study participants had proven telomere dysfunction. Patients were not selected for low leukocyte telomere length or telomere-related gene mutations, although about half of the study participants did have leukocyte telomere length <10th percentile for age. When combining signs and symptoms suggestive for telomere-associated pathology we found no evidence for their influence on outcome of danazol therapy. Albeit not statistically significant, when looking at patients who were

still alive and using danazol after 12 months, a nominally higher percentage had leukocyte telomere length <10th percentile for age compared to other participants. Whether treatment with danazol is beneficial in such a specific subgroup will be addressed in the ongoing TELO-SCOPE study.[23]

In recent years, the evidence for the role of androgens in the regulation of telomerase activity has increased. Telomere length in different cell lines can be modulated by sex hormones *in vitro*.[24–27] Oestrogen receptors act directly on the *TERT* (telomerase gene) promotor, whereas androgens influence expression of telomerase indirectly.[26,28] Higher circulating levels of testosterone metabolites and oestrogen have been associated with longer leukocyte telomere length in healthy volunteers. Furthermore, a Chinese case-control study found decreased serum testosterone levels in patients with IPF, as well as an association between shorter leukocyte telomere length and lower serum testosterone levels in patients with IPF.[29] A recent UK Biobank study found that IPF was more common in females with earlier menopause and premature ovarian failure, and in men with lower bioavailable testosterone concentrations. Furthermore, lower concentrations of sex hormones were associated with faster disease progression. For both sexes, lower concentrations of sex hormones were associated with shorter leukocyte telomere length.[30] In addition, anecdotal reports on the beneficial effect op androgen treatment in patients with pulmonary fibrosis have been published after the study by Townsley and colleagues.[19,31,32] All in all, androgen treatment does still seem to be a theoretically attractive treatment option for patients with IPF.

Presumed side effects of danazol were common in this study, as was the case in the study by Townsley and colleagues.[20] However, in our study a higher percentage of patients stopped treatment due to side effects (42% in our study versus 19% in the previous study). Although the side effect profile seemed similar, we hypothesize that in our study, because danazol was used as a treatment of last resort, side effects might have been less acceptable to patients and treating physicians. Second, most patients used danazol as an add-on treatment, and were already receiving treatment with antifibrotics. Some side effects of antifibrotics overlap with the side effects that we found.

Limitations other than those discussed above include the fact that this was not a randomised controlled trial, that there was missing data (partly related to the fact that patients with end-stage IPF did not come to the hospital as frequently), and the absence of longitudinal data on leukocyte telomere length.

CONCLUSION

In conclusion, in this study of fifty patients with IPF who showed evidence of disease progression despite treatment with pirfenidone or nintedanib, add-on treatment with danazol did not lead to slowing of lung function decline. Lung function decline pre and post treatment was worse than that in previous trials. Side effects necessitated stopping treatment in 42% of patients. Whether earlier treatment or treatment of specific subgroups of patients with IPF would be beneficial, remains to be determined.

REFERENCES

1. Lederer DJ, Martinez FJ. Idiopathic Pulmonary Fibrosis. *N Engl J Med*. 2018;378(19):1811-1823. doi:10.1056/NEJMra1705751 [doi]
2. Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2071-2082. doi:10.1056/NEJMoa1402584 [doi]
3. King Jr TE, Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2083-2092. doi:10.1056/NEJMoa1402582 [doi]
4. Armanios M. Telomerase and idiopathic pulmonary fibrosis. *Mutat Res*. 2012;730(1-2):52-58. doi:10.1016/j.mrfmmm.2011.10.013 [doi]
5. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;153(6):1194-1217. doi:10.1016/j.cell.2013.05.039 [doi]
6. Armanios M, Blackburn EH. The telomere syndromes. *Nat Rev*. 2012;13(10):693-704. doi:10.1038/nrg3246 [doi]
7. Hoffman TW, van Moorsel CHM, Borie R, Crestani B. Pulmonary phenotypes associated with genetic variation in telomere-related genes. *Curr Opin Pulm Med*. 2018;24(3):269-280. doi:10.1097/MCP.0000000000000475 [doi]
8. Cronkhite JT, Xing C, Raghu G, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2008;178(7):729-737. doi:10.1164/rccm.200804-550OC [doi]
9. Snetselaar R, van Moorsel CH, Kazemier KM, et al. Telomere length in interstitial lung diseases. *Chest*. 2015;148(4):1011-1018. doi:10.1378/chest.14-3078 [doi]
10. Dai J, Cai H, Li H, et al. Association between telomere length and survival in patients with idiopathic pulmonary fibrosis. *Respirology*. 2015;20(6):947-952. doi:10.1111/resp.12566 [doi]
11. Van Batenburg AA, Kazemier KM, Van Oosterhout MFM, et al. From organ to cell: Multi-level telomere length assessment in patients with idiopathic pulmonary fibrosis. *PLoS One*. 2020;15(1):e0226785. doi:10.1371/JOURNAL.PONE.0226785
12. Xin ZT, Beauchamp AD, Calado RT, et al. Functional characterization of natural telomerase mutations found in patients with hematologic disorders. *Blood*. 2007;109(2):524-532. doi:10.1182/blood-2006-07-035089
13. Ziegler P, Schrezenmeier H, Akkad J, et al. Telomere elongation and clinical response to androgen treatment in a patient with aplastic anemia and a heterozygous hTERT gene mutation. *Ann Hematol*. 2012;91(7):1115-1120. doi:10.1007/s00277-012-1454-x [doi]
14. Islam A, Rafiq S, Kirwan M, et al. Haematological recovery in dyskeratosis congenita patients treated with danazol. *Br J Haematol*. 2013;162(6):854-856. doi:10.1111/bjh.12432 [doi]
15. Khincha PP, Wentzensen IM, Giri N, Alter BP, Savage SA. Response to androgen therapy in patients with dyskeratosis congenita. *Br J Haematol*. 2014;165(3):349-357. doi:10.1111/bjh.12748 [doi]
16. Zlateska B, Ciccolini A, Dror Y. Treatment of dyskeratosis congenita-associated pulmonary fibrosis with danazol. *Pediatr Pulmonol*. June 2015. doi:10.1002/ppul.23235 [doi]
17. Kirschner M, Vieri M, Kricheldorf K, et al. Androgen derivatives improve blood counts and elongate telomere length in adult cryptic dyskeratosis congenita. *Br J Haematol*. 2021;193(3):669-673. doi:10.1111/BJH.16997

18. Clé D V, Catto LF, Darrigo LG, et al. Telomere Elongation and Clinical Improvement in Telomeropathy Patients: A Prospective Clinical Trial of Nandrolone in Telomeropathies. *Blood*. 2019;134(Supplement_1):2501-2501. doi:10.1182/BLOOD-2019-130844

19. Townsley DM, Dumitriu B, Liu D, et al. Danazol Treatment for Telomere Diseases. *N Engl J Med*. 2016;374(20):1922-1931. doi:10.1056/NEJMoa1515319 [doi]

20. Townsley DM, Dumitriu B, Young NS. Danazol Treatment for Telomere Diseases. *N Engl J Med*. 2016;375(11):1095-1096. doi:10.1056/NEJMc1607752 [doi]

21. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;30(10):e47.

22. Van der Auwera GA, Carneiro MO, Hartl C, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinforma*. 2013;43:11.10.1-33. doi:10.1002/0471250953.bi1110s43 [doi]

23. Hoffman TW, van der Vis JJ, Biesma DH, Grutters JC, van Moorsel CHM. Extrapulmonary manifestations of a telomere syndrome in patients with idiopathic pulmonary fibrosis are associated with decreased survival. *Respirology*. 2022;27(11):959-965. doi:10.1111/RESP.14264

24. MacKintosh JA, Pietsch M, Lutzky V, et al. TELO-SCOPE study: a randomised, double-blind, placebo-controlled, phase 2 trial of danazol for short telomere related pulmonary fibrosis. *BMJ Open Respir Res*. 2021;8(1):e001127. doi:10.1136/BMJRESP-2021-001127

25. Guo C, Armbruster BN, Price DT, Counter CM. In vivo regulation of hTERT expression and telomerase activity by androgen. *J Urol*. 2003;170(2 Pt 1):615-618. doi:10.1097/01.ju.0000074653.22766.c8 [doi]

26. Bayne S, Liu JP. Hormones and growth factors regulate telomerase activity in ageing and cancer. *Mol Cell Endocrinol*. 2005;240(1-2):11-22. doi:10.1016/j.mce.2005.05.009

27. Calado RT, Yewdell WT, Wilkerson KL, et al. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. *Blood*. 2009;114(11):2236-2243. doi:10.1182/blood-2008-09-178871 [doi]

28. Vieri M, Kirschner M, Tometten M, et al. Comparable Effects of the Androgen Derivatives Danazol, Oxymetholone and Nandrolone on Telomerase Activity in Human Primary Hematopoietic Cells from Patients with Dyskeratosis Congenita. *Int J Mol Sci*. 2020;21(19):1-12. doi:10.3390/IJMS21197196

29. Nanni S, Narducci M, Della Pietra L, et al. Signaling through estrogen receptors modulates telomerase activity in human prostate cancer. *J Clin Invest*. 2002;110(2):219-227. doi:10.1172/JCI15552 [doi]

30. Fang C, Huang H, Zhang Q, et al. Relation between sex hormones and leucocyte telomere length in men with idiopathic pulmonary fibrosis. *Respirology*. 2020;25(12):1265-1273. doi:10.1111/RESP.13871

31. Duckworth A, Ruth KS, Prague JK, et al. Study of the associations between short telomeres, sex hormones and pulmonary fibrosis. *medRxiv*. September 2022:2022.09.29.22280270. doi:10.1101/2022.09.29.22280270

32. Chambers DC, Lutzky VP, Apte SH, Godbolt D, Feenstra J, Mackintosh J. Successful treatment of telomeropathy-related interstitial lung disease with immunosuppression and danazol. *Respirol Case Reports*. 2020;8(6):e00607. doi:10.1002/RCR2.607

33. Dewald J, Attia S, Schriver E, Dudney T, Kravitz J. Progression of Pulmonary Fibrosis in a Patient With Telomere Disease Previously Treated With Danazol. *Chest*. 2017;152(4):A430. doi:10.1016/j.chest.2017.08.457

SUPPLEMENTARY DATA

Supplementary Table 1: Outcomes stratified by leukocyte telomere length below or above the 10th percentile for age

	Leukocyte telomere length <10th percentile for age (N=22)	**Leukocyte telomere length >10th percentile for age (N=21)**	***p*-value**
Any side effects (%)	14 (64)	13 (62)	1.00
Stopped because of side effects (%)	10 (45)	11 (52)	0.76
Any dose adjustments (%)	7 (32)	7 (33)	1.00
Still using danazol >1 year after start (%)	7 (32)	3 (14)	0.28
Mean FVC-decline after one year, mL (95% confidence interval)	491 (281 - 702)	468 (-30 - 967)	0.93

Supplementary Table 2: Outcomes stratified by presence or absence of any clinical suggestion of a telomere syndrome

	Any clinical suggestion of a telomere syndrome (N=18)	**No clinical suggestion of a telomere syndrome (N=32)**	***p*-value**
Any side effects (%)	12 (67)	17 (53)	0.39
Stopped because of side effects (%)	10 (56)	13 (41)	0.38
Any dose adjustments (%)	5 (28)	9 (28)	1.00
Still using danazol >1 year after start (%)	3 (17)	8 (25)	0.72
Mean FVC-decline after one year, mL (95% confidence interval)	392 (167 - 616)	500 (163 - 837)	0.65

Chapter 10

Towards treatable traits for pulmonary fibrosis

T.W. Hoffman, and J.C. Grutters

ABSTRACT

Interstitial lung diseases (ILD) are a heterogeneous group of disorders, of which many have the potential to lead to progressive pulmonary fibrosis. A distinction is usually made between primarily inflammatory ILD and primarily fibrotic ILD. As recent studies show that anti-fibrotic drugs can be beneficial in patients with primarily inflammatory ILD that is characterized by progressive pulmonary fibrosis, treatment decisions have become more complicated. In this perspective we propose that the 'treatable trait' concept, which is based on the recognition of relevant exposures, various treatable phenotypes (disease manifestations) or endotypes (shared molecular mechanisms) within a group of diseases, can be applied to progressive pulmonary fibrosis. These targets for medical intervention can be identified through validated biomarkers and are not necessarily related to specific diagnostic labels. Proposed treatable traits are: cigarette smoking, occupational, allergen or drug exposures, excessive (profibrotic) auto- or alloimmunity, progressive pulmonary fibrosis, pulmonary hypertension, obstructive sleep apnoea, tuberculosis, exercise intolerance, exertional hypoxia, and anxiety and depression. There are also several potential traits that have not been associated with relevant outcomes or for which no effective treatment is available at present: air pollution, mechanical stress, viral infections, bacterial burden in the lungs, surfactant-related pulmonary fibrosis, telomere-related pulmonary fibrosis, the rs35705950 *MUC5B* promoter polymorphism, acute exacerbations, gastro-oesophageal reflux, dyspnoea, and nocturnal hypoxia. The 'treatable traits' concept can be applied in new clinical trials for patients with progressive pulmonary fibrosis and could be used for developing new treatment strategies.

INTRODUCTION

The current classification of human diseases, which is based on disease manifestations in specific organ systems with different physiological, anatomical and histopathological correlates, does not seem to capture disease complexity for many types of diseases.[1,2] The definition of clinical phenotypes or endotypes, permitting the identification of distinct disease attributes that differentially respond to treatment in patients with the same type of disease, does not fully capture the biological complexities that lead to disease.[2] The 'treatable traits' concept has been proposed by Agusti for use in airway disease, and is distinct from endotyping or phenotyping.[1,3] Instead of requiring mutually exclusive phenotypes or endotypes, the 'treatable traits' concept can accommodate overlap between multiple traits in the same individual, regardless of a classifying diagnosis. Treatable traits should be clinically relevant, identifiable and measurable through the use of validated biomarkers, and treatable.[1,2] This article explores how the 'treatable traits' concept can be applied to pulmonary fibrosis, a subgroup of interstitial lung diseases (ILD).

Histopathologically, ILD are characterized by either predominant inflammation or fibrosis, and, in the past, it has been proposed that they can be placed on an inflammation-fibrosis spectrum.[4] Diseases on one end of the spectrum, such as organizing pneumonia (OP), were considered to be solely inflammatory diseases, to be treated with immunosuppressive drugs. Diseases on the other end of the spectrum, such as IPF, were considered to be dominant fibrotic diseases, to be treated with anti-fibrotic drugs instead. Albeit helpful, the spectrum does not always capture the underlying ILD pathophysiology. Patients with the same type of ILD can show a wide range of disease behaviour, varying from mild and stable to severe and rapidly progressive disease, including the potential for acute exacerbations of ILD. Patients with primarily inflammatory disease can also develop pulmonary fibrosis. In recent years there has been increased attention for the so-called progressive pulmonary fibrosis phenotype in patients with various types of ILD.[5] Several studies now suggest that anti-fibrotic drugs can be beneficial in patients with ILD that are usually thought to be on the inflammatory side of the spectrum such as ILD related to connective tissue disease (CTD-ILD), hypersensitivity pneumonitis (HP), sarcoidosis, and idiopathic non-specific interstitial pneumonia (NSIP)[6–9] Apart from the previously mentioned diseases, other diseases that can manifest as progressive pulmonary fibrosis include pleuraparenchymal fibroelastosis, fibrosing organizing pneumonia, desquamative interstitial pneumonia, occupational interstitial lung disease, such as asbestosis or pneumoconiosis, pulmonary Langerhans cell histiocytosis, pulmonary alveolar proteinosis, and unclassifiable interstitial lung disease.[10] A recent update of the Clinical Practice Guideline on diagnosis and treatment of IPF recommends anti-fibrotic treatment for patients with

progressive pulmonary fibrosis in ILD other than IPF.[10] Consequently, developing personalized treatment strategies has become even more important.

It has been stated previously that treatable traits might at some point be identified in patients with progressive pulmonary fibrosis, but a concrete framework has not been developed.[11] In this perspective we propose that the 'treatable traits' concept can be applied to progressive pulmonary fibrosis and propose concrete treatable traits, as well as potential treatable traits. We think that the 'treatable traits' concept better reflects the complexity of pulmonary fibrosis pathophysiology and can be especially useful for the development of novel treatment strategies for patients with progressive pulmonary fibrosis.

APPLICATION OF THE 'TREATABLE TRAITS' CONCEPT IN FIBROTIC ILD

We propose the following treatable traits for patients with progressive pulmonary fibrosis (Table 1): cigarette smoking, occupational, allergen or drug exposures, excessive (profibrotic) auto- or alloimmunity, progressive fibrosis, pulmonary hypertension, obstructive sleep apnoea, tuberculosis, exercise intolerance, exertional hypoxia, and anxiety and depression. As has also been suggested for bronchiectasis,[12] we have classified the traits into environmental factors, pulmonary disease, comorbidities and functional traits.

Environmental traits

Cigarette smoking is a known risk factor for IPF, with evidence of a dose-response relationship.[13] Furthermore, there are several other ILD with a very strong etiological link to cigarette smoking: such as pulmonary Langerhans cell histiocytosis, respiratory bronchiolitis-associated ILD, and desquamative interstitial pneumonia. For patients with IPF initial observations suggested that smoking is associated with a better prognosis, but this is likely related to the 'healthy-smoker effect', which represents less severe disease at diagnosis. When correcting for disease severity, survival actually seems to be worse in patients with IPF who are current smokers.[14] The detrimental effect of smoking on survival was recently demonstrated in patients with progressive pulmonary fibrosis due to non-IPF ILD.[15] There have been no randomized controlled trials investigating the effect of smoking cessation in patients with ILD, but smoking cessation alone has been associated with clear improvement in some patients with smoking-related ILD[13] and is likely beneficial for patients with all types of pulmonary fibrosis.

Numerous other exposures that can cause pulmonary fibrosis have been described, including radiation, asbestos, metal dust, wood dust, and various drugs.[46–51] Alternatively, many

Table 1: treatable traits in patients with pulmonary fibrosis.

Trait	Association with outcomes	How to detect	How to intervene	Future avenues for detection and treatment
Environmental				
Cigarette smoking	Probably associated with worse survival in patients with IPF and other progressive fibrotic ILD, [13–15] associated with disease progression in PLCH[16] and other smoking-related ILD	Smoking history	Smoking cessation	-
Occupational, allergen, or drug exposures	Ongoing exposure is probably related to survival in fibrotic HP[17–20] and to disease progression in pneumoconiosis[21] and drug-induced ILD[22]	Occupational, exposure, and drug history, serum IgG testing targeting potential antigens for HP[23]	Avoid relevant exposures	Development and validation of exposure questionnaire
Pulmonary				
Excessive (profibrotic) auto- or alloimmunity	CTD-ILD or HP has more favourable prognosis than IPF[24,25]	Established diagnosis of CTD, established diagnosis of HP, features suggestive of auto-immune disease, but no formal CTD diagnosis (features consistent with IPAF in clinical, serological or morphological domain)[26]	Immunosuppressive drugs[25,27]	Determine whether combination therapy of immunosuppressive drugs and antifibrotic treatment is warranted for certain patients, determine whether patients with certain features consistent with IPAF benefit from immunosuppressive treatment; investigate whether other circulating auto-antibodies can be used as a marker to give immunosuppressive therapy

Table 1: treatable traits in patients with pulmonary fibrosis. (continued)

Trait	Association with outcomes	How to detect	How to intervene	Future avenues for detection and treatment
Progressive fibrosis	Associated with increased mortality[28]	Two out of three of: worsening respiratory symptoms, physiological evidence of disease progression (absolute decline in FVC ≥5% of predicted within 1 year of follow up or absolute decline in DLCOc ≥10% of predicted within 1 year), or radiographical evidence of disease progression (increased extent or severity of traction bronchiectasis or bronchioloectasis, or new ground-glass opacity with traction bronchiectasis, or new fine reticulation, or increased extent or increased coarseness of reticular abnormality, or new or increased honeycombing, or increased lobar volume loss).[10]	Anti-fibrotic drugs[6–9], lung transplantation	Develop new radiological, histopathological, blood, BAL, or exhaled air biomarkers; develop novel strategies to replace fibrotic tissue with healthy tissue[29]
Comorbidities				
Pulmonary Hypertension	Associated with worse survival in patients with IPF[30]	Echocardiography, right heart catheterization	Consider inhaled treprostinil (associated with improved exercise capacity)[31]; consider PH-targeted therapy in patients with CTD-ILD and possible pulmonary arterial hypertension[32]; lung transplantation	Determine whether Treprostinil or inhaled nitric oxide leads to decreased mortality
Obstructive sleep apnoea	Associated with decreased survival in patients with IPF[33,34]	Polysomnography	CPAP[35,36]	Determine how screening for obstructive sleep apnoea can be implemented

Table 1: treatable traits in patients with pulmonary fibrosis. (continued)

Trait	Association with outcomes	How to detect	How to intervene	Future avenues for detection and treatment
Tuberculosis	Associated with decreased survival in patients with pneumoconiosis,[37,38] might be associated with progression of IPF[39]	Can be suggested by CT-scan abnormalitites,[40] diagnosis by sputum or bronchial washing mycobacterial culture, molecular diagnostic tests[41]	Tuberculosis treatment depending on drug-sensitivity pattern	Determine if standard treatment regimens should be extended, determine if latent tuberculosis should be screened for
Functional				
Exercise intolerance	Reduced quality of life[42]	6-minute walking test	Pulmonary rehabilitation[42]	Further development of specific pulmonary rehabilitation programs
Exertional hypoxia	Reduced exercise tolerance	Exercise testing (transcutaneous oxygen saturation ≤88% on 6-minute walking test)	Ambulatory oxygen suppletion[43]	Optimize oxygen-delivery system
Anxiety and depression	Reduced quality of life	Hospital Anxiety and Depression Scale[44]	Palliative care intervention including assessment, care plan, and community case conference[45]	Further development of interventions to treat anxiety and depression

BAL = bronchoalveolar lavage; CPAP = continuous positive airway pressure; CTD-ILD = connective tissue disease-associated ILD; DLCOc = diffusion capacity of the lung for carbon monoxide, corrected for blood haemoglobin level; FVC = forced vital capacity; HP = hypersensitivity pneumonitis; ILD = interstitial lung disease; IPAF = interstitial pneumonia with autoimmune features; IPF = idiopathic pulmonary fibrosis; PH = pulmonary hypertension; PLCH = pulmonary Langerhans cell histiocytosis

organic and inorganic compounds are also capable of inducing a lymphocyte-dependent immune response, which causes hypersensitivity pneumonitis. In these cases, it is not the offending antigen per se, but the local inflammatory response that can serve as a trigger for the fibrogenic response.[52] Continuous exposure to the offending agent has been associated with disease progression in HP, pneumoconiosis, and drug-induced ILD.[17–22] No randomized controlled trials of exposure avoidance have been performed, but removal of the offending exposure is very likely to be beneficial for all patients with pulmonary fibrosis related to specific exposures.

Pulmonary traits

Persistent inflammation can be a feed-forward mechanism in pulmonary fibrosis. Several types of pulmonary fibrosis, such as CTD-ILD and HP are known to be caused by excessive auto- or allo-inflammation.[25,27] Previous work has shown that alveolar macrophages from patients with several types of fibrotic lung disease show increased expression of the pro-Th2-inflammatory cytokine CCL 18, which seems to represent a feed-forward mechanism in the fibrotic process.[53] CCL18 expression has been associated with survival in patients with IPF and patients with CTD-ILD due to SSc.[54–56] Gene expression studies performed on peripheral blood also support the role of inflammatory processes in progression of IPF,[57–60] and in IPF lungs there is increased expression of genes related to inflammatory processes in fibrotic and non-fibrotic areas.[61] In a small cohort study, genes related to inflammatory processes were also upregulated in patients with fibrotic sarcoidosis and hypersensitivity pneumonitis.[62] Furthermore, it has been suggested that auto-inflammation might play a role in disease progression for patients with IPF, as increased numbers of circulating IgA auto-antibodies correlated with disease severity.[63]

A caveat here is that inflammation is a broad concept, and Inflammatory processes are a necessary part of the normal response to tissue damage and the normal fibrotic response.[64] Elevated markers of inflammation might simply represent more fibrosis, and do not necessarily imply a causative role for the inflammatory process in fibrosis progression. The presence of inflammatory cells in regions of tissue fibrosis can even be interpreted as their being part of an antifibrotic feedback loop.[65] The contribution of the immune system to the pathogenesis of pulmonary fibrosis is likely to be nuanced, as different immune cells can have either profibrotic or antifibrotic functions.[66] It will have to be determined what novel markers are able to distinguish a ‘normal’ inflammatory response from inflammation that is pathogenic and should be treated with anti-inflammatory drugs. At this time, anti-inflammatory treatment is known to be useful for patients with CTD-ILD, HP, and sarcoidosis, and can be considered for patients with idiopathic NSIP, but not in patients with IPF.[25,27,67,68]

In recent years the progressive pulmonary fibrosis phenotype has been defined and is increasingly recognized. Independent of the underlying diagnosis, progressive pulmonary fibrosis despite optimal treatment is associated with a worse prognosis.[28] Progressive pulmonary fibrosis is defined as at least two out of three of: worsening symptoms, radiographic progression, and physiological progression.[10] Predictors of progressive pulmonary fibrosis include greater impairment in lung function, more extensive disease on CT-imaging, and the presence of honeycombing or a UIP pattern on imaging.[5] Several trials have now shown a benefit of treatment with the antifibrotic agents pirfenidone and nintedanib in patients with progressive pulmonary fibrosis,[6–9] and this was consistent across subgroups of patients with different underlying diagnoses.[69]

Comorbidities

Many different comorbidities can be found in patients with pulmonary fibrosis, but we have now only included comorbid conditions for which we think specific screening is warranted at this point. This means that a specific test is available to diagnose these conditions, and that effective treatment is available. Other comorbid conditions might be added in the future. Pulmonary hypertension is commonly observed in patients with pulmonary fibrosis, with the prevalence depending on the underlying diagnosis and the characteristics of the studied cohort.[30] For example, in patients with IPF who are listed for lung transplantation, the prevalence of pulmonary hypertension varies from 18-51%.[30] In patients with IPF, pulmonary hypertension is associated with shorter survival.[30] International treatment guidelines recommend treatment with pulmonary hypertension specific medication for patients with connective tissue disease and (possible) group 1 pulmonary hypertension (i.e. pulmonary hypertension due to inflammatory vasculopathy).[32] The clinical practice guideline for IPF recommends against the use of pulmonary hypertension specific therapy for patients with IPF, and a recent trial failed to find a benefit of adding the pulmonary hypertension specific drug sildenafil to pirfenidone for patients with IPF[70,71]. A small randomized controlled trial suggests that inhaled nitric oxide might be beneficial in patients with pulmonary fibrosis complicated by pulmonary hypertension, but only used physical activity markers as outcome measures.[72] A recent large randomized controlled trial of inhaled Treprostinil has showed that this leads to increased exercise tolerance in patients with group 3 pulmonary hypertension (i.e. pulmonary hypertension secondary to lung disease),[31] but it is not known if this improves survival.

Obstructive sleep apnoea (OSA) has been observed in 32-85% of patients with IPF,[33] but the prevalence in patients with other types of pulmonary fibrosis is not well characterized. OSA in patients with IPF is associated with decreased survival[34], as well as the development of pulmonary hypertension.[33] Treatment of OSA with continuous positive airway pressure (CPAP) was associated with improved quality of life in patients with good treat-

ment adherence, and might lead to better survival.[35,36] How screening for OSA can best be implemented for patients with pulmonary fibrosis remains to be determined.

Tuberculosis is a very common disease worldwide, although the prevalence in most Western countries is low.[41] It has been shown that patients with pneumoconiosis are at an especially high risk to develop active tuberculosis, and this is associated with worse survival.[37,38] In patients with IPF, the prevalence of active tuberculosis seems to be higher than in the general population, and this might also be associated with worse survival.[39] The presence of active tuberculosis may be suggested by radiographic abnormalities such as nodular abnormalities or consolidations, but can be difficult to distinguish from the fibrotic disease.[40,73] It remains to be determined if standard treatment regimens for tuberculosis should be extended for patients with pulmonary fibrosis, and if patients with pulmonary fibrosis would benefit from screening for and treatment of latent tuberculosis.

Functional traits

Exercise intolerance has been associated with decreased quality of life in patients with ILD, and this can be improved by pulmonary rehabilitation.[42] Exertional hypoxia also leads to reduced exercise tolerance in patients with pulmonary fibrosis, and can be treated with supplemental oxygen during exercise.[43] It remains to be determined what the optimal pulmonary rehabilitation programme is and what the best oxygen delivery system is. A randomized controlled trial of oxygen suppletion via a portable concentrator in patients with IPF is planned[74,75]

Clinically relevant anxiety and depression are found in 12 and 7% of patients with interstitial lung disease,[44] and are associated with reduced quality of life.[76] A palliative care intervention that included a comprehensive assessment, care plan, and community case conference has been shown to improve anxiety and depression in patients with pulmonary fibrosis and caregivers.[45] Further development of care packages and trials of anti-anxiety medication in patients with pulmonary fibrosis are warranted.

Future avenues for detection and treatment of treatable traits

As indicated in the table, several markers to distinguish these traits already exist. However, novel biomarkers will need to be developed to better identify the traits and associated response to treatment. In recent years the term interstitial pneumonia with auto-immune features (IPAF) has been introduced in the context of research, and this might be a promising start to identify the inflammation trait, including distinguishing between normal or physiological inflammation and excessive or pathological inflammation.[26] Other promising avenues include the use of artificial intelligence for interpreting radiological imaging, as well as the study of new biomarkers in peripheral blood, BAL fluid, or exhaled air.[77,78,87,79–86]

Table 2: traits in patients with pulmonary fibrosis that are not (yet) treatable.

Trait	Association with outcomes	How to detect	Potential avenues for treatment
Environmental			
Air pollution	Associated with AE-IPF and progression of IPF[48,91–95]	Air quality monitoring, exposure history; no clear threshold for too much exposure	Possibly improve air quality, avoid exposure to bad quality air
Pulmonary			
Mechanical stress	Continuous mechanical stress is hypothesized to contribute to disease progression in patients with IPF[96,97], mechanical ventilation of patients with IPF is associated with high mortality[98,99]	Not clear	Avoid mechanical ventilation, possibly develop novel methods to decrease mechanical tension on alveoli
Viral infections	Potential role of human herpes viruses as a co-factor in initiation and progression of IPF[100,101]	Viral PCR on bronchoalveolar lavage fluid	Possibly antiviral treatment; no randomized controlled trials have been done yet
Bacterial burden in lungs	Bacterial burden in lower airways associates with disease progression in patients with IPF[51,102]	16s rRNA gene qPCR on bronchoalveolar lavage fluid	Possibly antibiotic treatment, vaccination; notably, treatment with cotrimoxazole or doxycycline had no effect on mortality or disease progression in patients with IPF[103–105]
Surfactant-related pulmonary fibrosis	Surfactant-related pulmonary fibrosis[106], higher risk of lung cancer in patients with *SFTPA2* gene mutations[107]	Mutations in *SFTPA1*, *SFTPA2*, *SFTPC*, *ABCA3*, *HPS, NKX2-1*	Development of novel treatments such as potentiators or gene-based therapy to correct surfactant processing[106], ABCA3 correction using cyclosporin A[108]
Telomere-related pulmonary fibrosis	Short leukocyte telomere length is associated with worse survival in patients with IPF or IPAF[109–111]; mutations in telomere-related genes are associated with a worse prognosis in patients with pulmonary fibrosis[112]	Telomere gene mutations, very short leukocyte telomere length[113]	Investigate anti-aging and telomere lengthening treatments such as dasatinib/quercetin,[114] danazol,[115] telomerase transfection[116]
rs35705950 *MUC5B* promoter polymorphism	Possibly associated with better survival in patients with IPF[117,118] and NSIP[119]	Genotyping of rs35705950 *MUC5B* promoter polymorphism	Investigate whether treatment with N-acetylcysteine,[120] P-2119,[121] or other mucolytics is effective

Table 2: traits in patients with pulmonary fibrosis that are not (yet) treatable. (continued)

Trait	Association with outcomes	How to detect	Potential avenues for treatment
Acute exacerbation	Very poor prognosis in various types of ILD[122]		Further investigation of factors predicting acute exacerbation of pulmonary fibrosis such as lymphocytosis in bronchoalveolar lavage fluid[123]; investigate novel treatments
Comorbidities			
Gastro-oesophageal reflux	Possibly associated with acute-exacerbations or disease progression in patients with IPF[124]	24-hour pH monitoring, patient history	Antacid therapy might be helpful and was conditionally recommended in IPF treatment guidelines[125], however there are increasing signals that this is not effective[126], and it is no longer recommended in updated guidelines,[10] laparoscopic fundoplication was not found to affect disease progression or mortality in patients with IPF in a small randomized controlled trial[127]
Functional			
Dyspnoea	Reduced quality of life[128]	Clinical history	Investigate whether benzodiazepines and or opioids can safely be used for symptom relief
Nocturnal hypoxia	Early mortality[129]	Polysomnography	Investigate efficacy of nocturnal oxygen suppletion

AE-IPF = acute exacerbation of IPF; ILD = interstitial lung disease; IPAF = interstitial pneumonia with autoimmune features; IPF = idiopathic pulmonary fibrosis; PCR = polymerase chain reaction

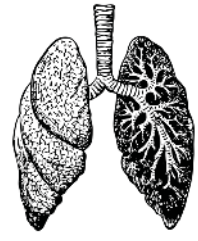
Several new insights have been derived from single-cell transcriptomic analysis of fibrotic lung tissue, and this could lead to the identification and targeting of novel pathways involved in the fibrotic process.[88] Molecular classifiers derived from gene expression signatures in transbronchial biopsies can potentially be used to determine which non-IPF ILD patients will have progressive pulmonary fibrosis, without having to wait for the fibrosis to actually be progressive.[89,90] Therapeutic trials based on these markers will help guide treatment of patients with pulmonary fibrosis in the future.

Development of novel treatments within the treatable traits framework

The treatable traits framework will also be useful for the development and evaluation of novel treatments for patients with progressive pulmonary fibrosis. Table 2 presents several possible traits that have been identified in patients with fibrotic ILD, but for which no clear association with relevant outcomes, clear method for detection, or proven treatment exists. This includes air pollution as an environmental trait, mechanical stress to the lungs, viral infections, bacterial burden in the lungs, surfactant-related pulmonary fibrosis, telomere-related pulmonary fibrosis, the rs35705950 *MUC5B* promoter polymorphism, and acute exacerbations of pulmonary fibrosis as pulmonary traits, gastro-oesophageal reflux as a comorbidity, and dyspnoea and nocturnal hypoxia as functional treats, although the latter could also be related to or grouped with OSA. An overview of all proposed treatable traits, as well as potential treatable traits, is provided in Figure 1.

Pulmonary traits
Excessive (profibrotic) auto- or alloimmunity
Progressive pulmonary fibrosis
Mechanical stress
Viral infections
Bacterial burden in the lungs
Surfactant-related pulmonary fibrosis
Telomere-related pulmonary fibrosis
rs35705950 *MUC5B* promoter polymorphism
Acute exacerbations

Environmental traits
Exposure to cigarette smoke
Occupational, allergen, or drug exposure
Exposure to air pollution



Comorbidities
Pulmonary hypertension
Obstructive sleep apnoea
Tuberculosis
Gastro-oesophageal reflux

Functional traits
Exercise intolerance
Exertional hypoxia
Anxiety and depression
Dyspnoea
Nocturnal hypoxia

Figure 1: proposed treatable traits for progressive pulmonary fibrosis, divided into pulmonary, environmental, functional, and comorbidity domains. Traits for which either a clear association with relevant outcomes, a clear method for measuring the trait, or a proven treatment is not available are marked in grey.

DISCUSSION

Treatable traits are not yet a proven concept for management of progressive pulmonary fibrosis, and much will need to be done in order for this to happen. Some key points are presented in Box 1. There are also several other caveats to the treatable traits approach. First, not all traits presented here might be treatable in the foreseeable future, although fascinating new developments such as high-content screening using in-vitro cell-based assays might give unexpected acceleration.[108] Of course, the proposed set of traits is not fixed, and traits could be changed, added, or removed depending on future discoveries. Second, if many traits are implicated in the same patient, this could lead to a prohibitively complicated cocktail of therapies. Third, the traits can potentially be present to different degrees at different stages of the disease, and determining at which degree treatment for a given trait should be started could be difficult in practice. Finally, adopting a new classification will always be confusing initially, and should only be done when the clinical benefits are large enough to justify this.[130] Despite this, we think it is still worthwhile to further develop the treatable traits concept for pulmonary fibrosis. This approach is quite flexible, and the treatable traits model can be very useful to capture evolution of the disease process with time in an individual patient.

- Create larger patient cohorts through international collaboration, and study markers for disease behaviour in non-IPF fibrotic ILD
- Investigate which markers can differentiate 'normal' inflammation related to the fibrotic process from 'excessive' inflammation that enhances the fibrotic process, and whether immunosuppressive therapy is beneficial in patients with IPAF
- Investigate in which patients immunosuppressive therapy is effective in addition to antifibrotic therapy
- Develop targeted therapies for fibrotic ILD patients with genetic abnormalities
- Investigate whether response to targeted therapies can be predicted by serum, BAL, or exhaled air biomarkers
- Investigate the value of molecular classifiers based on gene expression in transbronchial lung biopsies for identifying treatable traits
- Investigate the value of machine learning on radiological imaging for identifying treatable traits

Box 1: developments necessary for the use of treatable traits for progressive pulmonary fibrosis in clinical practice

CONCLUSION

In conclusion, there are many types of fibrotic lung disease, and there is large clinical variation in clinical presentation and disease course, even between patients with the same diagnosis under the current classification scheme. There are clear differences between the various diagnoses, but several processes seem to regularly play a role in patients with different types of progressive pulmonary fibrosis. These processes can be designated as treatable traits, which can be applied to all patients with progressive pulmonary fibrosis. Treatable traits are: cigarette smoking, occupational, allergen or drug exposures, excessive (profibrotic) auto- or alloimmunity, progressive fibrosis, pulmonary hypertension, obstructive sleep apnoea, tuberculosis, exercise intolerance, exertional hypoxia, and anxiety and depression. There are also several potential traits that have not been associated with relevant outcomes or for which no effective treatment is available at present: air pollution, mechanical stress, viral infections, bacterial burden in the lungs, surfactant-related pulmonary fibrosis, telomere-related pulmonary fibrosis, the rs35705950 *MUC5B* promoter polymorphism, acute exacerbations, gastro-oesophageal reflux, dyspnoea, and nocturnal hypoxia.

This article tries to provide a comprehensive framework for viewing all types of progressive pulmonary fibrosis. There are several advantages of the treatable traits model: it is a flexible model, obviates the need to identify a single or several ultimate causes for pulmonary fibrosis, allows for the co-existence of multiple traits within the same patient, and allows for the existence of different traits at different times during the disease course. Furthermore, using treatable traits would make it easier to include patients with very rare or unclassifiable forms of pulmonary fibrosis under the present classification system in future clinical trials. In addition, the use of combination treatment for patients with progressive pulmonary fibrosis can be directed by the concept of treatable traits. This can pave the way for precision medicine in pulmonary fibrosis.

REFERENCES

1. Agusti A, Bel E, Thomas M, et al. Treatable traits: Toward precision medicine of chronic airway diseases. *Eur Respir J*. 2016;47(2):410-419. doi:10.1183/13993003.01359-2015
2. McDonald VM, Fingleton J, Agusti A, et al. Treatable traits: a new paradigm for 21st century management of chronic airway diseases: Treatable Traits Down Under International Workshop report. *Eur Respir J*. 2019;53(5):1802058. doi:10.1183/13993003.02058-2018
3. Agustí A. Phenotypes and disease characterization in chronic obstructive pulmonary disease: Toward the extinction of phenotypes? *Ann Am Thorac Soc*. 2013;10(SUPPL). doi:10.1513/AnnalsATS.201303-055AW
4. Mikolasch TA, Garthwaite HS, Porter JC. Update in diagnosis and management of interstitial lung Disease. *Clin Med J R Coll Physicians London*. 2017;17(2):146-153. doi:10.7861/clinmedicine.17-2-146
5. Wijsenbeek M, Cottin V. Spectrum of Fibrotic Lung Diseases. *N Engl J Med*. 2020;8(Table 1):958-968. doi:10.1056/NEJMra2005230
6. Flaherty KR, Wells AU, Cottin V, et al. Nintedanib in progressive fibrosing interstitial lung diseases. *N Engl J Med*. 2019;381(18):1718-1727. doi:10.1056/NEJMoa1908681
7. Distler O, Highland KB, Gahlemann M, et al. Nintedanib for systemic sclerosis-associated interstitial lung disease. *N Engl J Med*. 2019;380(26):2518-2528. doi:10.1056/NEJMoa1903076
8. Maher TM, Corte TJ, Fischer A, et al. Pirfenidone in patients with unclassifiable progressive fibrosing interstitial lung disease: a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Respir Med*. 2020;8(2):147-157. doi:10.1016/S2213-2600(19)30341-8
9. Behr J, Prasse A, Kreuter M, et al. Pirfenidone in patients with progressive fibrotic interstitial lung diseases other than idiopathic pulmonary fibrosis (RELIEF): a double-blind, randomised, placebo-controlled, phase 2b trial. *Lancet Respir Med*. 2021;9(5):476-486. doi:10.1016/S2213-2600(20)30554-3
10. Raghu G, Remy-Jardin M, Richeldi L, et al. Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults: An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2022;205(9):E18-E47. doi:10.1164/RCCM.202202-0399ST
11. Wongkarnjana A, Scallan C, Kolb MRJ. Progressive fibrosing interstitial lung disease: treatable traits and therapeutic strategies . *Curr Opin Pulm Med*. 2020;xx(xx):xx-xx. doi:10.1097/MCP.0000000000000712
12. Boaventura R, Sibila O, Agusti A, Chalmers JD. Treatable traits in bronchiectasis. *Eur Respir J*. 2018;52(3). doi:10.1183/13993003.01269-2018
13. Margaritopoulos GA, Vasarmidi E, Jacob J, Wells AU, Antoniou KM. Smoking and interstitial lung diseases. *Eur Respir Rev*. 2015;24(137):428-435. doi:10.1183/16000617.0050-2015
14. Antoniou KM, Hansell DM, Rubens MB, et al. Idiopathic Pulmonary Fibrosis: Outcome in Relation to Smoking Status. *Am J Respir Crit Care Med*. 2007;177(2):190-194. doi:10.1164/RCCM.200612-1759OC
15. Platenburg MGJP, van der Vis JJ, Kazemier KM, Grutters JC, van Moorsel CHM. The detrimental effect of quantity of smoking on survival in progressive fibrosing ILD. *Respir Med*. 2022;194. doi:10.1016/J.RMED.2022.106760

16. Tazi A, De Margerie C, Naccache JM, et al. The natural history of adult pulmonary Langerhans cell histiocytosis: A prospective multicentre study. *Orphanet J Rare Dis*. 2015;10(1):1-10. doi:10.1186/S13023-015-0249-2

17. De Sadeleer LJ, Hermans F, De Dycker E, et al. Effects of Corticosteroid Treatment and Antigen Avoidance in a Large Hypersensitivity Pneumonitis Cohort: A Single-Centre Cohort Study. *J Clin Med*. 2018;8(1). doi:10.3390/JCM8010014

18. Pérez ERF, Swigris JJ, Forssén A V., et al. Identifying an inciting antigen is associated with improved survival in patients with chronic hypersensitivity pneumonitis. *Chest*. 2013;144(5):1644-1651. doi:10.1378/CHEST.12-2685

19. Gimenez A, Storrer K, Kuranishi L, Soares MR, Ferreira RG, Pereira CAC. Change in FVC and survival in chronic fibrotic hypersensitivity pneumonitis. *Thorax*. 2018;73(4):391-392. doi:10.1136/THORAXJNL-2017-210035

20. Nishida T, Kawate E, Ishiguro T, Kanauchi T, Shimizu Y, Takayanagi N. Antigen avoidance and outcome of nonfibrotic and fibrotic hypersensitivity pneumonitis. *ERJ Open Res*. November 2021:00474-02021. doi:10.1183/23120541.00474-2021

21. Barber C, Fishwick D. Pneumoconiosis. *Medicine (Baltimore)*. 2020;48(6):417-421. doi:10.1016/J.MPMED.2020.03.012

22. Ratwani A, Gupta B, Stephenson BW, Mani H, Brown AW. The Spectrum of Drug-Induced Interstitial Lung Disease. *Curr Pulmonol Reports 2019 84*. 2019;8(4):139-150. doi:10.1007/S13665-019-00233-Y

23. Raghu G, Wilson KC, Bargagli E, et al. Diagnosis of Hypersensitivity Pneumonitis in Adults. An Official ATS/JRS/ALAT Clinical Practice Guideline. *https://doi.org/101164/rccm202005-2032ST*. 2020;202(3):E36-E69. doi:10.1164/RCCM.202005-2032ST

24. Cottin V. Significance of connective tissue diseases features in pulmonary fibrosis. *Eur Respir Rev*. 2013;22(129):273-280. doi:10.1183/09059180.00003013

25. Salisbury ML, Myers JL, Belloli EA, Kazerooni EA, Martinez FJ, Flaherty KR. Diagnosis and treatment of fibrotic hypersensitivity pneumonia: Where we stand and where we need to go. *Am J Respir Crit Care Med*. 2017;196(6):690-699. doi:10.1164/rccm.201608-1675PP

26. Graney BA, Fischer A. Interstitial pneumonia with autoimmune features. *Ann Am Thorac Soc*. 2019;16(5):525-533. doi:10.1513/AnnalsATS.201808-565CME

27. Vacchi C, Sebastiani M, Cassone G, et al. Therapeutic Options for the Treatment of Interstitial Lung Disease Related to Connective Tissue Diseases. A Narrative Review. *J Clin Med*. 2020;9(2):407. doi:10.3390/jcm9020407

28. George PM, Spagnolo P, Kreuter M, et al. Progressive fibrosing interstitial lung disease: clinical uncertainties, consensus recommendations, and research priorities. *Lancet Respir Med*. 2020;8(9):925-934. doi:10.1016/S2213-2600(20)30355-6

29. Horowitz JC, Thannickal VJ. Mechanisms for the resolution of organ fibrosis. *Physiology*. 2019;34(1):43-55. doi:10.1152/physiol.00033.2018

30. Ruffenach G, Hong J, Vaillancourt M, Medzikovic L, Eghbali M. Pulmonary hypertension secondary to pulmonary fibrosis: clinical data, histopathology and molecular insights. *Respir Res 2020 211*. 2020;21(1):1-14. doi:10.1186/S12931-020-01570-2

31. Waxman A, Restrepo-Jaramillo R, Thenappan T, et al. Inhaled Treprostinil in Pulmonary Hypertension Due to Interstitial Lung Disease. *N Engl J Med*. 2021;384(4):325-334. doi:10.1056/NEJMOA2008470

32. Galiè N, Channick RN, Frantz RP, et al. Risk stratification and medical therapy of pulmonary arterial hypertension. *Eur Respir J*. 2019;53(1). doi:10.1183/13993003.01889-2018

33. Schiza S, Mermigkis C, Margaritopoulos GA, et al. Idiopathic pulmonary fibrosis and sleep disorders: no longer strangers in the night. *Eur Respir Rev*. 2015;24(136):327-339. doi:10.1183/16000617.00009114

34. Bosi M, Milioli G, Fanfulla F, et al. OSA and Prolonged Oxygen Desaturation During Sleep are Strong Predictors of Poor Outcome in IPF. *Lung*. 2017;195(5):643-651. doi:10.1007/s00408-017-0031-4

35. Mermigkis C, Bouloukaki I, Antoniou K, et al. Obstructive sleep apnea should be treated in patients with idiopathic pulmonary fibrosis. *Sleep Breath*. 2015;19(1):385-391. doi:10.1007/S11325-014-1033-6

36. Papadogiannis G, Bouloukaki I, Mermigkis C, et al. Patients with idiopathic pulmonary fibrosis with and without obstructive sleep apnea: Differences in clinical characteristics, clinical outcomes, and the effect of PAP treatment. *J Clin Sleep Med*. 2021;17(3):533-544. doi:10.5664/JCSM.8932

37. Tse LA, Yu ITS, Qiu H, Leung CC. Joint Effects of Smoking and Silicosis on Diseases to the Lungs. *PLoS One*. 2014;9(8):104494. doi:10.1371/JOURNAL.PONE.0104494

38. Hung CL, Su PL, Ou CY. Prognostic effect of tuberculosis on patients with occupational lung diseases: A 13-year observational study in a nationwide cohort. *Medicine (Baltimore)*. 2016;95(37). doi:10.1097/MD.0000000000004748

39. Novikova L, Ilkovich Y, Speranskaya A. Tuberculosis in patients with idiopathic pulmonary fibrosis. *Eur Respir J*. 2015;46(suppl 59):PA2046. doi:10.1183/13993003.CONGRESS-2015.PA2046

40. Lee YH, Cha SI, Lim JK, et al. Clinical and radiological features of pulmonary tuberculosis in patients with idiopathic pulmonary fibrosis. *Respir Investig*. 2019;57(6):544-551. doi:10.1016/J.RESINV.2019.08.001

41. Furin J, Cox H, Pai M. Tuberculosis. *Lancet*. 2019;393(10181):1642-1656. doi:10.1016/S0140-6736(19)30308-3

42. Dowman L, Hill CJ, May A, Holland AE. Pulmonary rehabilitation for interstitial lung disease. *Cochrane Database Syst Rev*. 2021;2021(1). doi:10.1002/14651858.CD006322.PUB4

43. Visca D, Mori L, Tsipouri V, et al. Effect of ambulatory oxygen on quality of life for patients with fibrotic lung disease (AmbOx): a prospective, open-label, mixed-method, crossover randomised controlled trial. *Lancet Respir Med*. 2018;6(10):759-770. doi:10.1016/S2213-2600(18)30289-3

44. Holland AE, Fiore JF, Bell EC, et al. Dyspnoea and comorbidity contribute to anxiety and depression in interstitial lung disease. *Respirology*. 2014;19(8):1215-1221. doi:10.1111/RESP.12360

45. Bajwah S, Ross JR, Wells AU, et al. Palliative care for patients with advanced fibrotic lung disease: a randomised controlled phase II and feasibility trial of a community case conference intervention. *Thorax*. 2015;70(9):830-839. doi:10.1136/THORAXJNL-2014-206583

46. Hanania AN, Mainwaring W, Ghebre YT, Hanania NA, Ludwig M. Radiation-Induced Lung Injury: Assessment and Management. *Chest*. 2019;156(1):150-162. doi:10.1016/j.chest.2019.03.033

47. Trethewey SP, Walters GI. The role of occupational and environmental exposures in the pathogenesis of idiopathic pulmonary fibrosis: A narrative literature review. *Med*. 2018;54(6). doi:10.3390/MEDICINA54060108

48. Johannson KA, Vittinghoff E, Morisset J, et al. Air Pollution Exposure Is Associated With Lower Lung Function, but Not Changes in Lung Function, in Patients With Idiopathic Pulmonary Fibrosis. *Chest*. 2018;154(1):119-125. doi:10.1016/j.chest.2018.01.015

49. Skeoch S, Weatherley N, Swift A, et al. Drug-Induced Interstitial Lung Disease: A Systematic Review. *J Clin Med*. 2018;7(10):356. doi:10.3390/jcm7100356

50. Chioma OS, Drake WP. Focus: Infectious Diseases: Role of Microbial Agents in Pulmonary Fibrosis. *Yale J Biol Med*. 2017;90(2):219.

51. O'Dwyer DN, Ashley SL, Gurczynski SJ, et al. Lung microbiota contribute to pulmonary inflammation and disease progression in pulmonary fibrosis. *Am J Respir Crit Care Med*. 2019;199(9):1127-1138. doi:10.1164/rccm.201809-1650OC

52. Vasakova M, Selman M, Morell F, Sterclova M, Molina-Molina M, Raghu G. Hypersensitivity pneumonitis: Current concepts of pathogenesis and potential targets for treatment. *Am J Respir Crit Care Med*. 2019;200(3):301-308. doi:10.1164/rccm.201903-0541PP

53. Prasse A, Pechkovsky D V., Toews GB, et al. A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18. *Am J Respir Crit Care Med*. 2006;173(7):781-792. doi:10.1164/rccm.200509-1518OC

54. Wiertz IA, Moll SA, Seeliger B, et al. Genetic Variation in CCL18 Gene Influences CCL18 Expression and Correlates with Survival in Idiopathic Pulmonary Fibrosis: Part A. *J Clin Med*. 2020;9(6):1940. doi:10.3390/jcm9061940

55. Caliskan C, Seeliger B, Jäger B, et al. Genetic Variation in CCL18 Gene Influences CCL18 Expression and Correlates with Survival in Idiopathic Pulmonary Fibrosis-Part B. *J Clin Med*. 2020;9(6):1993. doi:10.3390/jcm9061993

56. Hoffmann-Vold AM, Tennøe AH, Garen T, et al. High Level of Chemokine CCL18 Is Associated With Pulmonary Function Deterioration, Lung Fibrosis Progression, and Reduced Survival in Systemic Sclerosis. *Chest*; 2016:299-306. doi:10.1016/j.chest.2016.03.004

57. Bauer Y, Tedrow J, De Bernard S, et al. A novel genomic signature with translational significance for human idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2015;52(2):217-231. doi:10.1165/rcmb.2013-0310OC

58. Wang Y, Yella J, Chen J, McCormack FX, Madala SK, Jegga AG. Unsupervised gene expression analyses identify IPF-severity correlated signatures, associated genes and biomarkers. *BMC Pulm Med*. 2017;17(1):133. doi:10.1186/s12890-017-0472-9

59. Ammar R, Sivakumar P, Jarai G, Thompson JR. A robust data-driven genomic signature for idiopathic pulmonary fibrosis with applications for translational model selection. *PLoS One*. 2019;14(4). doi:10.1371/journal.pone.0215565

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

61. Luzina IG, Salcedo M V., Rojas-Peña ML, et al. Transcriptomic evidence of immune activation in macroscopically normal-appearing and scarred lung tissues in idiopathic pulmonary fibrosis. *Cell Immunol*. 2018;325:1-13. doi:10.1016/j.cellimm.2018.01.002

62. Lockstone HE, Sanderson S, Kulakova N, et al. Gene set analysis of lung samples provides insight into pathogenesis of progressive, fibrotic pulmonary sarcoidosis. *Am J Respir Crit Care Med*. 2010;181(12):1367-1375. doi:10.1164/rccm.200912-1855OC

63. Heukels P, Van Hulst JAC, Van Nimwegen M, et al. Enhanced Bruton's tyrosine kinase in B-cells and autoreactive IgA in patients with idiopathic pulmonary fibrosis. *Respir Res*. 2019;20(1):232. doi:10.1186/s12931-019-1195-7

64. Wallace WAH, Fitch PM, Simpson AJ, Howie SEM. Inflammation-associated remodelling and fibrosis in the lung - A process and an end point. *Int J Exp Pathol*. 2007;88(2):103-110. doi:10.1111/j.1365-2613.2006.00515.x

65. Luzina IG, Todd NW, Iacono AT, Atamas SP. Roles of T lymphocytes in pulmonary fibrosis. *J Leukoc Biol*. 2008;83(2):237-244. doi:10.1189/jlb.0707504

66. Desai O, Winkler J, Minasyan M, Herzog EL. The role of immune and inflammatory cells in idiopathic pulmonary fibrosis. *Front Med*. 2018;5:43. doi:10.3389/fmed.2018.00043

67. Grunewald J, Grutters JC, Arkema E V., Saketkoo LA, Moller DR, Müller-Quernheim J. Sarcoidosis. *Nat Rev Dis Prim*. 2019;5(1). doi:10.1038/s41572-019-0096-x

68. Wells AU, Kokosi M, Karagiannis K. Treatment strategies for idiopathic interstitial pneumonias. *Curr Opin Pulm Med*. 2014;20(5):442-448. doi:10.1097/MCP.0000000000000085

69. Wells AU, Flaherty KR, Brown KK, et al. Nintedanib in patients with progressive fibrosing interstitial lung diseases-subgroup analyses by interstitial lung disease diagnosis in the INBUILD trial: a randomised, double-blind, placebo-controlled, parallel-group trial. *Lancet Respir Med*. 2020;8(5):453-460. doi:10.1016/S2213-2600(20)30036-9

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

71. Behr J, Nathan SD, Wuyts WA, et al. Efficacy and safety of sildenafil added to pirfenidone in patients with advanced idiopathic pulmonary fibrosis and risk of pulmonary hypertension: a double-blind, randomised, placebo-controlled, phase 2b trial. *Lancet Respir Med*. 2021;9(1):85-95. doi:10.1016/S2213-2600(20)30356-8

72. Nathan SD, Flaherty KR, Glassberg MK, et al. A Randomized, Double-Blind, Placebo-Controlled Study of Pulsed, Inhaled Nitric Oxide in Subjects at Risk of Pulmonary Hypertension Associated With Pulmonary Fibrosis. *Chest*. 2020;158(2):637-645. doi:10.1016/J.CHEST.2020.02.016

73. Chung MJ, Goo JM, Im JG. Pulmonary tuberculosis in patients with idiopathic pulmonary fibrosis. *Eur J Radiol*. 2004;52(2):175-179. doi:10.1016/J.EJRAD.2003.11.017

74. Holland AE, Corte T, Chambers DC, et al. Ambulatory oxygen for treatment of exertional hypoxaemia in pulmonary fibrosis (PFOX trial): a randomised controlled trial. *BMJ Open*. 2020;10(12). doi:10.1136/BMJOPEN-2020-040798

75. Khor YH, Holland AE, Goh NSL, et al. Ambulatory Oxygen in Fibrotic Interstitial Lung Disease: A Pilot, Randomized, Triple-Blinded, Sham-Controlled Trial. *Chest*. 2020;158(1):234-244. doi:10.1016/J.CHEST.2020.01.049

76. Wijsenbeek MS, Holland AE, Swigris JJ, Renzoni EA. Comprehensive Supportive Care for Patients with Fibrosing Interstitial Lung Disease. *Am J Respir Crit Care Med*. 2019;200(2):152-159. doi:10.1164/RCCM.201903-0614PP

77. Inchingolo R, Varone F, Sgalla G, Richeldi L. Existing and emerging biomarkers for disease progression in idiopathic pulmonary fibrosis. *Expert Rev Respir Med*. 2019;13(1):39-51. doi:10.1080/17476348.2019.1553620

78. Hayton C, Terrington D, Wilson AM, Chaudhuri N, Leonard C, Fowler SJ. Breath biomarkers in idiopathic pulmonary fibrosis: A systematic review 11 Medical and Health Sciences. *Respir Res*. 2019;20(1). doi:10.1186/s12931-019-0971-8

79. Kim GHJ, Weigt SS, Belperio JA, et al. Prediction of idiopathic pulmonary fibrosis progression using early quantitative changes on CT imaging for a short term of clinical 18–24-month follow-ups. *Eur Radiol*. 2020;30(2):726-734. doi:10.1007/s00330-019-06402-6

80. Romei C, Tavanti LM, Taliani A, et al. Automated Computed Tomography analysis in the assessment of Idiopathic Pulmonary Fibrosis severity and progression. *Eur J Radiol*. 2020;124. doi:10.1016/j.ejrad.2020.108852

81. Sverzellati N, Silva M, Seletti V, et al. Stratification of long-term outcome in stable idiopathic pulmonary fibrosis by combining longitudinal computed tomography and forced vital capacity. *Eur Radiol*. 2020;30(5):2669-2679. doi:10.1007/s00330-019-06619-5

82. Jacob J, Bartholmai BJ, Rajagopalan S, et al. Predicting outcomes in idiopathic pulmonary fibrosis using automated computed tomographic analysis. *Am J Respir Crit Care Med*. 2018;198(6):767-776. doi:10.1164/rccm.201711-2174OC

83. Jacob J, Hirani N, Van Moorsel CHM, et al. Predicting outcomes in rheumatoid arthritis related interstitial lung disease. *Eur Respir J*. 2019;53(1). doi:10.1183/13993003.00869-2018

84. Jacob J, Bartholmai BJ, Egashira R, et al. Chronic hypersensitivity pneumonitis: Identification of key prognostic determinants using automated CT analysis. *BMC Pulm Med*. 2017;17(1). doi:10.1186/s12890-017-0418-2

85. Ungprasert P, Wilton KM, Ernste FC, et al. Novel Assessment of Interstitial Lung Disease Using the "Computer-Aided Lung Informatics for Pathology Evaluation and Rating" (CALIPER) Software System in Idiopathic Inflammatory Myopathies. *Lung*. 2017;195(5):545-552. doi:10.1007/s00408-017-0035-0

86. Ferrazza AM, Gigante A, Gasperini ML, et al. Assessment of interstitial lung disease in systemic sclerosis using the quantitative CT algorithm CALIPER. *Clin Rheumatol*. 2020;39(5):1537-1542. doi:10.1007/s10067-020-04938-3

87. Stainer A, Faverio P, Busnelli S, et al. Molecular Biomarkers in Idiopathic Pulmonary Fibrosis: State of the Art and Future Directions. *Int J Mol Sci*. 2021;22(12). doi:10.3390/IJMS22126255

88. Reyfman PA, Walter JM, Joshi N, et al. Single-Cell Transcriptomic Analysis of Human Lung Provides Insights into the Pathobiology of Pulmonary Fibrosis. *Am J Respir Crit Care Med*. 2019;199(12):1517-1536. doi:10.1164/RCCM.201712-2410OC

89. Pankratz DG, Choi Y, Imtiaz U, et al. Usual interstitial pneumonia can be detected in transbronchial biopsies using machine learning. *Ann Am Thorac Soc*. 2017;14(11):1646-1654. doi:10.1513/AnnalsATS.201612-947OC

90. Raghu G, Flaherty KR, Lederer DJ, et al. Use of a molecular classifier to identify usual interstitial pneumonia in conventional transbronchial lung biopsy samples: a prospective validation study. *Lancet Respir Med*. 2019;7(6):487-496. doi:10.1016/S2213-2600(19)30059-1

91. Winterbottom CJ, Shah RJ, Patterson KC, et al. Exposure to Ambient Particulate Matter Is Associated With Accelerated Functional Decline in Idiopathic Pulmonary Fibrosis. *Chest*. 2018;153(5):1221-1228. doi:10.1016/J.CHEST.2017.07.034

92. Sesé L, Nunes H, Cottin V, et al. Role of atmospheric pollution on the natural history of idiopathic pulmonary fibrosis. *Thorax*. 2018;73(2):145-150. doi:10.1136/THORAXJNL-2017-209967

93. Dales R, Blanco-Vidal C, Cakmak S. The Association Between Air Pollution and Hospitalization of Patients With Idiopathic Pulmonary Fibrosis in Chile: A Daily Time Series Analysis. *Chest*. 2020;158(2):630-636. doi:10.1016/J.CHEST.2020.02.017

94. Yoon HY, Kim SY, Kim OJ, Song JW. Nitrogen dioxide increases the risk of mortality in idiopathic pulmonary fibrosis. *Eur Respir J*. 2021;57(5). doi:10.1183/13993003.01877-2020

95. Johannson KA, Vittinghoff E, Lee K, et al. Acute exacerbation of idiopathic pulmonary fibrosis associated with air pollution exposure. *Eur Respir J*. 2014;43(4):1124-1131. doi:10.1183/09031936.00122213

96. Selman M, Pardo A. Revealing the Pathogenic and Aging-related Mechanisms of the Enigmatic Idiopathic Pulmonary Fibrosis. An Integral Model. *Am J Respir Crit Care Med*. 2014;189(10):1161-1172. doi:10.1164/rccm.201312-2221PP [doi]

97. Wu H, Yu Y, Huang H, Dai H, Chen J, Tang N. Progressive Pulmonary Fibrosis Is Caused by Elevated Mechanical Tension on Alveolar Stem Cells. 2020. doi:10.1016/j.cell.2019.11.027

98. Rush B, Wiskar K, Berger L, Griesdale D. The use of mechanical ventilation in patients with idiopathic pulmonary fibrosis in the United States: A nationwide retrospective cohort analysis. *Respir Med*. 2016;111:72-76. doi:10.1016/J.RMED.2015.12.005

99. Mallick S. Outcome of patients with idiopathic pulmonary fibrosis (IPF) ventilated in intensive care unit. *Respir Med*. 2008;102(10):1355-1359. doi:10.1016/J.RMED.2008.06.003

100. Molyneaux PL, Cox MJ, Wells AU, et al. Changes in the respiratory microbiome during acute exacerbations of idiopathic pulmonary fibrosis. *Respir Res*. 2017;18(1). doi:10.1186/s12931-017-0511-3

101. Duckworth A, Longhurst HJ, Paxton JK, Scotton CJ. The Role of Herpes Viruses in Pulmonary Fibrosis. *Front Med*. 2021;8:1137. doi:10.3389/FMED.2021.704222

102. Invernizzi R, Barnett J, Rawal B, et al. Bacterial burden in the lower airways predicts disease progression in idiopathic pulmonary fibrosis and is independent of radiological disease extent. *Eur Respir J*. January 2020:1901519. doi:10.1183/13993003.01519-2019

103. Wilson AM, Clark AB, Cahn T, et al. Effect of Co-trimoxazole (Trimethoprim-Sulfamethoxazole) vs Placebo on Death, Lung Transplant, or Hospital Admission in Patients With Moderate and Severe Idiopathic Pulmonary Fibrosis: The EME-TIPAC Randomized Clinical Trial. *JAMA*. 2020;324(22):2282-2291. doi:10.1001/JAMA.2020.22960

104. Martinez FJ, Yow E, Flaherty KR, et al. Effect of Antimicrobial Therapy on Respiratory Hospitalization or Death in Adults With Idiopathic Pulmonary Fibrosis: The CleanUP-IPF Randomized Clinical Trial. *JAMA*. 2021;325(18):1841-1851. doi:10.1001/JAMA.2021.4956

105. Chen CY, Chen CH, Wang CY, Lai CC, Chao CM, Wei YF. The effect of additional antimicrobial therapy on the outcomes of patients with idiopathic pulmonary fibrosis: a systematic review and meta-analysis. *Respir Res*. 2021;22(1):1-9. doi:10.1186/S12931-021-01839-0

106. van Moorsel CHM, van der Vis JJ, Grutters JC. Genetic disorders of the surfactant system: focus on adult disease. *Eur Respir Rev*. 2021;30(159). doi:10.1183/16000617.0085-2020

107. Wang Y, Kuan PJ, Xing C, et al. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am J Hum Genet*. 2009;84(1):52-59. doi:10.1016/j.ajhg.2008.11.010 [doi]

108. Forstner M, Lin S, Yang X, et al. High-Content Screening Identifies Cyclosporin A as a Novel ABCA3-Specific Molecular Corrector. *Am J Respir Cell Mol Biol*. 2022;66(4):382-390. doi:10.1165/RCMB.2021-0223OC

109. Dai J, Cai H, Li H, et al. Association between telomere length and survival in patients with idiopathic pulmonary fibrosis. *Respirology*. 2015;20(6):947-952. doi:10.1111/resp.12566 [doi]

110. Stuart BD, Lee JS, Kozlitina J, et al. Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. *LancetRespiratory Med*. 2014;2(7):557-565. doi:10.1016/S2213-2600(14)70124-9 [doi]

111. Newton CA, Oldham JM, Ley B, et al. Telomere length and genetic variant associations with interstitial lung disease progression and survival. *Eur Respir J*. 2019;53(4):1801641. Print 2019 Apr. doi:10.1183/13993003.01641-2018

112. Newton CA, Batra K, Torrealba J, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J*. 2016;48(6):1710-1720. doi:10.1183/13993003.00308-2016 [doi]

113. Hoffman TW, van Moorsel CHM, Borie R, Crestani B. Pulmonary phenotypes associated with genetic variation in telomere-related genes. *Curr Opin Pulm Med*. 2018;24(3):269-280. doi:10.1097/MCP.0000000000000475 [doi]

114. Justice JN, Nambiar AM, Tchkonia T, et al. Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. *EBioMedicine*. 2019;40:554-563. doi:10.1016/j.ebiom.2018.12.052

115. Townsley DM, Dumitriu B, Liu D, et al. Danazol Treatment for Telomere Diseases. *N Engl J Med*. 2016;374(20):1922-1931. doi:10.1056/NEJMoa1515319 [doi]

116. Povedano JM, Martinez P, Serrano R, et al. Therapeutic effects of telomerase in mice with pulmonary fibrosis induced by damage to the lungs and short telomeres. *Elife*. 2018;7. doi:10.7554/eLife.31299

117. Peljto AL, Zhang Y, Fingerlin TE, et al. Association Between the MUC5B Promoter Polymorphism and Survival in Patients With Idiopathic Pulmonary Fibrosis. *JAMA*. 2013;309(21):2232-2239. doi:10.1001/JAMA.2013.5827

118. Dudbridge F, Allen RJ, Sheehan NA, et al. Adjustment for index event bias in genome-wide association studies of subsequent events. *Nat Commun 2019 101*. 2019;10(1):1-10. doi:10.1038/s41467-019-09381-w

119. 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*. 2016;21(4):712-717. doi:10.1111/resp.12728 [doi]

120. Oldham JM, Ma SF, Martinez FJ, et al. TOLLIP, MUC5B, and the response to N-acetylcysteine among individuals with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2015;192(12):1475-1482. doi:10.1164/rccm.201505-1010OC

121. Hancock LA, Hennessy CE, Solomon GM, et al. Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice. *Nat Commun*. 2018;9(1). doi:10.1038/s41467-018-07768-9

122. Kolb M, Bondue B, Pesci A, et al. Acute exacerbations of progressive-fibrosing interstitial lung diseases. *Eur Respir Rev*. 2018;27(150). doi:10.1183/16000617.0071-2018

123. Hoffman TW, van Moorsel CHM, Kazemier KM, Biesma DH, Grutters JC, van Kessel DA. Humoral Immune Status in Relation to Outcomes in Patients with Idiopathic Pulmonary Fibrosis. *Lung*. 2021;199(6):667-676. doi:10.1007/S00408-021-00488-W

124. Johannson KA, Strambu I, Ravaglia C, et al. Antacid therapy in idiopathic pulmonary fibrosis: more questions than answers? *Lancet Respit Med*. 2017;5(7):591-598. doi:10.1016/S2213-2600(17)30219-9

125. Raghu G, Rochwerg B, Zhang Y, et al. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2015;192(2):e3-19. doi:10.1164/rccm.201506-1063ST [doi]

126. Tran T, Assayag D, Ernst P, Suissa S. Effectiveness of Proton Pump Inhibitors in Idiopathic Pulmonary Fibrosis: A Population-Based Cohort Study. *Chest*. 2021;159(2):673-682. doi:10.1016/J.CHEST.2020.08.2080

127. Raghu G, Pellegrini CA, Yow E, et al. Laparoscopic anti-reflux surgery for the treatment of idiopathic pulmonary fibrosis (WRAP-IPF): a multicentre, randomised, controlled phase 2 trial. *Lancet Respir Med*. 2018;6(9):707-714. doi:10.1016/S2213-2600(18)30301-1

128. Glaspole IN, Chapman SA, Cooper WA, et al. Health-related quality of life in idiopathic pulmonary fibrosis: Data from the Australian IPF Registry. *Respirology*. 2017;22(5):950-956. doi:10.1111/RESP.12989

129. Khor YH, Ng Y, Sweeney D, Ryerson CJ. Nocturnal hypoxaemia in interstitial lung disease: a systematic review. *Thorax*. 2021;76(12):1200-1208. doi:10.1136/THORAXJNL-2020-216749

130. Wells AU, Brown KK, Flaherty KR, Kolb M, Thannickal VJ. What's in a name? That which we call IPF, by any other name would act the same. *Eur Respir J*. 2018;51(5). doi:10.1183/13993003.00692-2018

Chapter 11

Summary and general discussion

This thesis aimed to explore clinical and genetic features of a telomere syndrome in patients with idiopathic pulmonary fibrosis (IPF). IPF is thought to represent a chronic disease process, where slowly accumulating lung damage in a susceptible lung eventually leads to pulmonary symptoms and finally overt disease.[1] In support of this concept, it has been found that so-called interstitial lung abnormalities on chest imaging are often progressive and can develop into full-blown pulmonary fibrosis.[2] These interstitial lung abnormalities therefore seem to represent an early stage of pulmonary fibrosis.[2] Interstitial lung abnormalities seem to be especially relevant in family members of patients with pulmonary fibrosis, and likely even more so when this is in the context of a telomere syndrome. Asymptomatic first-degree family members of patients with familial pulmonary fibrosis who have shorter leukocyte telomere length more often have interstitial lung abnormalities or interstitial lung disease.[3,4] Short telomeres in alveolar epithelial cells are also associated with interstitial lung abnormalities in a similar population.[5]

In **Chapter 2** we show that in patients with IPF, abnormalities were often seen on chest X-rays even prior to the initial symptoms of pulmonary fibrosis. In 96 of 409 (23%) patients with IPF a chest X-ray had been made a median of 50.5 months prior to the start of symptoms, and on 56 X-rays (58%) abnormalities were seen that potentially represented early stages of IPF. Even after the start of symptoms, the median time to a final diagnosis was 24 months. This illustrates that there is a significant potential for earlier detection and diagnosis of IPF. A computed tomography (CT)-scan should be advised for analysis of unexplained abnormalities on chest X-ray, and regular follow up should be considered if interstitial lung abnormalities are found. A study in the United States found that, in patients with interstitial lung abnormalities as seen on lung cancer screening CT-scans, the abnormalities were noted in only 64% of the reports. Furthermore, in 42% of the reports in which interstitial lung abnormalities were noted, the finding was only mentioned in the findings and not the impression section.[6] Therefore, increased awareness of early stages of pulmonary fibrosis, as seen on CT-scan or even chest X-ray, is required among radiologists.

A sobering finding of our study is that there was no association between survival and a longer time to diagnosis after the start of symptoms. This was in spite of the fact that approximately 80% of the patients were treated with either pirfenidone or nintedanib. This illustrates that, despite our best efforts, we still have little to offer in terms of prevention of disease progression and lengthening of survival. Investigations into the efficacy of current antifibrotic medication on disease progression of interstitial lung abnormalities, as well as potential new antifibrotic drugs, are needed. This will become increasingly relevant with the implementation of lung cancer screening programmes, and increased attention for screening of first-degree relatives of patients with pulmonary fibrosis.[3,7]

CLINICAL FEATURES OF A TELOMERE SYNDROME IN PATIENTS WITH IPF

It has become increasingly clear that patients with pulmonary fibrosis in the context of a telomere syndrome have a distinct clinical phenotype, characterized by worse survival.[8,9] Furthermore, shorter leukocyte telomere length in patients with IPF has been associated with worse survival.[10–12] However, genetic testing and leukocyte telomere length oftentimes are not available in clinical practice, and in some patients with a suspected telomere syndrome no genetic mutations are found. Simpler clinical parameters that can predict survival could therefore be useful. For example, recent work showed that a family history of pulmonary fibrosis associated with worse survival in patients with IPF.[13] Previous studies have investigated the prevalence of extrapulmonary features suggestive of a telomere syndrome in selected populations of patients with IPF, such as lung transplant candidates or patients with proven mutations in telomere-related genes.[8,9,14,15]

In **Chapter 3** we show that extrapulmonary features suggestive of a telomere syndrome were present in 27% of a cohort of 409 patients with IPF. These features included any or a combination of a clinical history (9% of patients; including haematological disease, liver disease, early greying of hair, nail dystrophy, skin abnormalities), a family history (10% of patients), or haematological laboratory abnormalities (19% of patients; including macrocytosis, anaemia, thrombopenia or leukopenia) suggestive of a telomere syndrome. The presence of any of these features was associated with shorter leukocyte telomere length and shorter survival (hazard ratio 1.58 (95% confidence interval 1.19-2.11); p=0.002 in a multivariate model correcting for age and lung function at diagnosis). The presence of any of these features was significantly more common in patients with IPF who had a family history of pulmonary fibrosis (38% of patients, versus 23% of patients with sporadic IPF; p=0.003). When only patients with sporadic IPF were included, there was no significant association between the presence of any feature suggestive of a telomere syndrome and leukocyte telomere length or survival. This is likely due to reduction in the sample size, as the presence of any feature suggestive of a telomere syndrome was still associated with survival when a family history of pulmonary fibrosis was added to the multivariate model for the whole cohort.

Our study confirms that features suggestive of a telomere syndrome are present in a relatively large proportion of patients with IPF, including patients with sporadic IPF. Furthermore, the presence of any of these features, which can be assessed using information available from the clinical history and standard laboratory tests, is associated with worse survival. Importantly, in the context of recent studies, it remains uncertain whether this association is mainly driven by patients with familial pulmonary fibrosis. Interestingly, in

contrast to an American study,[13] we did not find an association between survival and a family history of pulmonary fibrosis when using a multivariate model and found longer survival in patients with familial pulmonary fibrosis in a univariate model, likely because these patients were significantly younger at diagnosis. In contrast to the other study, patients with a family history of pulmonary fibrosis in our cohort had significantly better lung function parameters at diagnosis. Similar results were found in another European cohort of patients with familial and sporadic pulmonary fibrosis, which also did not report a significant association between a family history of pulmonary fibrosis and survival.[16] It therefore remains uncertain whether a family history of pulmonary fibrosis or clinical features suggestive of a telomere syndrome is the best predictor of survival.

An association between leukocyte telomere length and survival was not found in our study, which is in contrast to previous studies.[10–12] The reason for this remains elusive. A Spanish study suggests that the association between leukocyte telomere length and survival may be relevant for younger but not for older patients with IPF.[17] Another potential explanation is that most of our patients were treated with antifibrotic drugs, whereas this was not the case in the other cohorts.[10–12] In any case, the use of leukocyte telomere length as a prognostic biomarker for patients with IPF is still problematic. There is poor concordance between various available methods for measuring leukocyte telomere length.[18] We used the quantitative polymerase chain reaction (qPCR) method for measuring telomere length, the accuracy of which has been debated.[19] The qPCR method, however, was also used in other studies that investigated the relation between leukocyte telomere length and survival, and is, at the moment, the easiest and cheapest way to measure leukocyte telomere length. A poor correlation has been found between leukocyte and lung telomere length,[20] and an interesting area for future research would be measuring telomere length from bronchial biopsy or bronchial brush samples. This has been done in lung transplant recipients,[21,22] but not in patients with IPF.

As we did not have information on telomere gene mutations available for our cohort, future studies will have to determine the relative values of clinical and genetic information for determining the prognosis of patients with IPF, possibly combined with telomere length measurement.[23] Whether the features suggestive of a telomere syndrome that we observed are true manifestations of telomere dysfunction or simply represent a shared risk factor or more severe disease, especially in case of the laboratory abnormalities, can also not definitively be stated. It seems promising to explore telomere length in various tissue compartments in relation to clinical manifestations of a telomere syndrome in those tissue compartments.[20,24]

There has been increasing attention for comorbid conditions in patients with IPF, as some of these conditions can lead to reduced quality of life and also impact survival.[25,26] The prevalence of comorbid conditions in patients with IPF is related to shared risk factors (e.g. cigarette smoking, old age, mutations in telomere-related or surfactant-processing genes), as well as the fibrotic process in itself. It is unclear to what degree certain comorbid conditions in patients with IPF are related to telomere dysfunction. In some patients with a telomere syndrome, an immunodeficiency has been observed.[27,28] Interestingly, repeated injury to the pulmonary epithelium due to infections has been proposed as one of the pathophysiologic mechanisms that can lead to pulmonary fibrosis,[1] and respiratory tract infections might be a risk factor for progression or development of acute exacerbations of IPF.[29]

In **Chapter 4** we therefore sought to investigate the presence of humoral immunodeficiency in patients with IPF, in relation to leukocyte telomere length. However, the prevalence of humoral immunodeficiency was low. Only 6% of patients with IPF had serum immunoglobulin or IgG subclass levels below the lower limit of normal and 1% had a severely impaired response to pneumococcal vaccination. These factors were not associated with the development of acute exacerbations of IPF or leukocyte telomere length. Humoral immunodeficiency therefore does not seem to be a relevant comorbid condition in patients with IPF. We then investigated whether increased inflammatory markers were associated with acute exacerbations of IPF and survival. In the total cohort, the rate of acute exacerbations was 4.7% per year, and most patients who developed an acute exacerbation died. Patients who developed an acute exacerbation of IPF had significantly higher lymphocytes in bronchoalveolar lavage fluid at the time of diagnosis. At the time of an acute exacerbation, blood lymphocyte counts were significantly lower compared to at the time of diagnosis, which could represent recruitment of lymphocytes to the lungs. Higher lymphocytes in bronchoalveolar lavage fluid at the time of diagnosis were also associated with worse survival in a multivariate model (hazard ratio 1.87 for lymphocyte percentage >5 (95% confidence interval 1.028-2.71); p=0.005). Other factors associated with worse survival included higher blood neutrophil count, and serum IgA and IgG levels.

Taken together these findings support that inflammation may be a disease modifier in patients with IPF, and an increased inflammatory response might predispose patients to developing acute exacerbations of IPF. The role of inflammatory cells and cytokines has received more attention in recent years, and it seems that there is more to IPF pathogenesis than a purely fibrotic process.[30,31] However, our findings cannot definitively differentiate between cause and effect, as these increased inflammatory markers might simply represent a reaction to more repetitive micro-injuries with failing regeneration and subsequent severe pulmonary fibrosis, or a bystander phenomenon related to another factor

influencing IPF disease progression, such as chronic or recurrent infections.[32] Whether anti-inflammatory therapy can be useful for a subgroup of patients with IPF remains to be determined. Notably the results of the PANTHER-IPF trial, in which mortality was increased in patients who were treated with prednisolone, azathioprine and N-acetylcysteine compared to placebo,[33] have led to the discouragement of immunosuppressive therapy for patients with IPF. Future trials will therefore need to explore other immunosuppressants or other combinations of immunosuppressants and may benefit from selecting patients with increased inflammatory markers.

In **Chapter 5** we looked at the presence of extrapulmonary findings available from standard diagnostic CT imaging in relation to leukocyte telomere length and survival. Characteristics that were retrieved from CT-scans included coronary artery calcification score, bone mineral density, and the presence of diaphragmatic hernia. We found a high prevalence of comorbid cardiovascular disease, with 44% of patients having any type of cardiovascular comorbidity. This included 30% of all patients who had coronary artery disease. The presence of any type of cardiovascular disease was associated with decreased survival. Furthermore, higher coronary artery calcification score was also associated with decreased survival, especially when coronary artery calcification score was ≥8 (hazard ratio 1.71 (95% confidence interval 1.07-2.74); *p*-value 0.03 in a multivariate model that also corrected for a history of coronary artery disease). Neither a history of cardiovascular disease, coronary artery disease, or coronary artery calcification score was associated with leukocyte telomere length. The presence of cardiovascular comorbid conditions is a distinct clinical phenotype within IPF patients, but does not seem to be related to telomere dysfunction.

Other observations in our study included that 4% of patients had a history of osteoporosis, and 4% of patients had one or more vertebral compression fractures, as seen on CT-scan (some overlap between these two groups). There was no association between osteoporosis and leukocyte telomere length, or between bone mineral density, as measured on the CT-scan, and leukocyte telomere length. Therefore, osteoporosis is an infrequent comorbid condition in patients with IPF and does not seem to be related to telomere dysfunction. Furthermore, we investigated the prevalence of gastro-oesophageal reflux symptoms and diaphragmatic hernia as seen on CT-scan. Gastro-oesophageal reflux is a controversial risk factor for the development or progression of IPF, with conflicting results from previous studies.[34] In our study, we did not find an association between gastro-oesophageal reflux complaints or diaphragmatic hernia and acute exacerbations of IPF or survival. This argues against a significant role of gastro-oesophageal reflux in disease progression of IPF. This is in line with the results of several recent studies.[35–38] A recent update of the IPF Clinical Practice Guideline no longer recommends antacid medication for patients with IPF,[39] which represents a significant change from the previous guidelines.[40]

GENETIC FEATURES OF A TELOMERE SYNDROME IN PATIENTS WITH IPF

In **Chapter 6** the spectrum of pulmonary disease related to genetic abnormalities in telomere-related genes is reviewed. It is noted that genomic mutations in telomere-related genes *TERT*, *RTEL1*, *PARN*, *TERC*, *DKC1*, *TINF2*, and *NAF1* can cause pulmonary disease. This not only includes IPF, but also other types of pulmonary fibrosis, chronic obstructive pulmonary disease, hepatopulmonary syndrome, and pulmonary arteriovenous malformations. Pulmonary phenotypes associated with short telomere length and with single nucleotide polymorphisms (SNPs) in *TERT*, *TERC*, *OBFC1* and *RTEL1* overlap with phenotypes associated with disease-causing mutations in telomere genes. Interestingly, overlapping forms of pulmonary phenotypes within an individual or within families with a telomere-related genetic mutation are regularly found. However, patients with mutations in telomere-related genes and pulmonary disease have worse prognosis regardless of the phenotype at presentation. In recent years, mutations in several new telomere-related genes have been found to underly IPF.

In **Chapter 7** we describe a group of 60 patients who were referred for genetic screening because of suspected genetic abnormalities (in case of a family history of pulmonary fibrosis, clinical suspicion of a telomere syndrome, or a young age at presentation). Potentially disease-causing variants were identified in 32% of the patients. This is included variants in *TERT* and *RTEL1*, but also variants in *ACD* in three patients (two variants classified as pathogenic and one variant classified as variant of unknown significance). *ACD* encodes the shelterin component TPP1, which is responsible for the recruitment of telomerase to telomeres.[41] Previous studies had identified disease-causing mutations in *ACD* in patients with other telomere syndromes, namely aplastic anemia and Hoyeraal-Hreidarsson syndrome,[42,43] but not in patients with pulmonary fibrosis.

In **Chapter 8** we present the results of sequencing exon 6 of the *TINF2* gene in 158 patients with sporadic IPF. Mutations in the *TINF2* gene, which encodes the shelterin component TINF2, have been previously described in patients with familial pulmonary fibrosis and other telomere syndromes.[44,45] We identified one patient (0.6% of the cohort) with a variant in *TINF2* that lead to a Ser245Tyr substitution. The variant was predicted to be damaging by in-silico analyses. The same variant has been described in three patients with other types of telomere syndromes.[45–47] Our patient had pulmonary fibrosis, as well as hypogammaglobulinemia, which could also be a manifestation of a telomere syndrome. However, leukocyte telomere length was found to be normal in our patient, as well as in the other patients with the same mutation.[45–47] This either indicates that the variant that was identified is not pathogenic, as it does not lead to telomere shortening, or alterna-

tively, that mutations in telomere-related genes can also lead to disease through other mechanisms than telomere shortening.

Since the publication of our study, no new reports of the Ser245Tyr mutation have been published. Several recent studies provide even stronger support for a causal role of short telomeres in the development of IPF.[48] When the pathogenic c.2005T mutation in the *TERT*-gene appeared in a Dutch family, it took seven generations for telomeres in mutation carriers to become so short that the family was affected by pulmonary fibrosis.[49] Furthermore, it has been shown that, in families with telomere-related gene mutations, even non-carriers can develop pulmonary fibrosis, and this was related to short telomere length.[50] However, it must be stressed that it is not short telomeres per se that cause cellular senescence or apoptosis, but activation of the DNA damage response at telomeres.[51] Even in non-dividing cells, accumulation of damage to telomeres over time can lead to senescence, and this is not associated with short telomere length.[51] Moreover, it is not average telomere length, but the shortest telomere in a cell that determines the onset of senescence.[52] It is therefore conceivable that increased sensitivity to telomere damage by mutations in telomere-related genes can cause the onset of senescence in the absence of short average telomere length. However, this is more difficult to study than average telomere length. Previous studies did confirm the presence of DNA damage in the relevant cell types in patients with pulmonary fibrosis, and that this is a key event in the development of pulmonary fibrosis.[53,54]

Several new reports have been published on telomere-related gene mutations in patients with pulmonary fibrosis in recent years. Table 1 provides an overview of telomere-related genes and the genes in which mutations have been identified in patients with pulmonary fibrosis. Mutations in many telomere-related genes have now been identified in patients with various manifestations of a telomere syndrome, including pulmonary fibrosis, liver cirrhosis, bone marrow failure, and dyskeratosis congenita, supporting the concept of a spectrum of telomere dysfunction-related diseases.[55,56] Integrating genomics into management of IPF remains challenging.[57] Mutations in *TERC* seem to be associated with earlier disease onset than mutations in *PARN*.[9] However, information on genetic mutations does not always provide certainty; because of complex genetic inheritance, family members of patients with pulmonary fibrosis due to genetic mutations might still be at risk of pulmonary fibrosis, even if they are not mutation carriers themselves.[58] At present, there are no clear therapeutic implications when telomere-related gene mutations are identified for patients with pulmonary fibrosis, although short leukocyte telomere length can have treatment consequences for patients with bone marrow failure.[59] Of note, patients with telomere-related gene mutations that undergo lung transplantation are at a higher risk for haematological complications,[60] although post-transplant survival does not seem to

be impacted.[61,62] Whether these patients need intensified monitoring or reduced doses of post-transplantation immunosuppressive medication, remains to be determined.

Table 1: telomere-related genes in which mutations have been reported in patients with familial pulmonary fibrosis or IPF

Gene	Association with pulmonary fibrosis
TERF1	Not reported
TERF2	Not reported
TERF2IP	Not reported
TINF2	IPF, familial pulmonary fibrosis[44] (this thesis)
POT1	IPF, familial pulmonary fibrosis [63]
ACD	IPF, familial pulmonary fibrosis (this thesis)
CTC1	Not reported
STN1	Not reported
TEN1	Not reported
TERT	IPF, familial pulmonary fibrosis[64]
TERC	IPF, familial pulmonary fibrosis[64]
DKC1	IPF, familial pulmonary fibrosis[65]
NHP2	IPF, familial pulmonary fibrosis[66]
NOP10	IPF, familial pulmonary fibrosis[67]
GAR1	Not reported
NAF1	IPF, familial pulmonary fibrosis[68]
WRAP53	Not reported
RTEL1	IPF, familial pulmonary fibrosis[69]
PARN	IPF, familial pulmonary fibrosis[69]
ZCCHC8	IPF, familial pulmonary fibrosis[70]

TREATMENT OF PATIENTS WITH IPF IN THE CONTEXT OF A TELOMERE SYNDROME, WITHIN THE TREATABLE TRAITS FRAMEWORK

Current treatment options for patients with IPF are limited. Potential treatments include the antifibrotic medications pirfenidone and nintedanib, as well as lung transplantation for selected patients.[40] However, these treatments are only aimed at treating fibrosis, and do not target the causative pathophysiological process in patients with pulmonary fibrosis related to telomere dysfunction. A previous study suggested that the androgen danazol can lengthen leukocyte telomeres and reduce the need for blood transfusions in patients with aplastic anaemia or bone marrow failure in the context of a telomere syndrome. Furthermore, in patients who received danazol, lung function seemed to stabilize, instead of an observed decline prior to danazol treatment.[71] We therefore investigated whether danazol could slow disease progression in patients with IPF. In **Chapter 9** we describe the results of

a prospective observational study of patients with IPF who were treated with danazol as a last resort. Patients were offered treatment with danazol in case of disease progression despite treatment with pirfenidone and/or nintedanib or intolerance to antifibrotic drugs. We found that treatment with danazol did not lead to slowing of lung function decline. Secondary outcomes, including radiographic imaging and survival analyses, also did not point towards a beneficial effect of danazol treatment. The absence of an effect might be related due to the fact that our study population seemed to represent patients with an advanced stage of IPF, where therapeutic potential may be limited. Notably, the patients who used danazol for one year did have a slower lung function decline under danazol than prior to danazol. Alternatively, androgen treatment may only be useful in patients with proven telomere dysfunction. Future studies will have to determine whether earlier treatment with danazol or treatment of subgroups of patients with IPF is beneficial. In this context, the TELO-SCOPE study, an ongoing randomized controlled trial where patients with pulmonary fibrosis and leukocyte telomere length below the 10th percentile for age will receive treatment with danazol or placebo, is of interest.[72] However, it should be noted that the investigators of this study chose change in leukocyte telomere length, and not lung function, as their primary outcome, which is in contrast to most recent therapeutic studies for patients with IPF.

Novel treatments for telomere dysfunction in the lung are being investigated. Possible developments include the senolytic darcatinib/quercetin,[73] telomerase gene transfection,[74,75] delivery of TERT mRNA to relevant tissues,[76] telomerase activators,[77] or small-molecule enhancers of TERC.[78,79] These therapies might eventually be used to prevent development of pulmonary fibrosis in known telomere-related gene mutation carriers, or be used as an add-on therapy in patients with pulmonary fibrosis that is (partly) mediated by telomere dysfunction.

In **Chapter 10** we provide an overarching framework for the management of IPF and other fibrotic lung disease. This framework is based on the treatable traits concept, which was first developed for airway diseases.[80,81] This model allows for the identification of distinct but sometimes overlapping phenotypes or endotypes within a certain disease. It requires validated biomarkers, relevant outcome measures, and evidence-based treatments. We think that this approach can be very useful for patients with pulmonary fibrosis in view of several developments in the past years. First, it has become clear that patients with non-IPF progressive pulmonary fibrosis also benefit from treatment with antifibrotic drugs,[82–84] and these diseases now require a treatment approach that recognizes both the underlying cause of the fibrosis (e.g. exposure to an antigen in hypersensitivity pneumonitis), as well as the inherently progressive fibrosis. A recent update of the IPF Clinical Practice Guidelines includes a definition for progressive pulmonary fibrosis, and suggests

treatment with nintedanib for these patients.[39] Second, for patients with IPF and non-IPF ILD, several underlying causes have been identified, including telomere dysfunction and surfactant-processing disorders. Treatments targeting these mechanisms could be developed, but will need to be incorporated into a treatment framework that already includes antifibrotic drugs and other treatments that can be given irrespective of the underlying disease mechanism. Finally, there has been increased attention for symptom relief and palliative care interventions in patients with end-stage pulmonary fibrosis, and it is important to incorporate these interventions in a broader treatment framework.

Proposed treatable traits in patients with fibrotic ILD are: cigarette smoking, occupational exposure, allergen or drug exposures, excessive (profibrotic) auto- or alloimmunity, progressive fibrosis, pulmonary hypertension, obstructive sleep apnoea, exercise intolerance, exertional hypoxia, and anxiety and depression. There are also several potential traits that have not been associated with relevant outcomes or for which no effective treatment is available at present: air pollution, mechanical stress, viral infections, bacterial burden in the lungs, surfactant-related pulmonary fibrosis, telomere-related pulmonary fibrosis, the rs35705950 MUC5B promoter polymorphism, acute exacerbations, gastro-oesophageal reflux, dyspnoea, and nocturnal hypoxia. The 'treatable traits' concept can be applied in new clinical trials for patients with pulmonary fibrosis and could be used for developing new treatment strategies.

GENERAL CONSIDERATIONS AND PROSPECTS FOR FUTURE RESEARCH

The work presented in this thesis provides new insight into IPF as a part of the spectrum of telomere syndromes. It shows that potential manifestations of a telomere syndrome outside the lung are common in patients with IPF, with 9% and 19% of patients having a clinical history suggestive of a telomere syndrome, and laboratory abnormalities suggestive of a telomere syndrome, respectively. These factors are associated with shorter leukocyte telomere length and worse survival. It was also found that other hypothesized extrapulmonary manifestations of a telomere syndrome, namely humoral immunodeficiency and osteoporosis, were uncommon in patients with IPF, and did not associate with leukocyte telomere length. Coronary artery disease and a higher coronary artery calcification score are common, and indicate a worse prognosis, but were not associated with leukocyte telomere length and are unlikely to be manifestations of telomere dysfunction in patients with IPF. Figure 1 provides an overview of prognostic factors in patients with IPF over several domains, and indicates which factors are related to telomere dysfunction. Taken together, these findings support that IPF is more than a single organ disease.

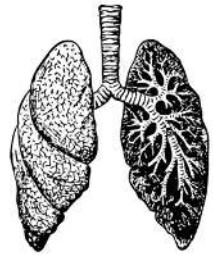
Furthermore, this thesis shows that mutations in various telomere genes can underly pulmonary disease, including mutations in *ACD* and *TINF2*. It is noted that mutations in most

Genetic factors
Telomere-related gene mutations
No MUC5B rs35705950 T allele

Environmental factors
Exposure to air pollution
Exposure to cigarette smoke

Demographic factors
Older age
Male sex

Family history
Family history of pulmonary fibrosis
Family history suggestive of a telomere syndrome



Functional measures
Decreased FVC
Decreased DLCO
Lower walking distance on 6MWT

Blood-based biomarkers
Short leukocyte telomere length
Macrocytosis, anaemia, or thrombopenia
Increased bronchoalveolar lavage fluid lymphocytes
Increased serum IgA
Increased serum IgG
Increased serum KL-6

Radiographic measures
Increased extent of fibrosis
Increased coronary artery calcification score

Comorbid conditions
Liver cirrhosis, haematological disease, early greying, nail dystrophy
Pulmonary hypertension
Cardiovascular disease
Malignancy
Obstructive sleep apnoea

Figure 1: Factors associated with a poor prognosis of patients with IPF, divided into several domains. Factors that are thought to be related to telomere dysfunction are highlighted in orange. FVC = forced vital capacity. DLCO = diffusion capacity of the lung for carbon monoxide. 6MWT = 6-minute walking test.

telomere-related genes that were first identified in patients with other manifestations of a telomere syndrome and no pulmonary fibrosis can also be found in patients with IPF. This illustrates that IPF is truly part of a spectrum of telomere dysfunction-related diseases. Specific treatments for these diseases remain to be further explored.

Several important questions remain. It has not been elucidated why the lungs are especially sensitive to telomere dysfunction, and why IPF is the most common manifestation of a telomere syndrome, and is often the first telomere syndrome phenotype to appear in pedigrees characterized by genetic anticipation.[27] One factor might be that average lung telomere length is relatively short compared to most tissues other than blood.[85] In addition, environmental exposures are likely to play a role, as pulmonary fibrosis is more likely to develop in carriers of telomere-related gene mutations or first-degree family members of patients with IPF who are exposed to cigarette smoke or other occupational or envi-

ronmental exposures.[4,14] Similarly, in mouse models of telomere dysfunction, exposure to bleomycin is required for the development of pulmonary fibrosis.[86] Another potential explanation would be that telomerase activity, which is variable among human stem cell compartments, and is extensively regulated,[87] is lower in type 2 alveolar epithelial cells compared to other stem cells. This has not been confirmed though, and an obvious reason why this would be the case is not apparent. Finally, in patients with dyskeratosis congenita, somatic reversion of pathogenic mutations has been found in the blood compartment, which might protect against bone marrow failure, and could explain some of the variance in disease manifestations in different tissues.[88,89] In any event, it is probable that whether a person develops pulmonary fibrosis depends on a combination of inherited telomere length, the functioning of telomerase and telomere protection proteins, and exposure to internal or external damaging factors. Future studies might shed more light on the factors that determine who develops pulmonary fibrosis by measuring telomere length in blood and lung cells, including measurement of the shortest telomeres as well as average telomere length,[90] measurement of telomere damage foci,[91] measurement of telomerase activity,[92] whole genome sequencing, and estimation of lifetime exposure using the exposome paradigm.[93] Novel treatments for patients with pulmonary fibrosis due to telomere dysfunction can be developed in the context of the treatable traits model.

REFERENCES

1. Selman M, Pardo A. Revealing the Pathogenic and Aging-related Mechanisms of the Enigmatic Idiopathic Pulmonary Fibrosis. An Integral Model. *Am J Respir Crit Care Med*. 2014;189(10):1161-1172. doi:10.1164/rccm.201312-2221PP [doi]
2. Hunninghake GM. Interstitial lung abnormalities: erecting fences in the path towards advanced pulmonary fibrosis. *Thorax*. 2019;74(5):506-511. doi:10.1136/THORAXJNL-2018-212446
3. Hunninghake GM, Quesada-Arias LD, Carmichael NE, et al. Interstitial Lung Disease in Relatives of Patients with Pulmonary Fibrosis. *Am J Respir Crit Care Med*. 2020;201(10):1240-1248. doi:10.1164/RCCM.201908-1571OC
4. Salisbury ML, Hewlett JC, Ding G, et al. Development and Progression of Radiologic Abnormalities in Individuals at Risk for Familial Interstitial Lung Disease. *Am J Respir Crit Care Med*. 2020;201(10):1230-1239. doi:10.1164/RCCM.201909-1834OC
5. Kropski JA, Pritchett JM, Zoz DF, et al. Extensive phenotyping of individuals at risk for familial interstitial pneumonia reveals clues to the pathogenesis of interstitial lung disease. *Am J Respir Crit Care Med*. 2015;191(4):417-426. doi:10.1164/rccm.201406-1162OC [doi]
6. Oldham JM, Adegunsoye A, Khera S, et al. Underreporting of interstitial lung abnormalities on lung cancer screening computed tomography. *Ann Am Thorac Soc*. 2018;15(6):764-766. doi:10.1513/ANNALSATS.201801-053RL
7. Hatabu H, Hunninghake GM, Richeldi L, et al. Interstitial lung abnormalities detected incidentally on CT: a Position Paper from the Fleischner Society. *Lancet Respir Med*. 2020;8(7):726-737. doi:10.1016/S2213-2600(20)30168-5
8. Borie R, Tabeze L, Thabut G, et al. Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. *Eur Respir J*. 2016;48(6):1721-1731. doi:10.1183/13993003.02115-2015 [doi]
9. Newton CA, Batra K, Torrealba J, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J*. 2016;48(6):1710-1720. doi:10.1183/13993003.00308-2016 [doi]
10. Stuart BD, Lee JS, Kozlitina J, et al. Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. *LancetRespiratory Med*. 2014;2(7):557-565. doi:10.1016/S2213-2600(14)70124-9 [doi]
11. Dai J, Cai H, Li H, et al. Association between telomere length and survival in patients with idiopathic pulmonary fibrosis. *Respirology*. 2015;20(6):947-952. doi:10.1111/resp.12566 [doi]
12. Newton CA, Oldham JM, Ley B, et al. Telomere length and genetic variant associations with interstitial lung disease progression and survival. *Eur Respir J*. 2019;53(4):1801641. doi:10.1183/13993003.01641-2018
13. Cutting CC, Bowman WS, Dao N, et al. Family History of Pulmonary Fibrosis Predicts Worse Survival in Patients With Interstitial Lung Disease. *Chest*. 2021;159(5):1913-1921. doi:10.1016/J.CHEST.2021.01.026
14. Diaz de Leon A, Cronkhite JT, Katzenstein AL, et al. Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations. *PLoS One*. 2010;5(5):e10680. doi:10.1371/journal.pone.0010680 [doi]

15. George G, Rosas IO, Cui Y, et al. Short telomeres, telomeropathy, and subclinical extrapulmonary organ damage in patients with interstitial lung disease. *Chest*. 2015;147(6):1549-1557. doi:10.1378/chest.14-0631

16. Krauss E, Gehrken G, Drakopanagiotakis F, et al. Clinical characteristics of patients with familial idiopathic pulmonary fibrosis (f-IPF). *BMC Pulm Med*. 2019;19(1):130. doi:10.1186/S12890-019-0895-6

17. Planas-Cerezales L, Arias-Salgado EG, Buendia-Roldan I, et al. Predictive factors and prognostic effect of telomere shortening in pulmonary fibrosis. *Respirology*. 2019;24(2):146-153. doi:10.1111/resp.13423 [doi]

18. Montpetit AJ, Alhareeri AA, Montpetit M, et al. Telomere length: a review of methods for measurement. *Nurs Res*. 2014;63(4):289-299. doi:10.1097/NNR.0000000000000037 [doi]

19. Gutierrez-Rodrigues F, Santana-Lemos BA, Scheucher PS, Alves-Paiva RM, Calado RT. Direct comparison of flow-FISH and qPCR as diagnostic tests for telomere length measurement in humans. *PLoS One*. 2014;9(11). doi:10.1371/JOURNAL.PONE.0113747

20. Snetselaar R, van Batenburg AA, van Oosterhout MFM, et al. Short telomere length in IPF lung associates with fibrotic lesions and predicts survival. *PLoS One*. 2017;12(12):e0189467. doi:10.1371/journal.pone.0189467 [doi]

21. Faust HE, Golden JA, Rajalingam R, et al. Short lung transplant donor telomere length is associated with decreased CLAD-free survival. *Thorax*. 2017;72(11):1052-1054. doi:10.1136/thoraxjnl-2016-209897 [doi]

22. Mackintosh JA, Yerkovich ST, Tan ME, Samson L, Hopkins PMA, Chambers DC. Airway Telomere Length in Lung Transplant Recipients. *Front Immunol*. 2021;12:1299. doi:10.3389/FIMMU.2021.658062

23. Kannengiesser C, Borie R, Renzoni EA. Pulmonary fibrosis: Genetic analysis of telomere-related genes, telomere length measurement-or both? *Respirology*. 2019;24(2):97-98. doi:10.1111/RESP.13456

24. Daniali L, Benetos A, Susser E, et al. Telomeres shorten at equivalent rates in somatic tissues of adults. *Nat Commun*. 2013;4:1597. doi:10.1038/ncomms2602 [doi]

25. Raghu G, Amatto VC, Behr J, Stowasser S. Comorbidities in idiopathic pulmonary fibrosis patients: a systematic literature review. *Eur Respir J*. 2015;46(4):1113-1130. doi:10.1183/13993003.02316-2014

26. Caminati A, Lonati C, Cassandro R, et al. Comorbidities in idiopathic pulmonary fibrosis: an underestimated issue. *Eur Respir Rev*. 2019;28(153). doi:10.1183/16000617.0044-2019

27. Armanios M. Telomerase and idiopathic pulmonary fibrosis. *Mutat Res*. 2012;730(1-2):52-58. doi:10.1016/j.mrfmmm.2011.10.013 [doi]

28. Wagner CL, Hanumanthu VS, Conover Talbot C, et al. Short telomere syndromes cause a primary T cell immunodeficiency. *J Clin Invest*. 2018;128(12):5222-5234. doi:10.1172/JCI120216

29. Collard HR, Ryerson CJ, Corte TJ, et al. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. *Am J Respir Crit Care Med*. 2016;194(3):265-275. doi:10.1164/rccm.201604-0801CI [doi]

30. Desai O, Winkler J, Minasyan M, Herzog EL. The role of immune and inflammatory cells in idiopathic pulmonary fibrosis. *Front Med*. 2018;5:43. doi:10.3389/fmed.2018.00043

31. Heukels P, Moor CC, von der Thüsen JH, Wijsenbeek MS, Kool M. Inflammation and immunity in IPF pathogenesis and treatment. *Respir Med*. 2019;147:79-91. doi:10.1016/j.rmed.2018.12.015

32. Molyneaux PL, Maher TM. The role of infection in the pathogenesis of idiopathic pulmonary fibrosis. *Eur Respir Rev*. 2013;22(129):376-381. doi:10.1183/09059180.00000713

33. Raghu G, Anstrom KJ, King TE, Lasky JA, Martinez FJ. Prednisone, Azathioprine, and *N* -Acetylcysteine for Pulmonary Fibrosis. *N Engl J Med*. 2012;366(21):1968-1977. doi:10.1056/NEJMoa1113354

34. KA J, I S, C R, et al. Antacid therapy in idiopathic pulmonary fibrosis: more questions than answers? *Lancet Respir Med*. 2017;5(7):591-598. doi:10.1016/S2213-2600(17)30219-9

35. Raghu G, Pellegrini CA, Yow E, et al. Laparoscopic anti-reflux surgery for the treatment of idiopathic pulmonary fibrosis (WRAP-IPF): a multicentre, randomised, controlled phase 2 trial. *Lancet Respir Med*. 2018;6(9):707-714. doi:0.1016/S2213-2600(18)30301-1

36. Bedard Methot D, Leblanc E, Lacasse Y. Meta-analysis of Gastroesophageal Reflux Disease and Idiopathic Pulmonary Fibrosis. *Chest*. 2019;155(1):33-43. doi:10.1016/j.chest.2018.07.038

37. Fidler L, Sitzer N, Shapera S, Shah PS. Treatment of Gastroesophageal Reflux in Patients With Idiopathic Pulmonary Fibrosis: A Systematic Review and Meta-Analysis. *Chest*. 2018;153(6):1405-1415. doi:10.1016/j.chest.2018.03.008

38. Jo HE, Corte TJ, Glaspole I, et al. Gastroesophageal reflux and antacid therapy in IPF: analysis from the Australia IPF Registry. *BMC Pulm Med*. 2019;19(1):84-019-0846-2. doi:10.1186/s12890-019-0846-2 [doi]

39. Raghu G, Remy-Jardin M, Richeldi L, et al. Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults: An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2022;205(9):E18-E47. doi:10.1164/RCCM.202202-0399ST

40. Raghu G, Rochwerg B, Zhang Y, et al. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2015;192(2):e3-19. doi:10.1164/rccm.201506-1063ST [doi]

41. Rajavel M, Mullins MR, Taylor DJ. Multiple facets of TPP1 in telomere maintenance. *Biochim Biophys Acta*. 2014;1844(9):1550-1559. doi:10.1016/j.bbapap.2014.04.014 [doi]

42. Guo Y, Kartawinata M, Li J, et al. Inherited bone marrow failure associated with germline mutation of ACD, the gene encoding telomere protein TPP1. *Blood*. 2014;124(18):2767-2774. doi:10.1182/blood-2014-08-596445 [doi]

43. Kocak H, Ballew BJ, Bisht K, et al. Hoyeraal-Hreidarsson syndrome caused by a germline mutation in the TEL patch of the telomere protein TPP1. *Genes Dev*. 2014;28(19):2090-2102. doi:10.1101/gad.248567.114 [doi]

44. Alder JK, Stanley SE, Wagner CL, Hamilton M, Hanumanthu VS, Armanios M. Exome sequencing identifies mutant TINF2 in a family with pulmonary fibrosis. *Chest*. December 2014. doi:10.1378/chest.14-1947 [doi]

45. Walne AJ, Vulliamy T, Beswick R, Kirwan M, Dokal I. TINF2 mutations result in very short telomeres: analysis of a large cohort of patients with dyskeratosis congenita and related bone marrow failure syndromes. *Blood*. 2008;112(9):3594-3600. doi:10.1182/blood-2008-05-153445 [doi]

46. Vulliamy T, Beswick R, Kirwan MJ, Hossain U, Walne AJ, Dokal I. Telomere length measurement can distinguish pathogenic from non-pathogenic variants in the shelterin component, TIN2. *Clin Genet*. 2012;81(1):76-81. doi:10.1111/j.1399-0004.2010.01605.x [doi]

47. Miano M, Lanciotti M, Giardino S, Dufour C. Ser245Tyr TINF2 mutation in a long-term survivor after a second myeloablative SCT following late graft failure for Aplastic Anaemia. *Blood Cells Mol Dis*. 2015;55(2):187-188. doi:0.1016/j.bcmd.2015.01.002

48. Duckworth A, Gibbons MA, Allen RJ, et al. Telomere length and risk of idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease: a mendelian randomisation study. *Lancet Respir Med*. 2021;9(3):285-294. doi:10.1016/S2213-2600(20)30364-7

49. van der Vis JJ, van der Smagt JJ, Hennekam FAM, Grutters JC, van Moorsel CHM. Pulmonary Fibrosis and a TERT Founder Mutation With a Latency Period of 300 Years. *Chest*. 2020;158(2):612-619. doi:10.1016/J.CHEST.2020.03.069

50. van der Vis JJ, van der Smagt JJ, van Batenburg AA, et al. Pulmonary fibrosis in non-mutation carriers of families with short telomere syndrome gene mutations. *Respirology*. 2021;26(12):1160-1170. doi:10.1111/RESP.14145

51. Rossiello F, Jurk D, Passos JF, d'Adda di Fagagna F. Telomere dysfunction in ageing and age-related diseases. *Nat Cell Biol 2022 242*. 2022;24(2):135-147. doi:10.1038/s41556-022-00842-x

52. Hemann MT, Strong MA, Hao LY, Greider CW. The Shortest Telomere, Not Average Telomere Length, Is Critical for Cell Viability and Chromosome Stability. *Cell*. 2001;107(1):67-77. doi:10.1016/S0092-8674(01)00504-9

53. McDonough JE, Martens DS, Tanabe N, et al. A role for telomere length and chromosomal damage in idiopathic pulmonary fibrosis. *Respir Res*. 2018;19(1):1-8. doi:10.1186/S12931-018-0838-4

54. Batenburg AA van, Kazemier KM, Oosterhout MFM van, et al. Telomere shortening and DNA damage in culprit cells of different types of progressive fibrosing interstitial lung disease. *ERJ Open Res*. 2021;7(2):00691-02020. doi:10.1183/23120541.00691-2020

55. Holohan B, Wright WE, Shay JW. Cell biology of disease: Telomeropathies: An emerging spectrum disorder. *J Cell Biol*. 2014;205(3):289-299. doi:10.1083/jcb.201401012 [doi]

56. Grill S, Nandakumar J. Molecular mechanisms of telomere biology disorders. *J Biol Chem*. 2021;296:100064. doi:10.1074/JBC.REV120.014017

57. Adegunsoye A, Vij R, Noth I. Integrating Genomics Into Management of Fibrotic Interstitial Lung Disease. *Chest*. 2019;155(5):1026-1040. doi:10.1016/j.chest.2018.12.011

58. Borie R, Renzoni E. Pulmonary fibrosis associated with telomere-related gene mutations: A complex inheritance. *Respirology*. 2021;26(12):1098-1100. doi:10.1111/RESP.14168

59. Alder JK, Hanumanthu VS, Strong MA, et al. Diagnostic utility of telomere length testing in a hospital-based setting. *Proc Natl Acad Sci U S A*. 2018;115(10):E2358-E2365. doi:10.1073/pnas.1720427115

60. Borie R, Kannengiesser C, Hirschi S, et al. Severe hematologic complications after lung transplantation in patients with telomerase complex mutations. *J Heart Lung Transplant*. 2015;34(4):538-546. doi:10.1016/j.healun.2014.11.010 [doi]

61. Planas-Cerezales L, Arias-Salgado EG, Berastegui C, et al. Lung Transplant Improves Survival and Quality of Life Regardless of Telomere Dysfunction. *Front Med*. 2021;8:920. doi:10.3389/FMED.2021.695919

62. Alder JK, Sutton RM, Iasella CJ, et al. Lung transplantation for idiopathic pulmonary fibrosis enriches for individuals with telomere-mediated disease. *J Hear Lung Transplant*. 2022;41(5):654-663. doi:10.1016/J.HEALUN.2021.11.008

63. Kelich J, Aramburu T, van der Vis JJ, et al. Telomere dysfunction implicates POT1 in patients with idiopathic pulmonary fibrosis. *J Exp Med*. 2022;219(5). doi:10.1084/JEM.20211681/213140

64. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med*. 2007;356(13):1317-1326. doi:10.1056/NEJMoa066157

65. Kropski JA, Mitchell DB, Markin C, et al. A novel dyskerin (DKC1) mutation is associated with Familial Interstitial Pneumonia. *Chest*. February 2014. doi:10.1378/chest.13-2224 [doi]

66. Benyelles M, O□Donohue MF, Kermasson L, et al. NHP2 deficiency impairs rRNA biogenesis and causes pulmonary fibrosis and Høyeraal-Hreidarsson syndrome. *Hum Mol Genet*. 2020;29(6):907-922. doi:10.1093/HMG/DDAA011

67. Kannengiesser C, Kannengiesser C, Kannengiesser C, et al. First heterozygous NOP10 mutation in familial pulmonary fibrosis. *Eur Respir J*. 2020;55(6). doi:10.1183/13993003.02465-2019

68. Stanley SE, Gable DL, Wagner CL, et al. Loss-of-function mutations in the RNA biogenesis factor NAF1 predispose to pulmonary fibrosis-emphysema. *Sci Transl Med*. 2016;8(351):351ra107. doi:10.1126/scitranslmed.aaf7837 [doi]

69. Stuart BD, Choi J, Zaidi S, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. *Nat Genet*. 2015;47(5):512-517. doi:10.1038/ng.3278 [doi]

70. Gable DL, Gaysinskaya V, Atik CC, et al. ZCCHC8, the nuclear exosome targeting component, is mutated in familial pulmonary fibrosis and is required for telomerase RNA maturation. *Genes Dev*. 2019;33(19-20):1381-1396. doi:10.1101/GAD.326785.119

71. Townsley DM, Dumitriu B, Liu D, et al. Danazol Treatment for Telomere Diseases. *N Engl J Med*. 2016;374(20):1922-1931. doi:10.1056/NEJMoa1515319 [doi]

72. MacKintosh JA, Pietsch M, Lutzky V, et al. TELO-SCOPE study: a randomised, double-blind, placebo-controlled, phase 2 trial of danazol for short telomere related pulmonary fibrosis. *BMJ Open Respir Res*. 2021;8(1):e001127. doi:10.1136/BMJRESP-2021-001127

73. Justice JN, Nambiar AM, Tchkonia T, et al. Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. *EBioMedicine*. 2019;40:554-563. doi:10.1016/j.ebiom.2018.12.052

74. Povedano JM, Martinez P, Serrano R, et al. Therapeutic effects of telomerase in mice with pulmonary fibrosis induced by damage to the lungs and short telomeres. *Elife*. 2018;7. doi:10.7554/eLife.31299

75. Jaijyan DK, Selariu A, Cruz-Cosme R, et al. New intranasal and injectable gene therapy for healthy life extension. *Proc Natl Acad Sci U S A*. 2022;119(20). doi:10.1073/PNAS.2121499119

76. Ramunas J, Yakubov E, Brady JJ, et al. Transient delivery of modified mRNA encoding TERT rapidly extends telomeres in human cells. *FASEB J*. 2015;29(5):1930-1939. doi:10.1096/FJ.14-259531

77. Le Saux CJ, Davy P, Brampton C, et al. A Novel Telomerase Activator Suppresses Lung Damage in a Murine Model of Idiopathic Pulmonary Fibrosis. *PLoS One*. 2013;8(3). doi:10.1371/JOURNAL.PONE.0058423

78. Nagpal N, Wang J, Zeng J, et al. Small-Molecule PAPD5 Inhibitors Restore Telomerase Activity in Patient Stem Cells. *Cell Stem Cell*. 2020;26(6):896-909.e8. doi:10.1016/J.STEM.2020.03.016

79. Shukla S, Schmidt JC, Goldfarb KC, Cech TR, Parker R. Inhibition of telomerase RNA decay rescues telomerase deficiency caused by dyskerin or PARN defects. *Nat Struct Mol Biol*. 2016;23(4):286-292. doi:10.1038/nsmb.3184 [doi]

80. Agusti A, Bel E, Thomas M, et al. Treatable traits: Toward precision medicine of chronic airway diseases. *Eur Respir J*. 2016;47(2):410-419. doi:10.1183/13993003.01359-2015

81. Boaventura R, Sibila O, Agusti A, Chalmers JD. Treatable traits in bronchiectasis. *Eur Respir J*. 2018;52(3). doi:10.1183/13993003.01269-2018

82. Flaherty KR, Wells AU, Cottin V, et al. Nintedanib in progressive fibrosing interstitial lung diseases. *N Engl J Med*. 2019;381(18):1718-1727. doi:10.1056/NEJMoa1908681

83. Wells AU, Flaherty KR, Brown KK, et al. Nintedanib in patients with progressive fibrosing interstitial lung diseases-subgroup analyses by interstitial lung disease diagnosis in the INBUILD trial: a randomised, double-blind, placebo-controlled, parallel-group trial. *Lancet Respir Med*. 2020;8(5):453-460. doi:10.1016/S2213-2600(20)30036-9

84. Maher TM, Corte TJ, Fischer A, et al. Pirfenidone in patients with unclassifiable progressive fibrosing interstitial lung disease: a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Respir Med*. 2020;8(2):147-157. doi:10.1016/S2213-2600(19)30341-8

85. Demanelis K, Jasmine F, Chen LS, et al. Determinants of telomere length across human tissues. *Science*. 2020;369(6509). doi:10.1126/SCIENCE.AAZ6876

86. Povedano JM, Martinez P, Flores JM, Mulero F, Blasco MA. Mice with Pulmonary Fibrosis Driven by Telomere Dysfunction. *Cell Rep*. 2015;12(2):286-299. doi:10.1016/j.celrep.2015.06.028 [doi]

87. Roake CM, Artandi SE. Regulation of human telomerase in homeostasis and disease. *Nat Rev Mol Cell Biol 2020 217*. 2020;21(7):384-397. doi:10.1038/s41580-020-0234-z

88. Jongmans MCJ, Verwiel ETP, Heijdra Y, et al. Revertant somatic mosaicism by mitotic recombination in dyskeratosis congenita. *Am J Hum Genet*. 2012;90(3):426-433. doi:10.1016/J.AJHG.2012.01.004

89. Schratz KE, Gaysinskaya V, Cosner ZL, et al. Somatic reversion impacts myelodysplastic syndromes and acute myeloid leukemia evolution in the short telomere disorders. *J Clin Invest*. 2021;131(18). doi:10.1172/JCI147598

90. Lai TP, Zhang N, Noh J, et al. A method for measuring the distribution of the shortest telomeres in cells and tissues. *Nat Commun 2017 81*. 2017;8(1):1-14. doi:10.1038/s41467-017-01291-z

91. Mender I, Shay JW. Telomere Dysfunction Induced Foci (TIF) Analysis. *Bio-protocol*. 2015;5(22). doi:10.21769/BIOPROTOC.1656

92. Tomlinson CG, Sasaki N, Jurczyluk J, Bryan TM, Cohen SB. Quantitative assays for measuring human telomerase activity and DNA binding properties. *Methods*. 2017;114:85-95. doi:10.1016/J.YMETH.2016.08.002

93. Vrijheid M. The exposome: a new paradigm to study the impact of environment on health. *Thorax*. 2014;69(9):876-878. doi:10.1136/THORAXJNL-2013-204949

Appendices

NEDERLANDSE SAMENVATTING

Idiopathische pulmonale fibrose (IPF) is een ernstige longziekte die wordt gekenmerkt door steeds verdere verlittekening van de longen. Patiënten met IPF krijgen vaak steeds meer last van benauwdheid en komen uiteindelijk aan de ziekte te overlijden. De enige genezende behandeling is longtransplantatie, maar deze is niet voor elke patient mogelijk. Behandeling met de antifibrotische medicijnen pirfenidon en nintedanib kan achteruitgang remmen, maar kan verlittekening niet stoppen of terugdraaien. Er is steeds meer aandacht voor het vroegtijdig opsporen van IPF of voorstadia van IPF. Hopelijk kan er zo eerder worden gestart met een behandeling om verlittekening te voorkomen. In **Hoofdstuk 2** wordt beschreven dat zelfs jaren voordat patiënten met IPF symptomen van de ziekte krijgen, er vaak al afwijkingen kunnen worden gezien op röntgenfoto's die om andere redenen waren gemaakt. Dit bevestigt dat IPF een ziekte is die zich vaak langzaam ontwikkelt, en dat er potentie is voor het eerder opsporen van (voorstadia van) de ziekte. Verder werd gevonden dat er geen betere overleving was van patiënten bij wie sneller na het begin van de symptomen de diagnose werd gesteld, ondanks dat de meeste patiënten wel werden behandeld met een antifibrotisch medicijn. Dit laat zien dat er nog veel werk moet worden verricht voordat eerdere opsporing van longfibrose ook voor betere overleving van patiënten zal zorgen.

In een deel van de patiënten komt IPF of een andere vorm van longfibrose voor in de familie. IPF is dus soms erfelijk. Het kan dat deze patiënten, maar ook patiënten zonder longfibrose in de familie, afwijkingen hebben in genen die betrokken zijn bij het onderhoud van telomeren. Telomeren zijn de uiteinden van chromosomen, en normaliter worden deze na elke celdeling iets korter. Wanneer telomeren te kort zijn, kan een cel niet verder delen en raakt deze in een soort slaaptoestand of gaat deze te gronde. In de long betekent dit dat schade die ontstaat als gevolg van constante blootstelling aan virussen, bacteriën, luchtvervuiling, of andere factoren, niet goed kan worden hersteld en er verlittekening ontstaat. In het algemeen is de overleving van patiënten met IPF afwijkingen in telomeergenen slechter dan in patiënten met IPF zonder afwijkingen in telomeergenen. Patiënten met een zogenaamd telomeersyndroom kunnen ook ziekten buiten de long hebben, zoals verlittekening van de lever, bloedarmoede, beenmergfalen, en nagel- of huidafwijkingen. In **Hoofdstuk 3** wordt beschreven dat ongeveer een kwart van de patiënten met IPF mogelijke uitingen van een telomeersyndroom heeft buiten de long. Dit is bijvoorbeeld een voorgeschiedenis van bloedarmoede of verlittekening van de lever, een familiegeschiedenis van beenmergfalen of verlittekening van de lever, of laboratoriumafwijkingen in een van de verschillende bloedlijnen. Patiënten die een of meer van deze uitingen hebben, hadden een slechtere overleving dan patiënten die dit niet hebben. Deze informatie kan

dus worden gebruikt om een inschatting van de prognose te maken, zelfs als specifieke informatie over afwijkingen in telomeergenen niet beschikbaar is.

In **Hoofdstuk 4** en **Hoofdstuk 5** worden mogelijke andere uitingen van een telomeersyndroom buiten de long onderzocht. Stoornissen in de afweer, botontkalking, en kransslagaderverkalking lijken allemaal niet geassocieerd te zijn met kortere telomeren. Afweerstoornissen en botontkalking komen maar weinig voor bij patiënten met IPF. Kransslagaderverkalking komt wel vaker voor, in ongeveer 30% van de patiënten. Ondanks dat dit niet een uiting van een telomeersyndroom lijkt te zijn, is dit wel geassocieerd met een slechtere overleving. Een zogeheten kransslagader-calcificatie-score kan vrij gemakkelijk worden bepaald aan de hand van de scan die van de longen wordt gemaakt voor het stellen van de diagnose van IPF. Patiënten bij wie deze score hoog is, hebben een slechtere overleving.

De verschillende uitingen van een telomeersyndroom lijken zich op een spectrum te bevinden. In **Hoofdstuk 6** wordt een overzicht gegeven van de verschillende uitingen van een telomeersyndroom in de long. Het blijkt dat dit niet alleen IPF is, maar ook andere vormen van longfibrose, emfyseem, en afwijkingen aan de longvaten komen voor. Afwijkingen in verschillende telomeergenen kunnen allemaal tot vergelijkbare ziekte lijden, terwijl dezelfde afwijking soms to verschillende ziekten kan lijden. In **Hoofdstuk 7** wordt aangetoond dat in sommige patiënten met IPF mutaties in het *ACD*-gen ten grondslag liggen aan de ziekte. Mutaties in dit telomeergen werden eerder al aangetoond in patiënten met een andere vorm van een telomeersyndroom (bloedarmoede). In **Hoofdstuk 8** wordt een patiënt beschreven met een afwijking in het telomeergen *TINF2*. Deze patiënt had naast IPF ook een afweerstoornis. Opvallend was dat de lengte van telomeren in het bloed van deze patiënt normaal was. Dit laat zien dat telomeerlengte waarschijnlijk niet de enige belangrijke factor is. Ook telomeren die een normale lengte hebben, maar om een andere reden onvoldoende stabiel zijn, kunnen tot vergelijkbare problemen leiden als te korte telomeren.

Er is nog geen specifieke behandeling beschikbaar voor patiënten met IPF in het kader van een telomeersyndroom. Er zijn wel aanwijzingen dat het middel danazol patiënten met bloedarmoede in het kader van een telomeersyndroom kan helpen, en mogelijk ook verlittekening van de long kan remmen. In **Hoofdstuk 9** worden de resultaten beschreven van behandeling van patiënten met IPF met danazol. In deze studie wordt, voor de hele groep patiënten, geen effect gezien van behandeling met danazol op de achteruitgang van longfunctie, en ook secundaire uitkomstmaten zoals overleving en radiografische beelden, geven geen aanwijzingen voor een positief effect van danazol. De afwezigheid van dit effect zou er mee te maken kunnen hebben dat alleen patiënten in een laat stadium van de

ziekte behandeling met danazol kregen, nadat ze ondanks behandeling met antifibrotische middelen achteruit gingen. Een andere belangrijke bevinding is dat 42% van de patiënten binnen een jaar stopte met danazol vanwege bijwerkingen. Patiënten die danazol wel ten minste een jaar gebruikten, hadden een langzamere achteruitgang van hun longfunctie dan voordat ze het middel gebruikten. Dit zou kunnen betekenen dat eerdere of meer langdurige behandeling nodig is voordat danazol een positief effect heeft. Of behandeling met danazol in een eerder stadium van de ziekte of in specifieke subgroepen van patiënten met IPF inderdaad zinvol is, zal uit toekomstig onderzoek moeten blijken.

Het wordt steeds duidelijker dat er veel overlap is tussen onderliggende oorzaken van IPF en andere vormen van longfibrose. Daarnaast is recent aangetoond dat de antifibrotische medicijnen ook bij andere vormen van longfibrose dan IPF lijken te werken. Het wordt daarom ingewikkelder om te bepalen welke behandeling of behandelingen het meest geschikt zijn voor een specifieke patiënt met longfibrose. In **Hoofdstuk 10** wordt het zogeheten 'treatable traits' model toegepast op longfibrose. Binnen dit model kunnen er verschillende onderliggende oorzaken of risicofactoren van longfibrose worden onderzocht met behulp van gevalideerde tests, zoals een test in het bloed, een scan, of een vragenlijst. Afhankelijk van de uitslag van een test kan een bepaalde behandeling worden gestart om een specifieke 'trait' te behandelen. Meerdere onderliggende oorzaken of risicofactoren kunnen aanwezig zijn in dezelfde patiënt en dus kunnen ook meerdere soorten behandeling nodig zijn. Dit model is een belangrijke stap op weg naar gepersonaliseerde behandeling voor patiënten met longfibrose.

Toekomstige onderzoeken kunnen hopelijk beter inzicht geven in de precieze oorzaak van IPF in patiënten met een telomeersyndroom. Waarom krijgt de ene patiënt met een afwijking in een telomeergen IPF, en de andere patiënt een andere longziekte of zelfs een ziekte buiten de longen? Omgevingsfactoren lijken in ieder geval een rol te spelen. Het systematisch nagaan van telomeerlengte in verschillende weefsels, de activiteit van verschillende mechanismen betrokken bij het onderhoud van telomeren, en blootstellingen aan (mogelijke) schadelijke stoffen kunnen hier hopelijk antwoord op geven. Daarnaast kunnen uiteindelijk nieuwe behandelingen voor longfibrose in het kader van een telomeersyndroom worden ontwikkeld binnen het 'treatable traits' model.

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Thijs Willem Hoffman was born on 13 November 1993 in Rheden, The Netherlands. He graduated with distinction from Katholieke Scholengemeenschap De Breul and Junior College Utrecht in 2011. Thereafter, he started medical school at Utrecht University. In 2012, he started working as a student researcher on the subject of immunodeficiency and lung transplantation at the Department of Pulmonology of St. Antonius Hospital under the supervision of Prof. Dr. Grutters and Dr. van Kessel. He obtained a Bachelor's degree in Medicine in 2014, and continued to study for his Master's degree in Medicine. In 2017 he worked in Lusaka, Zambia for 8 months at the Department of Infectious Diseases at University Teaching Hospital. When he returned, he started the work on this thesis at the Department of Pulmonology of St. Antonius Hospital under the supervision of Prof. Dr. Biesma, Dr. van Kessel, Dr. van Moorsel, and Prof. Dr. Grutters. In 2018 he obtained his Master's degree in Medicine in and subsequently started working as an intern in the Department of Pulmonology of St. Antonius Hospital. In 2020 he started his residency in Pulmonology at St. Antonius Hospital, under the supervision of Dr. Schramel.

LIST OF PUBLICATIONS

Hoffman TW, van Es HW, Biesma DH, Grutters JC. Potential interstitial lung abnormalities on chest X-rays prior to symptoms of idiopathic pulmonary fibrosis. BMC Pulm Med. 2022; 22:329

Hoffman TW, Grutters JC. Towards treatable traits for pulmonary fibrosis. J Pers Med. 2022; 12(8):1275

Ziko LM, Hoffman TW, Fwoloshi S, Chanda D, Nampungwe YM, Patel D, Bobat H, Moonga A, Chirwa L, Hachaambwa L, Mateyo KJ. Aetiology and prognosis of community-acquired pneumonia at the Adult University Teaching Hospital in Zambia. PLoS One. 2022; 17(7): e0271449

Hoffman TW, van der Vis JJ, Biesma DH, Grutters JC, van Moorsel CHM. Extrapulmonary manifestations of a telomere syndrome in patients with idiopathic pulmonary fibrosis are associated with decreased survival. Respirology. 2022; 27(11): 959-965

Hoffman TW, Meek B, Rijkers GT, van Kessel DA. Serologic response to a third dose of an mRNA-based SARS-CoV-2 vaccine in lung transplant recipients. Transpl Immunol. 2022; 72: 101599

Hoffman TW, van Es HW, Grutters JC, Mateyo KJ. Multidisciplinary diagnosis of interstitial lung disease in Zambia using videoconferencing. Pan Afr Med J. 2022; 41: 183

Hoffman, TW, Meek, B, Rijkers, GT van Kessel, DA. Poor Serologic Response to 2 Doses of an mRNA-based SARS-CoV-2 Vaccine in Lung Transplant Recipients. Transplantation. 2022; 106(1): e103-e104

Hoffman, TW, van Moorsel, CHM, Kazemier, KM, Biesma, DH, Grutters, JC, van Kessel, DA. Humoral immune status in relation to outcomes in patients with idiopathic pulmonary fibrosis. Lung. 2021; 199(6): 667-676

Hoffman, TW, Hemke, AC, Zanen, P, Luijk, B, Hoek, RA, Verschuuren, EA, van Kessel, DA. Waiting List Dynamics and Lung Transplantation Outcomes After Introduction of the Lung Allocation Score in The Netherlands. Transplant Direct. 2021; 7(10): e760

Shahin S, Hoffman TW, van Es HW, Grutters JC, Mateyo KJ. Congenital tracheobronchomegaly (Mounier-Kuhn syndrome) in a 28-year-old Zambian male: a case report. Pan Afr Med J. 2021; 40: 153

Scharringa S, Hoffman TW, van Kessel DA, Rijkers GT. Vaccination and their importance for lung transplant recipients in a COVID-19 world. Expert Rev Clin Pharmacol. 2021; 14(11): 1413-1425

Hoffman TW, Meek B, Rijkers GT, Grutters JC, van Kessel DA. Pneumococcal Conjugate Vaccination Followed by Pneumococcal Polysaccharide Vaccination in Lung Transplant Candidates and Recipients. Transplant Direct. 2020;6(6): e555

Holzer L, Hoffman TW, van Kessel DA, Rijkers GT. Pneumococcal vaccination in lung transplant patients. Expert Rev Vaccines. 2020: 19(3): 227-234

Hoffman TW, van der Vis JJ, van der Smagt JJ, Massink MPG, Grutters JC, van Moorsel CHM. Pulmonary fibrosis linked to variants in the ACD gene, encoding the telomere protein TPP1. Eur Respir J. 2019; 54(6): 1900806

Hoffman TW, van Moorsel CHM, Borie R, Crestani B. Pulmonary phenotypes associated with genetic variation in telomere-related genes. Curr Opin Pulm Med. 2018; 24(3): 269-280

Hoffman TW, van Kessel DA, van Tol MJD, Vidarsson G, Jol-van der Zijde EC, Rijkers GT, van Velzen-Blad H. An unusual presentation of a patient with severe hypogammaglobulinemia. Clin Case Rep. 2018; 6(12): 2416-2423

Klay D, Hoffman TW, Harmsze AM, Grutters JC, van Moorsel CHM. Systematic review of drug effects in humans and models with surfactant-processing disease. Eur Respir Rev. 2018; 27(149): 170135

Fwoloshi S, Chomba M, Ngalamika O, Hoffman TW, Mulenga L, Mweemba A. Guillain-Barre syndrome in a patient with HIV managed with plasma exchange: a case report from Zambia. J Trop Med Health. 2018: 133

Fwoloshi S, Hachaambwa LM, Chiyenu KO, Chirwa L, Hoffman TW, Ngalamika O, Bailey S. Screening for diabetes mellitus among tuberculosis patients: findings from a study at a tertiary hospital in Lusaka, Zambia. Can J Infect Dis Med Microbiol. 2018: 3524926

Hoffman TW, van Kessel DA, Rijkers GT. Impact of using different response criteria for pneumococcal polysaccharide vaccination for assessment of humoral immune status. J Clin Immunol. 2018; 38(2): 149-152

Van Kessel DA, Hoffman TW, van Velzen-Blad H, Meek B, van Mens S, Grutters JC, Rijkers GT. Case report on a defective antibody response against pneumococcal serotype 9V in a patient with a single episode of pneumonia. Pneumonia. 2017; 9: 16

van Kessel DA, Hoffman TW, Kwakkel-van Erp JM, Oudijk JD, Zanen P, Rijkers GT, Grutters JC. Long-term follow up of humoral immune status in adult lung transplant recipients. Transplantation. 2017; 101(10): 2477-2483

van Kessel DA, Hoffman TW, van Velzen-Blad H, van de Graaf EA, Grutters JC, Rijkers GT. Immune status assessment in adult lung transplant candidates. Transpl Immunol. 2017; 40: 31-34

van Kessel DA, Hoffman TW, van Velzen-Blad H, Zanen P, Grutters JC, Rijkers GT. Long-term clinical outcome of antibody replacement therapy in humoral immunodeficient adults with respiratory tract infections. EBioMedicine. 2017; 18: 254-260

Hoffman TW, Grutters JC. Case 8-2016: A man with recurrent fevers, hypoxemia, and lung infiltrates. N Engl J Med. 2016; 375(4): 392

Hoffman TW, van der Vis JJ, van Oosterhout MF, van Es HW, van Kessel DA, Grutters JC, van Moorsel CHM. TINF2 gene mutation in a patient with pulmonary fibrosis. Case Rep Pulmonol. 2016; 2016: 1310862

Van Moorsel CHM, 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: 304186

Hoffman TW, van Kessel DA, van Velzen-Blad H, Grutters JC, Rijkers GT. Antibody replacement therapy in primary antibody deficiencies and iatrogenic hypogammaglobulinemia. Expert Rev Clin Immunol. 2015; 11(8): 921-933

van Kessel DA, Hoffman TW, van Velzen-Blad H, Zanen P, Rijkers GT, Grutters JC. Response to pneumococcal vaccination in mannose-binding lectin-deficient adults with recurrent respiratory tract infections. Clin Exp Immunol. 2014; 177(1): 272-279

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