Biomarkers of progressive lung fibrosis

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Abstract

Interstitial lung diseases (ILD) encompass a heterogeneous group of immuno-inflammatory and fibrotic diseases of the lung parenchyma. The most common and severe ILD is idiopathic pulmonary fibrosis (IPF), a chronic progressive fibrotic lung disease of unknown aetiology associated with poor prognosis. A substantial proportion of individuals with ILDs other than IPF also develop progressive fibrosis with clinical, radiological, and genetic similarities, suggesting a shared final common pathway across progressive fibrotic ILDs irrespective of aetiology. Study of shared mechanisms of progression has the potential to aid prognostication, enable a precise approach to therapeutic strategies and allow stratification into clinical trials. Biomarkers are objectively measured and reproducible characteristics that enable stratification of disease phenotypes. The aim of this thesis was to examine and characterise the role of clinical biomarkers in fibrotic lung diseases to enable early identification of progressive fibrotic phenotypes.

An evidence synthesis of blood biomarkers as prognosticators in IPF highlighted several biomarkers with prognostic potential and identified priorities for future blood biomarker research. The first individual participant data (IPD) meta-analysis in IPF of matrix metalloproteinase-7 demonstrated baseline measurements were independently associated with disease outcomes. To evaluate the role of physiological variables as prognostic biomarkers and as surrogate trial endpoints, the largest analysis of interventional trial placebo arms in IPF was performed using robust IPD methodology. Baseline and three-month change in physiological variables, particularly FVC were independently associated with disease outcomes, supporting their role as prognostic biomarkers. The association

between short-term change in FVC and disease outcomes were replicated in individuals receiving anti-fibrotics using pooled analysis of pirfenidone and nintedanib treatment arms. Moreover, a difference in FVC change over three-months between treatment and placebo arms was observed, supporting three-month FVC as a surrogate endpoint in future IPF trials.

An ongoing prospective multi-centre observational cohort study (INJUSTIS) to assess longitudinal disease behaviour and the role of biomarkers in other fibrotic lung diseases was established. Interim analysis suggested a significant proportion of individuals with non-IPF fibrotic ILD had progressive phenotypes that were comparable with disease behaviour in IPF. Lung function, particularly FVC change over three-months was independently associated with poorer outcomes. The role of home spirometry in fibrotic ILD was assessed, and though measurements were accurate and reliable when compared with hospital spirometry, daily FVC measurements were unable to predict mortality at earlier timepoints. An exploratory blood biomarker analysis performed in individuals with extremes of IPF offered further support for the role of CA-125 as a prognostic biomarker and identified several biomarkers and biological pathways for more focussed assessment in the complete INJUSTIS cohort.

Taken collectively, the data presented in this thesis strongly support an important role for biomarkers in fibrotic ILD to identify progressive fibrotic phenotypes and enable personalised approaches to patient management. Whilst the COVID-19 pandemic was severely disruptive, the work presented forms the basis for further study of biomarkers in progressive pulmonary fibrosis.

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The work presented in this thesis would not have been possible without several individuals to whom I owe much gratitude. Firstly, I would like to thank Professor Gisli Jenkins who trusted me throughout my research and offered unwavering support with an enthusiasm for science that is contagious. I would like to sincerely thank Dr. Iain Stewart for his patience, encouragement, attention to detail, and for always supporting me whenever I felt like giving up! I thank Dr Gauri Saini for offering me the opportunity to undertake research in the first instance, and I am indebted to Professor Simon Johnson for his advice and wisdom towards the end of my PhD.

I would also like to take this opportunity to thank Lucy Howard for her unrelenting dedication and support with the INJUSTIS study. I would like to express my gratitude to all the members at the Nottingham Respiratory Research Unit, particularly Glenn Hearson, Rebecca Braybrooke, and Laura Matthews, who were always available to assist and respond to my never-ending requests! I also would like to thank my fellow research fellows, Queenie and Ayushman, who were just great fun, shared my difficult moments and lent me an ear whenever I needed to rant!

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Declaration of work performed

The work presented in this thesis was supported by several individuals. This section details the contribution of others involved.

My involvement with the INJUSTIS study began with the design and set up, which involved writing the protocol, participant information sheets, clinical research form (CRF) and seeking ethical approval. The initial set up, particularly seeking ethical approval, and writing patient information sheets was supported by Lucy Howard. Once ethical approval was obtained, I have been involved with screening and recruiting participants, performing study visits, leading site initiation visits (SIV), coordinating recruiting sites and being the general first point of contact for any clinical study-related enquiries. Lucy Howard was the project coordinator, and attended SIVs, helped with managing sites, kept a recruitment log, updated ethics, and performed the majority of patient visits at the University Hospitals of Nottingham. All lab work for the INJUSTIS study including processing of samples and distributing lab kits was performed by the lab team at the Biomedical Research Centre (BRC). I performed the bronchoscopies with support from a respiratory consultant and provided the samples to the lab team for further processing. Database support (Medrio) and data checking was provided by Glenn Hearson, with data entered locally by both me and Lucy. A steering committee consisting of the chief investigator, statistician, lab team and database manager was established, and provided oversight, meeting quarterly. For the analysis presented in Chapter 7, samples were sent to Olink laboratories who carried out the lab work and provided results in an excel spreadsheet.

For the systematic reviews and meta-analyses presented in Chapters 2, 3 and 4, I put together the research questions including the PROSPERO protocols, devised the search terms with support from a librarian, performed the searches, shortlisted suitable studies, extracted the data and performed the statistical analysis. The assistance of a second reviewer was sought for literature searching and data extraction as per systematic review best practice. For both IPD meta-analyses I contacted the authors directly and wrote the statistical proposals for data sharing platforms. The contracts team at the University of Nottingham supported with data sharing agreements.

All data cleaning and statistical analyses presented in this thesis were performed by me, with oversight from Dr Iain Stewart. This includes the aggregate data meta-analysis, two IPD meta-analyses, INJUSTIS baseline demographics analysis, Olink biomarker analysis and home spirometry analysis.

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Publications arising from this thesis

1. **Khan F**, Stewart I, Howard L, et al. The Its Not JUST Idiopathic pulmonary fibrosis Study (INJUSTIS): description of the protocol for a multicentre prospective observational cohort study identifying biomarkers of progressive fibrotic lung disease*. BMJ Open Respiratory Research* 2019;6:

2. **Khan F**, Stewart I, Fabbri L, et al. Systematic review and meta-analysis of anakinra, sarilumab, siltuximab and tocilizumab for COVID-19. *Thorax* 2021; 76:907-919

3. **Khan F**, Howard L, Hearson G et al. Clinical Utility of Home versus Hospital Spirometry in Fibrotic ILD: Evaluation Following INJUSTIS Interim Analysis. *Annals of the American Thoracic Society* 2021 Sep 17. doi: 10.1513/AnnalsATS.202105-612RL.

4. **Khan F**, Stewart I, Saini G et al. An individual participant data meta-analysis of prognostic blood biomarkers in IPF. *Eur Respiratory Journal* 2021.

5. **Khan F**, Stewart I, Moss S et al. Three-month FVC: a potential trial endpoint for IPF based on individual participant data meta-analysis. *American Journal of Respiratory and Critical Care Medicine, In press.*

6. Fabbri L, Moss S, **Khan F** et al. Parenchymal lung disease following COVID-19 and viral pneumonitis hospitalisation: A systematic review and meta-analysis. *Under revision, Thorax.*

7. Wild JM, Porter JC, Molyneaux PL…**Khan F** et al. Understanding the burden of interstitial lung disease post-COVID-19: the UK Interstitial Lung Disease-Long COVID Study (UKILD-Long COVID). *BMJ Open Respiratory Research* 2021;**8:**e001049

Abstracts as poster presentations

1. **Khan F**, Howard L, Hearson G et al. Home Spirometry monitoring in fibrotic ILD: an interim analysis from the INJUSTIS study. *BTS Winter Meeting* 2021. (High scoring abstract)

2. **Khan F**, Stewart I, Saini G et al. A systematic review and individual participant data meta-analysis of MMP-7 and outcomes in IPF. *European Respiratory Society Annual Congress* 2021

3. **Khan F**, I Stewart, Howard L et al. An exploratory analysis of blood biomarkers in idiopathic pulmonary fibrosis based on interim data from the INJUSTIS study. *American Thoracic Society Conference 2022. Accepted, awaiting presentation*

Chapter 1 Introduction

1.1 Introduction to thesis

Interstitial lung diseases (ILD) are parenchymal diseases associated with substantial heterogeneity. In this thesis, I aim to examine and characterise the role of clinical biomarkers in fibrotic lung disease to enable early identification of progressive fibrotic phenotypes. This chapter presents an introduction to fibrotic ILD including its epidemiology, clinical features, pathogenesis, and treatment modalities. The second section of the introduction summarises our current understanding of biomarkers and examines their possible role in ILD. The chapter concludes with a thesis hypothesis and more specific aims.

1.2 Introduction to ILD

1.2.1 Classification of ILDs

Interstitial Lung Diseases (ILD) encompass a heterogeneous group of immuno-inflammatory and fibrotic diseases of the lung parenchyma sharing clinical, radiographic, and pathophysiological manifestations. Current classifications (Figure 1-1) broadly divide parenchymal diseases by aetiology into those with known causes such as those related to connective tissue, environmental exposures, drugs, and systemic disease, and those that are unknown such as idiopathic interstitial pneumonias (IIP)[1].

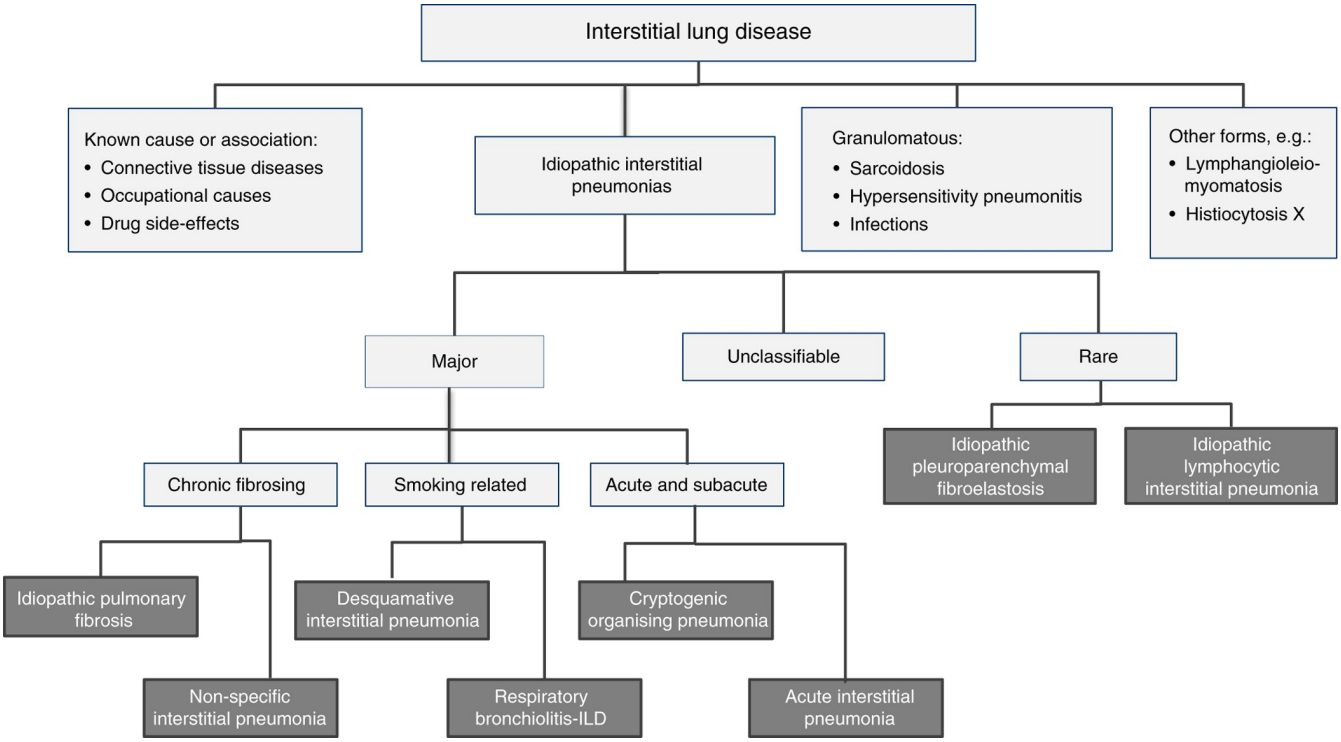


Figure 1-1 - Classification of interstitial lung diseases. Adapted from ATS/ERS 2013 guidelines

The most common and severe subtype of IIP is idiopathic pulmonary fibrosis (IPF), a chronic progressive fibrotic lung disease of unknown aetiology with poor prognosis[2]. Disease trajectory is variable, ranging from slow progression to rapid loss of lung function and death[3]. Whilst IPF is considered the archetypal progressive fibrotic ILD, a proportion of individuals with other ILDs from known causes also develop progressive fibrotic phenotypes during their disease[4]. Examples of conditions characterised by progressive pulmonary fibrosis include asbestosis[5], fibrotic hypersensitivity pneumonitis (HP)[6], rheumatoid arthritis associated ILD (RA-ILD)[7], systemic sclerosis-associated ILD (SSc-ILD)[8], sarcoidosis[9] and unclassifiable ILD (uILD)[10]. Although no uniform criteria currently exist, a progressive fibrotic phenotype is often associated with similar biological and clinical behaviours as IPF, suggesting individuals with progressive fibrosis could be "lumped" together regardless of aetiology, particularly for the purposes of clinical research and treatment.

1.2.2 Epidemiology

IPF is the most prevalent fibrotic ILD affecting approximately three million individuals worldwide[11-15]. Incidence is estimated at 3-9 cases per 100,000 per year in Europe and Northern America and has steadily risen over time[12 16]. The lowest rates globally appear to be in Asia with a reported incidence of 1.2-4.16 per 100,000 people, and Scandinavia, with an incidence between 1.3 and 4.3 per 100,000[16]. IPF affects men disproportionately and tends to occur in older adults with 85% of new diagnoses in UK made in individuals above the age of 70 years [11 3]. Median survival from diagnosis in individuals not receiving anti-fibrotic therapy is approximately three to five years[12], with many dying from progressive respiratory failure.

The epidemiology of progressive fibrotic phenotypes in ILDs other than IPF is less well known, possibly due to the heterogeneous nature of the aetiology and complexity of diagnosis[3]. A survey of 486 physicians worldwide (243 pulmonologists, 203 rheumatologists, 40 internists), coupled with data from US insurance claims, estimated that 18-32% of individuals diagnosed with ILDs other than IPF develop a progressive fibrotic phenotype[17].

RA-ILD, a well-known complication of rheumatoid arthritis (RA), occurs in approximately 5-10% of RA sufferers, with an increased risk with prolonged duration of RA[18]. Notably RA-ILD shares risk factors with IPF including male gender, older age, and smoking history[7 19]. Whilst epidemiological studies are scarce, a large longitudinal population-based study of RA-ILD

estimated median survival from diagnosis of only 2.6 years[7], which is comparable to IPF survival.

Estimating the incidence of HP is influenced by global variations in disease definitions, differing diagnostic criteria and geographical variation of inciting antigens. In the UK, the incidence has been relatively stable over the previous two decades and is estimated at approximately 0.9 cases per 100,000 person-years[20]. It remains unknown what proportion of individuals with HP develop progressive fibrotic phenotypes. Nonetheless, consistent with other fibrotic lung diseases, the median survival is poor, particularly when an inciting antigen cannot be identified and is estimated at approximately five years[21].

Asbestosis shares several risk factors with IPF and other fibrotic ILDs, including age and male gender[22]. The worldwide incidence of asbestosis is generally on the decline due to a reduction in occupational exposures, although asbestos use remains high in Russia and Asia[23]. In an analysis of the SWORD (Surveillance of Work-related and Occupational Respiratory Disease) database, the incidence of asbestosis in the UK was estimated at 1.3 per 100,000 per year, increasing with age and peaking between 75-79 years[22]. Once asbestosis is diagnosed, survival may be shortened by 8 years[24], although progression is reported to be dependent on the amount of retained asbestos fibres in the lung[25].

Unclassifiable ILD (uILD) contributes to a significant burden of ILD in clinical practice and represents between 10% and 38% of all ILDs, with variable definitions and heterogeneity in

diagnostic algorithms across different ILD centres[26 27]. Age appears to be a risk factor, with the prevalence of uILD relative to other ILDs disproportionately increased in the elderly population[28]. Due to diagnostic heterogeneity, survival is difficult to predict, but has been estimated as 46-70% at 5 years[10 26].

ILDs where progression of fibrosis seems more indolent relative to other fibrotic ILDs, include sarcoidosis and SSc-ILD. Whilst most individuals with sarcoidosis enter spontaneous remission, approximately 20% develop pulmonary fibrosis, but unlike IPF, fibrotic activity does not seem to progress inexorably once initiated[9]. Ten-year survival in fibrotic sarcoid is around 85%, better than other progressive fibrotic ILDs[29]. ILD is reported to develop in 35-90% of individuals with systemic-sclerosis[3], and accounts for one-third of all disease-related deaths[30]. Median survival from a high resolution computer tomography (HRCT) diagnosis of ILD is 11.2 years[8], a prognosis considerably better than other fibrotic ILDs, possibly because of a higher prevalence of a non-specific interstitial pneumonia (NSIP) pattern[31].

The remainder of this thesis will focus on progressive fibrotic ILDs namely IPF, fibrotic HP, RA-ILD, asbestosis and uILD.

1.2.3 Clinical features of ILD

A diagnosis of fibrotic ILD is suspected in adults presenting with unexplained progressive breathlessness often with chronic dry cough and fine crackles on clinical examination. Rate of symptom progression can vary between individuals, but may be present for up to five

years before a formal diagnosis is reached, with individuals often misdiagnosed with chronic obstructive pulmonary disease or heart failure in the interim[32]. Diagnoses are challenging and are typically made with increased diagnostic confidence following multidisciplinary team (MDT) discussions involving clinicians, thoracic radiologists and histopathologists[33]. In a multicentre evaluation of inter-multidisciplinary team agreement, agreement between MDTs across seven countries was good for a diagnosis of IPF, but low for HP[34]. This low agreement in HP may be explainable, in part, by the absence of consensus diagnostic criteria. It would therefore not be unreasonable to conclude that significant variations in therapy for the same underlying disease exist across different centres, highlighting a limitation of the current ILD classification.

1.2.4 Diagnostic methods

Reduced forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide (DL_{CO}) are typical investigation findings. Chest radiographs, which may be normal in up to 10% in early disease[35], tend to demonstrate reduced lung volumes with bilateral reticular infiltrates. HRCT is considered the cornerstone of diagnosis and is described in more detail below. Once an interstitial process is recognised, a focused and detailed history and examination to identify associated causes such as connective tissue disease, autoimmune conditions and diseases related to drug, smoking and occupational exposures are performed. Further investigations may include, but are not limited to, serological testing, muscle enzymes, serum precipitins, bronchoalveolar lavage (BAL) fluid and lung biopsy.

1.2.4.1 Imaging

HRCT is central to confirming a diagnosis of ILD and recognising specific radiological patterns that may identify underlying causes. The hallmark histological pattern of IPF is usual interstitial pneumonia (UIP), characterised radiologically by patchy reticular opacities which are bilateral, subpleural and with basal predominance. Reticular changes are often associated with traction bronchiectasis and honeycombing (clustered cystic air spaces). International consensus criteria for the diagnosis of IPF incorporating HRCT features have been developed with four diagnostic categories[2]. "UIP pattern" and "probable UIP" are distinguished by the presence or absence of honeycombing. An "indeterminate UIP" pattern is assigned for HRCT imaging that demonstrates features of fibrosis but does not meet UIP or probable UIP criteria and is not explicitly indicative of a specific aetiology. The presence of extensive ground glass opacification, lung cysts, nodules, consolidation and marked mosaic attenuation with air trapping suggest an "alternative diagnosis".

Studies have demonstrated UIP on HRCT has a 90-100% positive predictive value for UIP on subsequent histology[36-38], although notably inter-observer agreement for a UIP pattern amongst thoracic radiologists is only moderate[39]. Whilst guidelines recommend the consideration of histological confirmation for the diagnosis of IPF in the absence of honeycombing[2], in clinical practice surgical lung biopsy rates are much lower due to associated risks[40]. A high proportion of individuals with probable UIP and about half of those with indeterminate UIP are also likely to have histopathological UIP if biopsied[38]. Therefore, a diagnosis of UIP is typically made on HRCT findings alone in the correct clinical context.

Whilst characteristically associated with IPF, a radiological UIP pattern with or without honeycombing is commonly shared with other fibrotic ILDs[41]. Individuals with HP demonstrate significant heterogeneity in radiological features which can range from predominantly inflammatory changes such as ground glass opacification, mosaic attenuation and centrilobular nodules to fibrosis with reticulation, traction bronchiectasis, and honeycombing. Distinguishing radiologically between IPF and fibrotic HP can be particularly challenging, and the "headcheese sign" has been suggested as a more specific radiological finding[42]. Nonetheless, parenchymal fibrosis may be present in approximately 85% of HP sufferers, of which a quarter may follow a UIP distribution[43].

Radiological changes in RA-ILD include reticulation, ground-glass opacities, consolidation, honeycombing and nodules. All patterns of ILD are known to occur in patients with RA-ILD, but the most common is a UIP pattern, with a prevalence as high as 75%[44], and like IPF, tends to be highly specific for histopathological UIP[45]. Four major patterns have been described, namely UIP, nonspecific interstitial pneumonia (NSIP), obliterative bronchiolitis, and organising pneumonia[46].

Asbestosis is characterised by pleural plaques and often a UIP pattern, which is frequently indistinguishable from IPF[47]. Diagnosis therefore relies on an accurate occupational history, which can be challenging due to subjectivity and inaccuracies in patient recall. When significant exposure to asbestos cannot be recognised, other diagnoses including IPF are made. Thresholds to define clinically significant exposure can often vary between individual clinicians and respiratory centres, leading to inconsistent diagnostic characterisation. When

the thorough evaluation of suspected ILD is unable to yield a confident diagnosis, individuals are diagnosed with uILD[48], with UIP observed in around 75% of individuals[49].

1.2.4.2 Biopsy

Consistent with the radiological categorisation of IPF, histopathological guidelines recommend a similar approach of UIP, probable UIP, indeterminate for UIP and alternative diagnosis[2]. A UIP pattern is characterised by honeycombing alone or patchy fibrosis with architectural distortion in a predominantly subpleural and/or paraseptal distribution in the presence of fibroblastic foci (proliferating fibroblasts and myofibroblasts).

Histological patterns in other fibrotic ILDs can be diverse, but often mimic a UIP-like pattern with fibrotic changes accompanied by architectural distortion[50 51]. Inter-observer agreement between pathologists for the diagnosis of CTD-ILD and HP tends to be poor, with a Cohen's kappa coefficient of 0.22 and 0.2 respectively[34]. Poor inter-observer agreement alongside shared histopathological changes across fibrotic ILDs reiterate the limitations of the current ILD classification. In an observational study of consecutive IPF participants, almost half were reclassified as HP over a six year follow up period[52]. In view of this diagnostic heterogeneity, individuals with similar fibrotic diseases may be provided with different diagnostic labels, even after surgical lung biopsy, which is likely to influence further management and the choice of therapy offered.

1.2.4.3 Progression of disease

Current methods of evaluating disease progression in clinical practice rely on the development of fibrosis, using a combination of symptoms, pulmonary function tests and thoracic imaging. An FVC decline ≥ 10% over 12 months is a surrogate for mortality, and is therefore commonly used to define disease progression, and as an endpoint in IPF clinical trials[53-57]. The role of lung function is not limited to IPF, having been shown to demonstrate prognostic value in other fibrotic ILDs[58-61]. Whilst FVC change over 12 months remains a commonly used prognostic marker, the course of disease for an individual patient remains impossible to predict at the point of initial presentation[15]. The accurate and early prediction of disease course is essential for ongoing clinical care, including appropriately counselling patients and enabling personalised approaches to therapy.

1.2.5 Pathogenesis

Despite significant advances, our understanding of fibrotic lung disease pathogenesis remains incomplete. IPF, formerly considered an inflammatory driven disease with parenchymal fibrosis a late sequalae, is now considered to be an epithelial driven disease[62]. The current paradigm for the pathogenesis of IPF suggests a complex interplay of a dysfunctional epithelium and aberrant wound healing leading to chronic fibro-proliferation following repeated epithelial micro-injury from environmental factors in genetically susceptible individuals. Whilst individual fibrotic ILDs have disease specific triggers with characteristic clinical, radiological, and pathological features, it is postulated that progressive fibrosis promotes a self-sustaining and vicious amplification loop that drives

progressive tissue remodelling independent of aetiology and external stimulation[63]. The remainder of this section will focus on IPF and will attempt to explore commonalities and distinctions in pathogenesis across progressive fibrotic ILDs.

1.2.5.1 Risk factors

1.2.5.1.1 Environmental

Exposures in IPF are largely unknown, though exposure to metal, wood and silica dusts, atmospheric pollutants such as ozone (O3), nitrogen dioxide (NO2) and particulate matter, and exposures related to agriculture and livestock have all been associated with the risk of developing IPF [64-66]. The relationship between smoking and the development of pulmonary fibrosis remains uncertain, with studies demonstrating conflicting results[67-70].

An important differentiating factor between a diagnosis of IPF or an alternate fibrotic ILD is the identification of an inciting exposure. Repeated exposure to small environmental particles (< 5um) are understood to provoke an exaggerated immune response in HP[50], with the most commonly implicated antigens including thermophilic actinobacteria, fungi, avian antigens, industrial isocyanates and non-tuberculous mycobacteria[50 71]. Similarly, heavy and prolonged asbestos exposure is linked to the development of asbestosis[47], with a 25 fibre/ml-years exposure threshold commonly implicated, although this remains contentious with individual cases reported following much lower lifetime exposures[72]. In an epidemiological study, annual deaths from IPF were related to previous UK asbestos imports, raising the suspicion that a proportion of IPF deaths were indeed due to

unrecognised asbestos exposure and emphasising the difficulties in separating IPF and asbestosis[73].

1.2.5.1.2 Gastro-oesophageal reflux disease

In recent years, interest has grown around the micro-aspiration of gastro-oesophageal contents in IPF pathogenesis [74 75], culminating in antacid medications receiving a conditional recommendation in the latest iteration of the international guidelines[76]. A meta-analysis of 18 case-control studies, found gastro-oesophageal reflux disease (GORD) was associated with IPF[77], but following adjustment for smoking history, no association was observed, suggesting smoking may be a confounder. Furthermore, in a post-hoc analysis of landmark clinical trials, antacid therapy failed to improve clinical outcomes [78]. Consequently, it has been postulated that the presence of a hiatus hernia causing both acid and non-acid reflux, could be a risk factor for developing IPF and subsequent disease progression[79], though further study is required.

1.2.5.1.3 Microbial agents

Evidence for the role of microbial agents in IPF aetiology is accumulating. A viral aetiology has been suggested given the presence of viral signatures in the lungs of IPF individuals, although their precise contribution to the initiation and progression of disease remains unclear[80]. Detected viruses in IPF lungs include Epstein-Barr virus, cytomegalovirus, hepatitis C and herpes simplex virus[81]. Adjunctive antiviral therapy has demonstrated potential benefit in attenuating disease progression, though data are limited[82].

A higher bacterial burden has been reported in the lower airways of IPF and is associated with poor disease outcomes, [83] [84] though the EME-TIPAC study exploring the efficacy of co-trimoxazole in reducing mortality and/or hospitalisation found no benefit[85]. Notably, the microbial composition in HP does not predict survival and is distinct from IPF, suggesting microbial alterations may be disease specific[86].

1.2.5.1.4 COVID-19

Severe acute respiratory syndrome causing coronavirus-2 (SARS-CoV-2) was first identified in Wuhan, China and was soon declared a global pandemic by the World Health Organisation (WHO)[87]. The clinical presentations associated with COVID-19 are vast, ranging from asymptomatic disease to severe acute respiratory distress syndrome (ARDS), multi-organ failure and death. Longitudinal data obtained from survivors suggests a substantial proportion have ongoing breathlessness associated with long term impairment of lung function and CT evidence of pulmonary fibrosis, months after the initial illness[88]. Similar fibrotic abnormalities have been noted after previous coronavirus outbreaks including severe acute respiratory syndrome causing coronavirus (SARS-CoV) and Middle Eastern Respiratory Syndrome (MERS)[89] [90]. It currently remains unknown whether post-COVID lung abnormalities will be time-dependent and reversible, or whether they will develop into a persistent or even progressive fibrotic phenotype. Further research to understand the trajectory of disease, risk factors for progression and potential therapeutic options is underway, with results eagerly awaited[91].

1.2.5.1.5 Genetics

An increasing body of evidence has identified genetic variants associated with both familial and sporadic pulmonary fibrosis[92]. Genetic variants can be divided into those that are rare (MAF <1%) but highly penetrant with a large effect size[93], and those that are common (mean allele frequency [MAF] >5%) but have a smaller effect size. Phenotypic and genomic markers commonly overlap, with different ILD manifestations observed in individuals with identical mutations, reiterating the need for better understanding of underlying pathobiological pathways[94].

The occurrence of ILD in two or more first-degree relatives constitutes the syndrome of familial pulmonary fibrosis (FPF)[94]. Studies of individuals with FPF have identified several rare genetic variants, implicating maintenance of telomere length and surfactant dysfunction[95 96]. Telomeres are the caps of chromosomes, progressively shortening with each successive cell division, before ultimately activating cell death[97]. Genetic mutations in six telomere-related genes, telomerase reverse transcriptase (TERT), telomerase RNA component (TERC), regulator of telomere elongation helicase 1 (RTEL1), poly(A) specific ribonuclease (PARN), dyskerin (DKC1), TRF-1 interacting protein 2 (TINF2), all of which lead to shortened telomere lengths, are enriched in familial and sporadic IPF[98]. TERT mutations are the most frequently identified rare variants associated with pulmonary fibrosis, being present in approximately 15% of FPF, and 1-3% of sporadic cases[93 97], and are associated with reduced survival and greater FVC decline[99 100]. Shortened telomeres that are associated with accelerated progression have been demonstrated in other fibrotic ILDs, suggesting telomere biology is a key contributor to the pathogenesis of fibrotic ILD, regardless of

aetiology[98]. In individuals with HP, a substantial proportion have rare, protein-altering variants in telomere related genes which are associated with reduced survival[101]. Similarly, short telomeres in HP are associated with the extent of radiological fibrosis, histological pattern, and reduced survival[102]. Similarly, in RA-ILD, a whole exome sequence (WES) revealed an excess of mutations in telomere-maintenance genes such as TERT, RTELI1 and PARN, with shortened telomere lengths compared with controls[103].

Other rare genetic variants found less frequently but carrying large effect include genes encoding surfactant protein C (SFTPC) and surfactant protein A2 (SFTPA2)[104 105]. Surfactant gene mutations are understood to increase endoplasmic reticulum (ER) stress and activate the unfolded protein response (UPR), predisposing to subsequent lung fibrosis[93]. Although studies have provided evidence for the role of surfactant protein mutations in familial fibrosis, the frequency of mutations in sporadic cases is only 1%[106 107].

Common genetic variants that increase susceptibility to IPF and other fibrotic ILDs have been identified in genome wide association studies (GWAS)[108-113]. A common gain-of-function single nucleotide polymorphism (SNP) in the mucin 5B (MUC5B) promoter region (rs35705950), is considered the strongest risk factor accounting for one-third of the risk of developing IPF[114]. Unlike other common variants, the MUC5B variant has a strong disease effect, with each minor allele copy conferring a five-fold increased disease risk[115 116]. Importantly, the MUC5B variant is observed in up to one-fifth of unaffected individuals, suggesting other genetic variants alone or in combination contribute to the development of disease[110]. Notably, the minor allele for MUC5B has been found in other fibrotic ILDs

suggesting shared genetic risk factors. In two independent cohorts with HP, the MUC5B minor allele was present in increased frequency (24.3% and 32.3%) compared with healthy controls (10.7%) [102], and its presence was associated with increased radiological fibrosis, and a trend towards poorer survival. In a further study, the presence of the MUC5B minor allele predisposed to asbestosis, but was not associated with survival[117]. A study of participants with RA-ILD found the MUC5B minor allele was around five-times more likely compared with controls, with no association between the minor allele and a diagnosis of RA alone[118]. A particularly strong association between the MUC5B minor allele and radiological UIP was observed, suggesting it may be a risk factor for the development of UIP, a pattern shared by progressive fibrotic ILDs such as IPF, asbestosis, RA-ILD and HP, rather than IPF alone. This notion is further supported by the lack of a known association between the MUC5B minor allele and sarcoidosis[119], or SSc-ILD[120] , the latter typically characterised by NSIP[121].

Other genetic variants implicating various pathways including host defence, cellular barrier function and mTOR signalling, have been identified as risk factors for IPF. Genetic variants include DSP (desmoplakin), AKAP13 (A-kinase anchoring protein 13), TOLLIP (toll-interacting protein), SPPL2C (signal peptide peptidase-like 2C), FAM13A (family with sequence similarity, member A), ATP11A (ATPase, class VI, type IIA, DPP9 (dipeptidyl-peptidase 9), KANSL1 (KAT8 regulatory NSL complex, subunit 1) and recently identified signals near MAD1L1, DEPTOR and KIF15[108 110 112 113].

1.2.5.2 Cells and mediators

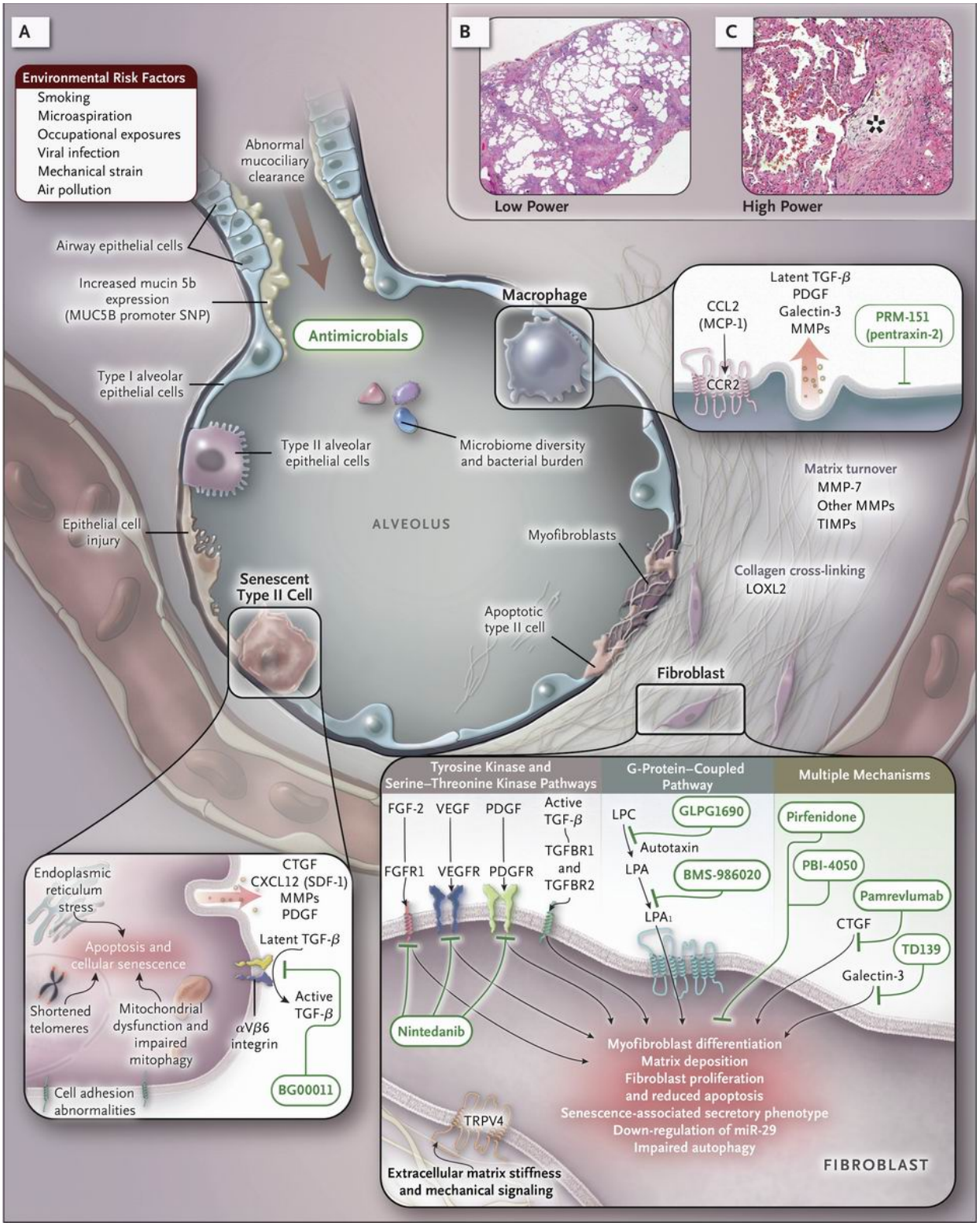


Figure 1-2 - Pathobiological features of Idiopathic Pulmonary Fibrosis

(Taken from Lederer DJ, Martinez FJ. Idiopathic Pulmonary Fibrosis. The New England journal of medicine 2018; 378(19):1811-23

1.2.5.2.1 Epithelium

In normal lung, following epithelial injury and damage to type 1 alveolar epithelial cells (AEC1), type 2 alveolar epithelial cells (AEC2) proliferate and restore alveolar integrity through several mechanisms (Figure 1-2). Increasing genetic, proteomic, and histological evidence suggests a dysfunctional, genetically susceptible, and fragile alveolar epithelium with reduced ability to respond to repetitive local micro-injuries, the origin of which remains elusive, is crucial to the pathogenesis of fibrosis[122 123]. Several genetic variants implicated in IPF, are either expressed in the alveolar epithelium (MUC5B, DSP, AKAP13) [112], or lead to molecular changes in epithelial cells (TERT, TERC, PARN). Moreover, blood proteins (surfactant protein-D, matrix metalloproteinase-7, cancer antigen 125) associated with epithelial cell dysfunction are elevated in individuals with IPF and appear to correlate with disease progression[124]. Histologically, epithelial damage is believed to lead airway basal cells to undergo a process known as bronchiolisation of alveolar spaces, resulting in aberrant proliferation and distortion in the architecture of alveolar spaces[125].

A dysfunctional epithelium promotes fibrosis through several mechanisms. An imbalance between cellular demand for protein synthesis, and endoplasmic reticulum (ER) capacity to synthesise and process the protein leads to ER stress, and activation of the unfolded protein response (UPR)[126]. The UPR has several consequences including inhibiting protein translation, targeting proteins for degradation, and activating numerous intracellular apoptotic pathways designed to restore normality in ER work[127]. Alongside cell apoptosis, ER stress and activation of the UPR has profibrotic effects, including enhancing the activation of myofibroblasts and promoting the release of profibrotic cytokines.

Dysfunctional epithelial cells further contribute to fibrogenesis by secreting transforming growth factor-beta 1 (TGF-β1), the most potent pro-fibrotic mediator in IPF[125]. TGF-β1 is synthesised as a small latent complex requiring activation before it's biological effects can be exerted [128 129]. The best characterised mechanism of activation is by αvβ6 integrin, which is increased considerably in injured epithelia, again underlining the importance of the epithelium in fibrogenesis[122]. Once activated, TGF-β1 promotes epithelial cell apoptosis, epithelial to mesenchymal transition (EMT), collagen synthesis, differentiation of fibroblasts to myofibroblasts, and production of other profibrotic and angiogenic mediators. TGF-β1 also induces the expression, secretion and activation of matrix metallo-proteinases which are proteins implicated in IPF[122].

EMT is the molecular process, induced by ER stress and enhanced by specific growth factors, whereby epithelial cells are reprogrammed into mesenchymal cells. EMT occurs in development, carcinogenesis and may play a role in fibrogenesis in response to sustained inflammation and injury[130]. Mesenchymal cells contribute to wound repair and tissue remodelling acting as the major effector cells of pulmonary fibrosis and are discussed in later sections.

With the role of epithelial cell dysfunction well established in IPF, similar epithelial abnormalities following initial insult are likely to be central to the progression of other fibrotic ILDs. EMT has been described in HP[131], and elevated levels of TGF-β1 have been noted in other fibrotic lung diseases such as progressive RA-ILD, HP and asbestosis[132 133]. Moreover, ER stress, a precursor to the fibrotic response in IPF, is understood to play an

important pathogenic role in non-pulmonary fibrotic conditions such as chronic kidney disease, hepatic fibrosis and inflammatory bowel disease, suggesting shared fibrotic pathways across organ-specific diseases[134].

1.2.5.2.2 Mesenchyme

Mesenchymal cells, principally fibroblasts and myofibroblasts are the major effector cells of pulmonary fibrosis, synthesising much of the matrix. Under normal circumstances mesenchymal cells make up a minority of pulmonary cells but are important for the development and repair of the lung. However, in pathological fibrosis the mesenchymal cell population expands considerably and myofibroblasts synthesise vast quantities of disorganised and dense extracellular matrix, including type 1 collagen[135 136]. Mechanical characteristics of ECM are possibly the most important regulators of myofibroblast activity, with stiffer matrix enhancing activity in a positive feedback loop[137].

The origins of myofibroblasts are likely to be important and may be potentially targetable. Although the precise origin of lung myofibroblast cells remains controversial, four possible sources have been proposed. The most established explanation for the increase in myofibroblasts is the differentiation and expansion of interstitial fibroblasts. Activated fibroblasts help remodel and re-establish the extracellular matrix (ECM) and following persistent exposure to TGF-β1 and other profibrotic mediators, transdifferentiate into αSMA-expressing myofibroblasts[138]. The second potential origin of myofibroblasts is from the migration and trans-differentiation of fibrocytes. Fibrocytes are bone-marrow derived

cells that respond to TGF-β1 and though contentious, may contribute to lung fibrosis by secreting profibrotic cytokines, and producing type 1 and III collagens [139]. Thirdly, emerging evidence has shown myofibroblasts may originate from the migration, trans-differentiation and expansion of microvascular mesenchymal cells known as pericytes, although further study is needed[140]. The final source of myofibroblasts is from EMT as previously highlighted.

1.2.5.2.3 Inflammation and immunity

Despite the pathogenic paradigm of fibrosis shifting from a predominantly inflammatory disease to an epithelial-driven disease, it is likely innate and adaptive immune processes actuate existing fibrotic responses. Following epithelial injury, macrophages attempt wound repair by secreting pro-inflammatory cytokines such as TNFα, IL-1 and IL-6. Pro-inflammatory M1 macrophages later convert into M2 macrophages that secrete PDGF and pro-fibrotic growth factors and contribute to the formation of extracellular matrix[141].

Persistent injury leads to the recruitment of neutrophils in response to IL-8, the predominant neutrophilic chemoattractant cytokine. Neutrophils are innate immune cells acting as key players in the acute phase of inflammation producing reactive oxygen species (ROS), further worsening epithelial damage, with resultant epithelial cell apoptosis[125]. Neutrophils may contribute to fibrosis via regulation of ECM turnover by the release of neutrophil elastase (NE), which has both pro-fibrotic and anti-fibrotic properties. NE, elevated in IPF lungs, breaks down collagen-IV and elastin, alleviating fibrosis, but also activates TGF-β1 and promotes fibroblast proliferation and myofibroblast differentiation,

thus enhancing fibrosis[141]. Mice deficient in neutrophil elastase seem to be protected from bleomycin and asbestos induced lung fibrosis suggesting shared mechanisms of disease[142]. Increased neutrophils seem to correlate with severity of fibrosis in both HP and asbestosis[143] [144]. Free radicals are also implicated in the mechanism of asbestosis, with asbestos fibres inducing epithelial cell injury and apoptosis[145].

In the past decade, the adaptive immune system, particularly Th-2 and Th-17 T-cells have been shown to be integral to fibrogenesis[146]. Th-2 T cell derived cytokines such as IL-4, IL-5, IL-13 and TGF-β1, promote macrophage recruitment and myofibroblast activation and proliferation[147]. Similarly, Th-17 cells produce cytokines such as IL-17 and IL-22, which are understood to induce the proliferation of fibroblasts and secretion of collagen, via a TGF-β1-dependent mechanism[148]. Immunopathological mechanisms are thought to be central in fibrotic ILDs such as HP. Whilst the acute form is mediated by immune complexes and Th-1 activity, growing evidence suggests individuals with HP demonstrate increased CD4+ T cells with skewed Th-2 T cell differentiation and cytokine release regulating the fibrotic response[149] [150]. Interestingly, cigarette smoke is thought to be protective in the development of HP, with nicotine acting on lymphocytes to decrease the reactivity of the Th-1 and Th-17 lineages[151]. However, nicotine increases Th-2 activity and may explain why smokers who develop HP often follow a chronic proliferative course[152]. There may be a role for Th-2 T cells in RA-ILD pathogenesis[153], with limited evidence for elevated CD4+ T cells in lung tissue[154].

1.2.6 Acute exacerbation

Acute exacerbations (AE) of fibrotic ILD are characterised by significant respiratory deterioration alongside evidence of new onset widespread alveolar abnormalities,[155] with histological evidence of diffuse alveolar damage (DAD). Retrospective data suggest AEs are unpredictable, although more frequent in older, non-smoking individuals with more advanced disease. Risk factors are poorly defined, with a need for biomarkers to identify those particularly at-risk. AEs of IPF are associated with a high in-hospital mortality and a median survival of 3-4 months[156]. Like IPF, post-exacerbation mortality rates in other fibrotic ILDs are reported to range from 33-83%, with in-hospital mortality rates of 50-100% in CTD-ILDs and 75-100% in HP[157 158]. In a recent retrospective observational study, the frequency of exacerbations was lower in individuals with non-IPF group compared with IPF, but short-term survival (90 days) was comparable[159]. Comparable outcomes suggest mutual pathological processes may be responsible for AEs across fibrotic ILDs, and further investigation is required. Evidence for the management of AEs is limited, with supportive care and corticosteroids recommended based on anecdotal evidence[155 160]. Studies evaluating antifibrotics in preventing exacerbations have shown contrasting results in both IPF and non-IPF ILD [161 162 163 164].

1.2.7 Treatment of Fibrotic ILDs

1.2.7.1 Immune suppression

Corticosteroids are widely instituted as first line therapy for symptomatic fibrotic ILD, despite the lack of evidence to authenticate their use[165]. Importantly, immunosuppressants

were frequently used to treat IPF, until the pivotal PANTHER study, which demonstrated prednisolone and azathioprine were associated with increased mortality and hospitalisation[166]. Participants were administered relatively high doses of prednisolone (0.5mg/kg/day) and the findings of this trial should not be interpreted to support an absence of inflammation in IPF (as discussed in section 1.2.5.2.3). Immunomodulatory agents are commonly trialled in the management of fibrotic ILDs, based on extrapolation from the Fibrosing Alveolitis in Scleroderma Trial (FAST) and Scleroderma Lung Studies (SLS)[167-169]. In SLS I, one year of oral cyclophosphamide in SSc-ILD resulted in a modest but significant mean FVC difference of 2.53% compared with placebo, with parallel improvements in dyspnoea and quality of life (QoL) scores[168]. Mycophenolate (MMF) was better tolerated in comparison with cyclophosphamide in SLS II, with similar improvements in lung function and dyspnoea[169]. The use of MMF in the SENSCIS study was associated with a slower decline in FVC at 52 weeks in both placebo and nintedanib arms[170]. No randomised trials of MMF have been performed in other fibrotic ILDs, but small retrospective studies have supported an association with lung function improvement[171 172]. Other immunomodulatory agents typically used in the management of ILD in the absence of trial evidence, include azathioprine, methotrexate, and rituximab. In view of the current therapeutic uncertainties and poor prognosis of fibrotic ILDs, there is an urgent need for evidence based and well-tolerated therapies[173].

1.2.7.2 Antifibrotics

Treatment advances have been made recently, with the approval of anti-fibrotic therapy in the UK for an MDT confirmed diagnosis of IPF and predicted FVC of 50-80%[174]. Whilst

antifibrotics are prescribed with the aim to decelerate disease progression, pooled analyses of clinical trials suggest there may be additional mortality and QoL benefits[162 175]. Pirfenidone was approved in 2011 for the treatment of IPF following the CAPACITY and ASCEND studies[53 176], where pirfenidone reduced FVC decline compared with placebo. The precise mechanism of action remains unknown, but pirfenidone has been shown to have anti-inflammatory, antioxidative and antiproliferative properties[177]. Nintedanib was approved in Europe in 2015 following the outcome of three clinical studies, TOMORROW, INPULSIS-1, and INPULSIS-2[54 178], all showing nintedanib significantly reduced the rate of FVC decline compared with placebo. Nintedanib, a potent, oral intracellular tyrosine kinase inhibitor acts on platelet derived growth factor receptors, fibroblast growth factor receptors and vascular endothelial growth factor receptors as well as non-receptor members of the Src family; critical signalling pathways involved in the proliferation, migration, and differentiation of lung fibroblasts and myofibroblasts[179].

Mechanistic similarities between IPF and other progressive fibrotic ILDs suggest that the currently available antifibrotics may be viable therapeutic options across fibrotic ILDs, with several recent clinical trials supporting this hypothesis [57 164 170 180]. The landmark INBUILD study explored the efficacy of nintedanib in a heterogeneous group of progressive fibrotic lung diseases and found nintedanib slowed FVC decline at 52 weeks compared with placebo (-80.8ml vs. -187.8ml; difference 107ml; 95% CI 65.4-148.5)[164]. Notably, the mean FVC decline in the placebo arm was comparable to that reported in IPF placebo arms. The SENSCIS trial which led to the U.S Food and Drug Administration (FDA) approval of nintedanib in SSc-ILD, evaluated the safety and efficacy of nintedanib in participants with

SSc-ILD [170 181]. Although the effect was modest, nintedanib slowed the rate of FVC decline compared with placebo, (-52.4ml per year in nintedanib group vs. -93.3ml per year in placebo; difference, 41.0 ml per year, 95% CI, 2.9-79.0). Interestingly, the magnitude of the effect of nintedanib on lung function was amplified in those concomitantly taking mycophenolate, suggesting combining antifibrotics with existing therapies to complement therapeutic effects may be worth exploring further. A further study evaluated pirfenidone in progressive fibrotic uILD and demonstrated reduced FVC decline at 24 weeks compared with placebo, but no meaningful impact was noted on hospital admissions, exacerbations, mortality and QoL measures[57].

In each of these studies, study participants were enriched for progressors based on lung function decline, radiological deterioration, and worsening symptoms over the preceding 6-24 months. Since both pirfenidone and nintedanib are unable to reverse existing fibrosis, it will be of crucial importance to identify individuals with a progressive phenotype earlier in their disease course before they develop irreversible fibrosis. This will enable prompt intervention with anti-fibrotic therapy, and stratification into clinical trials. Conversely, there are likely to be several individuals with fibrotic ILD who have an indolent disease course, where a watch-and-wait approach is likely to be of greater benefit than intervention with drugs that have several interactions and side effects. Current methods for separating stable vs. progressive disease are unsatisfactory and rely on the development of irreversible fibrosis. Earlier and more precise objective measures that predict disease behaviour and response to therapy are an urgent priority, to enable personalised approaches to managing fibrotic ILD.

1.3 Introduction to Biomarkers

1.3.1 What are biomarkers?

Biomarkers are defined as characteristics that are reproducible, accurate, objectively measured and evaluated as indicators of normal biologic processes, pathogenic processes, or pharmacological responses to a therapeutic intervention[182]. Whilst the term 'biomarkers' has traditionally been reserved for measurable proteins in body fluids or tissues, biomarkers are surrogate markers for any clinically meaningful variable. There has been some progress in the development of biomarkers in IPF[124 183], but there remains a significant gap in our understanding of biomarkers in other fibrotic ILDs.

Biomarkers can be broadly categorised into two major types: biomarkers of exposure which are used in risk prediction, and biomarkers of disease, which are used in the diagnosis and monitoring of disease progression[184]. Biomarkers of exposure tend to measure characteristics assumed to modify the risk of developing disease. The principal benefit of a biomarker of exposure over a history of exposure is the ability to the estimate the actual "internal" dose of the exposure, improving the precision of measurement. Genetic variants are examples of exposure biomarkers which may be related to disease susceptibility, with most diseases typically a composite of genetic and environmental factors. Genetic biomarkers exist prior to the development of disease, and independently to other exposures, and can therefore be particularly useful biomarkers, particularly when combined with other associations to improve precision.

Biomarkers of disease enable earlier diagnosis or identification of the outcome of interest to be determined at a more primitive stage of disease. In the context of fibrotic ILD, disease biomarkers have the potential to increase diagnostic confidence (diagnostic), and thereafter measure disease severity and predict disease progression (prognostic) and monitor response to therapy (theranostic). Determining an individual's disease trajectory, likely response to therapy and long-term prognosis can be particularly challenging in ILD. With the approval of pirfenidone and nintedanib, patients and their clinicians now have a choice of drugs. Biomarkers to predict disease progression and responsiveness to individual therapies will contribute to personalised medicine by facilitating decisions regarding treatment initiation and discontinuation, thus ensuring patients receive the right treatment at the right time. Moreover, biomarkers have the potential to reclassify ILDs according to distinct molecular pathways (endotypes), enabling the identification of novel therapeutics targeting specific mechanisms of disease rather than clinical phenotypes of disease[185]. Importantly, biomarkers may be utilised in clinical trials to reduce disease heterogeneity and improve endpoint precision, leading to more streamlined studies.

1.3.2 Current biomarkers in ILD

1.3.2.1 Imaging

Radiological risk factors that predict poorer outcomes in fibrotic ILDs have been identified. The presence of increased reticulation and traction bronchiectasis in HP may help stratify those with a progressive phenotype, having been shown in numerous studies to be important determinants of survival in HP [186 187]. Salisbury et al phenotyped participants with

HP into three groups based on radiological appearances: no fibrosis, non-honeycomb fibrosis, and honeycomb fibrosis. Those without fibrosis had a reasonable median survival (14.73 years) compared with individuals with evidence of fibrosis (7.95 years). The presence of honeycombing was associated with a median survival (2.76 years) equivalent to that of a matched IPF cohort (2.81 years) [187]. In a further study, distinct HRCT patterns, particularly extensive traction bronchiectasis were shown to confer a 5-year survival of just 30% [188 189].

The prevalence of radiological UIP in RA-ILD may be as high as 75% [44], and consistent with IPF, tends to be highly specific for histopathological UIP[45]. Studies exploring the prognostic outcomes of RA-UIP have demonstrated contrasting results, with several studies showing UIP to predict poor prognosis in RA-ILD[44 190 191], whilst others have not[58]. In a landmark study, the presence of UIP in RA-ILD was associated with a median survival of 3.2 years (vs. 6.6 years in non-UIP) which was similar to survival in the IPF cohort[192]. When examined more specifically, the extent of traction bronchiectasis and honeycombing were independent radiological predictors of worse survival. Participants with RA-UIP also had a longer duration of rheumatoid arthritis (RA), suggesting non-UIP may progress to UIP over time. It remains unclear whether RA-UIP and non-UIP are separate disease entities or reflect disease progression.

An observational study in fibrotic ILDs found honeycombing was present in 42%, 41.9%, 37.6% and 28.6% of participants with HP, CTD-ILD, IPF and uILD, respectively[193]. Importantly, the mean survival was shorter among those with honeycombing, with mortality rates in HP and uILD similar to IPF, suggesting the presence of honeycombing was representative of a

progressive fibrotic phenotype. In the IPF cohort, no mortality association was observed with honeycombing, possibly because IPF represents a typically progressive fibrotic condition.

Together these radiological parallels suggest a common pathobiological mechanism linked to traction bronchiectasis, honeycombing and a UIP pattern may exist across diverse ILD subtypes and identify a progressive fibrotic phenotype correlated with poor survival. Thus, the morphological pattern may be a more useful prognostic determinant than ILD subtypes. Early studies evaluating the use of computer-based quantitative analyses software (CALIPER) have shown promise in predicting disease progression and survival[194]. Whilst imaging is the cornerstone for diagnosis, differentiation, and prognostication of fibrotic ILDs, deeper insights into progressive disease will arise from combining with other investigations such as lung physiology, blood biomarkers and genetics.

1.3.2.2 Genetics

Genetic variants known to be involved in IPF such as telomere-related variants and the MUC5B minor allele, and their association with poorer survival in fibrotic ILD have been discussed. Whilst the identification of genetic polymorphisms hold promise in ILD, genetic testing is yet to be adopted in the clinical environment. An understanding of the interaction between genetic risk variants and environmental exposures to influence disease pathogenesis is needed.

From a management perspective, it is possible genetic subgroups respond differently to distinct therapies. In a post-hoc genotype-stratified survival analysis of participants from the PANTHER study[166], the use of N-acetylcysteine was found to be beneficial in those with a TT genotype for rs3750920 (TOLLIP), but harmful in those with a CC genotype, suggesting differential responses to therapy based on TOLLIP genotype[195]. Further analysis indicated immunosuppressants were particularly harmful in those with telomere lengths below an age-adjusted 10th percentile[196]. If the pharmacogenetic interaction between telomere length and immunosuppressants is independent of IPF, then it is possible that individuals with other fibrotic ILDs and short telomere lengths may also be harmed by these therapies. Further genotype stratified clinical trials and longitudinal studies in carefully phenotyped individuals at high risk of progressive fibrosis are necessary. Insights from such studies have the potential to enable pathway-specific targeted therapies and contribute to precision medicine in ILD.

1.3.2.3 Pulmonary Function Tests

Physiological biomarkers such as forced vital capacity (FVC) or diffusing capacity of the lung for carbon monoxide (DL_{CO}) remain the backbone of the evaluation of ILD patients, having been used to assess disease severity and predict survival[197-199]. The FVC, a measure of the maximal amount of air exhaled after deep inspiration, is typically reduced in ILD, a condition characterised by a restrictive ventilatory defect. Longitudinal change in serial FVC measures is accepted to reflect disease progression in IPF[55], with an FVC decline ≥ 10% over 12 months considered a surrogate for mortality, and a commonly used endpoint in IPF clinical

trials[53 54]. Identifying optimal FVC thresholds at earlier timepoints has the potential to transform clinical care and early phase clinical trials by acting as surrogate endpoints. The DL_{CO} measures the ability of the lungs to transfer gas from inhaled air to pulmonary capillaries and is typically measured by a single-breath test involving gases such as helium or methane. The role of lung function is not limited to IPF, having been shown to demonstrate prognostic value in other fibrotic ILDs[58-61].

However, physiological biomarkers have multiple limitations. Lung function indices are confounded by inter-laboratory variability, participant cooperation and co-existing obstructive lung disease which may comparatively preserve lung volumes and thus underestimate disease progression[200]. Moreover, although reduced lung volumes are associated with increased mortality, the FVC poorly correlates with breathlessness or quality of life scores[201]. Similarly, the presence of pulmonary hypertension, a common sequalae of ILD, may confound DL_{CO} measurements, resulting in the overestimation of disease progression. Additionally, the absence of a standardised staging threshold hampers usefulness in predicting prognosis. Whilst serial changes in lung physiology may improve predictive power[61 197], in a condition with a poor survival, earlier predictors are crucial.

1.3.2.4 Home Spirometry

The remote monitoring of physiological variables has become essential in many chronic conditions following the advancement of technology and falling costs of devices[202 203].

Potential advantages, other than empowering patients with accepting responsibility for their own health, include earlier detection of abnormalities, predicting prognosis, and monitoring response to therapeutics. Management decisions in ILD are made based on routine hospital spirometry typically performed at 3-6 monthly intervals, alongside symptoms and other clinical investigations. Home spirometry offers the opportunity for more frequent lung function measurement, thus minimising measurement variation whilst also enabling longitudinal modelling and offering greater insight into disease behaviour.

Studies evaluating the use of home spirometry in IPF, have found changes in FVC as early as three months can accurately predict disease progression and survival[183]. Home spirometry values seem to correlate with hospital-based spirometry, with good participant adherence rates, and have the potential to act as biomarkers by providing an earlier and more accurate determination of disease behaviour[204 205]. Whilst the role of home spirometry in fibrotic ILDs other than IPF has not been elucidated, it may be similarly beneficial, in view of the comparable natural history.

Home spirometry may have an important role in clinical trials, which are currently limited by the increasing numbers of participants required to be adequately powered and the absence of an early endpoint, with FVC change at 12 months typically the primary endpoint. The potential for increased sensitivity of home spirometry suggests home FVC could be used as an efficacy endpoint in future clinical trials at earlier time points with reduced sample size requirements, thus streamlining future clinical trials and accelerating output of therapeutics.

1.3.2.5 Six-minute walk distance

The six-minute walk test (6MWT) is an inexpensive and practical measure of functional status in respiratory disease that can be performed without the need for advanced training or specialised equipment. Both the total distance walked, and episodes of desaturation have been independently associated with survival in IPF[206 207]. In a large well-defined population of participants with IPF, a baseline distance < 250m and a 24 week decline in walk-distance > 50m were associated with increased mortality[208]. An important limitation of the 6MWT is the inability of the test to provide insight into the mechanisms of exercise limitation, particularly as patients frequently suffer with multiple comorbidities, such as peripheral arterial disease, musculoskeletal problems, frailty, and cognitive dysfunction, that may influence the outcome[209 210]. It is therefore evident that more precise biomarkers reflecting distinct molecular phenotypes predictive of disease trajectory are desperately required.

1.3.2.6 Blood biomarkers

Several blood derived biomarkers have been explored in IPF, often in retrospective studies with relatively small sample sizes and without replication of findings in validation cohorts or separate prospective studies. Furthermore, studies have been limited by inconsistent biomarker assays, analyses using data-dependent biomarker thresholds and an array of outcomes limiting the generalisability of findings. Blood derived biomarkers have several advantages in characterising pulmonary fibrosis and can be categorised in IPF according to likely pathogenic pathways, broadly including biomarkers associated with alveolar epithelial cell dysfunction, biomarkers associated with ECM remodelling and fibroproliferation, and

biomarkers associated with immune dysregulation (Figure 1-3). It is also plausible that combinations of blood biomarkers will add granularity to our understanding of the pathogenesis and prognosis of IPF, and further studies evaluating their utility are needed. The following section summarises the most frequently studied blood biomarkers in IPF, with a detailed review of prognostic blood biomarkers presented later in the thesis.

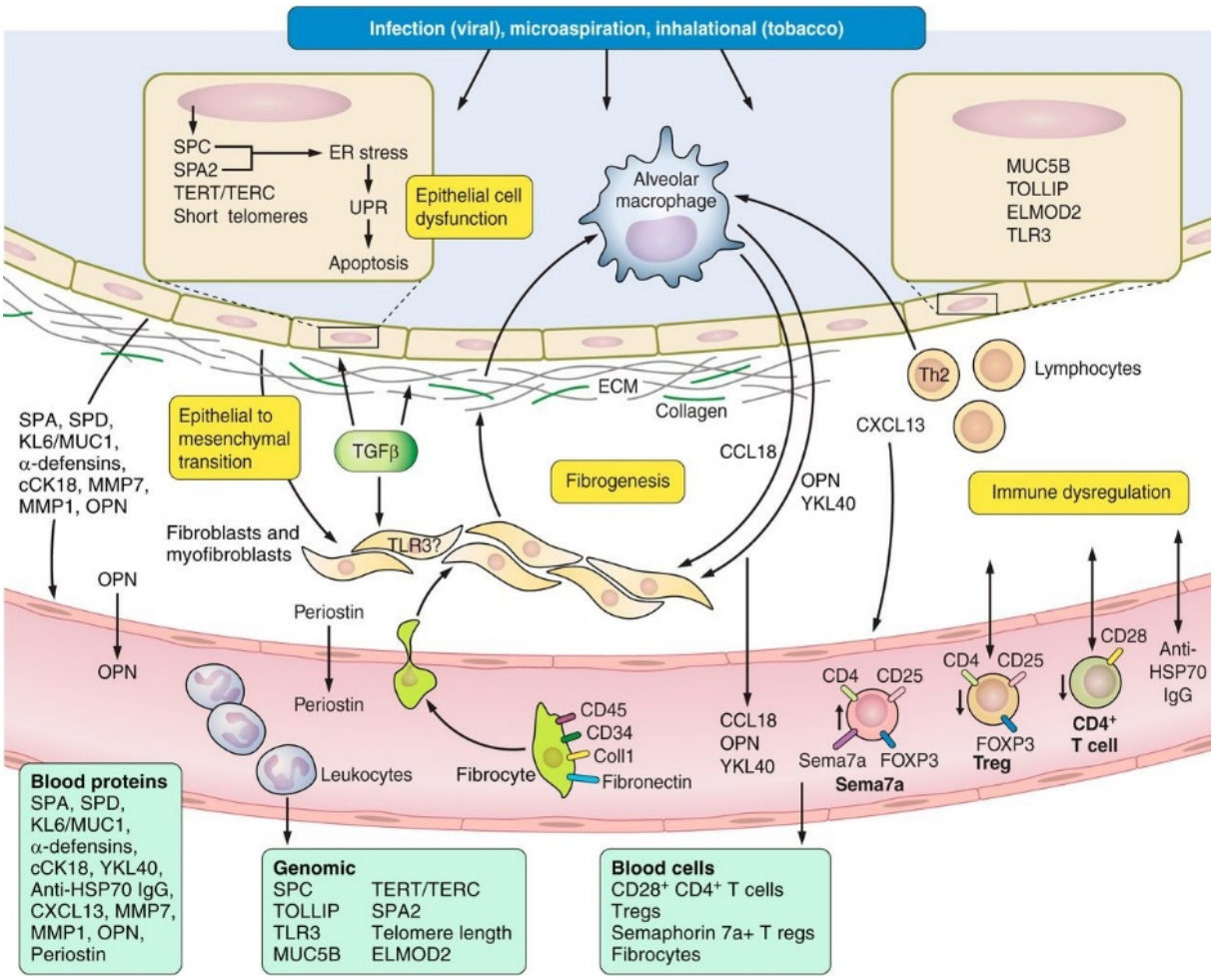


Figure 1-3 - Core mechanisms and candidate molecular biomarkers for IPF.

(Taken from Ley B, Brown KK, Collard HR. Molecular biomarkers in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2014; 307(9):681-91

1.3.2.6.1 Alveolar epithelial cell dysfunction

Biomarkers associated with epithelial cell dysfunction include Krebs von den Lungen-6 (KL-6), surfactant protein-A and -D, matrix metalloproteinases-7 (MMP-7), cancer antigen 125

(CA-125) and carbohydrate antigen 19-9 (CA19-9). KL-6, a mucin-like glycoprotein expressed in regenerating AEC2's is released in epithelial injury, promoting the migration and proliferation of fibroblasts[211]. Surfactant proteins (SP) synthesised and secreted by AEC2s, facilitate transport and function of surfactant lipids, therefore reducing alveolar surface tension and preventing lung collapse. Abnormal surfactant proteins increase ER stress, triggering the apoptosis of AEC2s and initiating fibrosis[211-213]. SP-D levels differ between healthy controls and asymptomatic first-degree relatives of sufferers with familial interstitial pneumonia, suggesting those at-risk develop abnormalities in these proteins before the onset of symptomatic disease[214]. MMPs are zinc-containing endopeptidases that degrade multiple components of ECM and are described in further detail later[215].

1.3.2.6.2 ECM remodelling and fibroproliferation

Biomarkers associated with ECM remodelling and fibroproliferation include ECM neoepitopes, lysyl oxidase like 2(LOXL2), periostin and osteopontin. Matrix degradation fragments, known as neoepitopes are generated by MMP activity and released into the circulation[216]. LOXL proteins play a key role in ECM remodelling and fibrogenesis by promoting collagen cross-linking and are crucial for fibroblast to myofibroblast transdifferentiation[217 218]. Periostin is an ECM protein that promotes ECM deposition, mesenchymal cell proliferation and parenchymal fibrosis[213], and is produced by bronchial epithelial cells in response to IL-13[219]. Osteopontin, a pro-inflammatory and profibrotic cytokine involved in tissue repair, induces upregulation of MMP-7 expression[220], and has been shown to be increased in IIPs[221].

1.3.2.6.3 Immune dysregulation

Numerous biomarkers associated with innate and adaptive immune dysfunction have been identified in IPF, including CC chemokine ligand 18 (CCL-18), chinitase-3-like protein 1 (YKL-40), anti-heat shock protein (HSP-70) antibodies and C-X-C motif chemokine 13 (CXCL13). YKL-40, a chitinase-like protein produced by alveolar macrophages and AEC2s, regulates cell proliferation. The precise mechanism of YKL-40 is poorly understood, but in animal models has mitogenic effects on lung fibroblasts[222]. Increased YKL-40 levels are found in alveolar macrophages and bronchial epithelia adjacent to fibrotic areas in IPF[223]. Increased YKL-40 have also been reported in other fibrotic ILDs such as HP and asbestosis[224-226]. CCL-18 is a small protein derived from alveolar macrophages that acts as a chemo-attractant and stimulates collagen production in fibroblasts, independent of TGF-β signalling pathways[215 227]. In fibrotic ILDs increased numbers of CCL-18-positive macrophages are found, with increased CCL-18 gene expression[228]. CXCL13 is a critical agent for B-cell trafficking in inflammatory foci and lymphoid aggregates, with dysregulated B cells implicated in IPF pathogenesis[211].

1.3.2.6.4 Blood biomarkers in other fibrotic ILDs

There is limited evidence for blood biomarkers in other fibrotic ILDs. Retrospective studies have identified possible associations between blood biomarkers identified in IPF and clinical outcomes in other fibrotic ILDs, suggesting shared pathogenic pathways across progressive fibrotic ILDs. Further prospective studies are urgently needed to identify blood derived biomarkers that underpin progressive fibrotic ILD irrespective of aetiology.

1.4 Hypothesis and aims

A substantial proportion of individuals with ILDs other than IPF develop progressive fibrosis with clinical, radiological, and genetic parallels with IPF suggesting a shared final common pathway across progressive fibrotic ILDs irrespective of aetiology. Study of shared mechanisms of progression has the potential to aid prognostication, enable a targeted approach to therapeutic strategies and allow stratification within clinical trials. The aim of this thesis is to examine and characterise the role of clinical biomarkers in fibrotic lung diseases to enable the precise identification of progressive fibrotic phenotypes.

Hypothesis: There are shared pathways of progressive fibrosis across interstitial lung diseases that can be characterised and measured with biomarkers

The hypothesis was tested by addressing the following specific aims:

1) To evaluate the role of serum proteins as biomarkers in pulmonary fibrosis
2) To determine the role of blood biomarkers as therapeutic targets
3) To describe the baseline features and longitudinal disease behaviours of a cohort with mixed fibrotic ILD
4) To assess the role of demographics and physiological variables as biomarkers of clinical progression in pulmonary fibrosis
5) To perform an exploratory analyses of blood biomarkers to identify novel analytes and their biological pathways associated with disease progression

1.5 Chapter outline

Chapter 2 presents a concise appraisal of published studies examining the association between blood biomarkers and clinical endpoints in untreated IPF. The intention of this systematic review is to summarise understanding of IPF blood biomarkers, whilst identifying research gaps for future study. The chapter includes the first study to utilise individual participant data to meta-analyse the association between matrix-metalloproteinase 7 and disease outcomes.

Chapter 3 explores the role of interleukin inhibitors for treating COVID-19, to ascertain whether blood biomarkers hold potential as therapeutic targets. Specifically, I perform a systematic review and meta-analysis of interleukin inhibitors, to explore their association with disease outcomes. Findings from this chapter are likely to aid understanding of pulmonary fibrosis which shares risk factors with COVID-19, as well as the role of interleukin inhibition in influencing the development and trajectory of post-COVID fibrosis.

Chapter 4 is the largest study of pooled placebo and treatment arms from IPF interventional trials, where I examine the role of demographic and physiological variables, as prognostic biomarkers in fibrotic ILD. Moreover, this is the largest study to evaluate the role of three-month physiological biomarkers as potential surrogate endpoints in ILD clinical trials by exploring their association with clinical outcomes. Associations of physiological variables and disease outcomes are investigated later in this thesis in a mixed cohort of fibrotic ILD to identify commonalities and differences across ILD subtypes.

Chapter 5 presents details of the Its not Just Idiopathic Pulmonary Fibrosis Study (INJUSTIS), an ongoing observational cohort study of 250 participants with fibrotic lung disease. The INJUSTIS cohort will be used to evaluate longitudinal disease behaviour in fibrotic ILDs, whilst identifying biomarkers that are associated with a progressive fibrotic phenotype. Alongside a description of the study, I provide a recruitment update and describe the study population, including demographics and longitudinal physiology and quality of life data.

Chapter 6 utilises interim data from the INJUSTIS study and is presented in two sections. In the first, I examine the association between demographic factors, questionnaire scores and physiology, to ascertain their role as prognostic biomarkers in fibrotic ILD. In the second part, I investigate the feasibility of home spirometry and evaluate its potential as a prognostic marker and earlier endpoint in future fibrotic ILD interventional trials.

Chapter 7 reports details of a discovery proteomic analysis from 24 participants with IPF recruited into the INJUSTIS study. The aim is to measure biomarkers identified in Chapter 3 in extremes of IPF, whilst also performing an unbiased analysis to identify novel analytes. The findings from this analysis will guide biomarker analytic strategy in the complete INJUSTIS cohort once the remaining participants have been recruited.

Chapter 8 summarises the main findings presented in this thesis, describes the clinical and research implications, and identifies priorities for further research.

Chapter 2 Evidence synthesis of blood biomarkers as prognosticators in IPF, an archetypal ILD

2.1 Introduction

Blood derived biomarkers have been extensively investigated in IPF, but none have reached the threshold for implementation in clinical practice. Biomarker studies have faced several limitations, including insufficient sample sizes with lack of power calculations, lack of adjustment for important covariates and inconsistent endpoints, thus often yielding results that have been unreliable and ungeneralisable. The purpose of this chapter is to systematically collate, appraise and synthesise blood biomarker studies to offer a concise and unbiased overview of the association between blood biomarkers and clinical endpoints in untreated IPF. Since IPF reflects the prototypic progressive fibrotic lung disease, biomarkers demonstrating prognostic potential will be specifically evaluated in other fibrotic ILDs as part of the INJUSTIS study described later in this thesis, to explore the hypothesis that there are shared disease pathways between fibrotic ILDs. Furthermore, an additional aim of this chapter is to identify research gaps in our understanding of biomarkers, which can then be used to inform further study in pulmonary fibrosis.

The protocol for the study can be found on PROSPERO (registration number: CRD42019120402). The key findings from this chapter have been published as a manuscript "A systematic review of blood biomarkers with individual participant data meta-analysis of matrix-metalloproteinase-7 in IPF" in the European Respiratory Journal (ERJ). Findings have also been presented as a poster presentation at the European Respiratory Society (ERS) Congress 2021.

Following initial searches and data extraction, it became apparent there were sufficient studies of MMP-7 to enable meta-analysis using individual participant data (IPD). The methodology and results for this sub-study have been described separately in the second part of the chapter.

2.1.1 Aims of study

1) To qualitatively synthesise evidence from studies exploring the relationship between baseline blood biomarkers and/or three-month change and clinical outcomes in IPF
2) To use individual participant data to quantitively synthesise the association between baseline, and three-month change in MMP-7, and clinical outcomes in IPF
3) To identify blood biomarkers with robust evidence that can be investigated in other fibrotic ILDs to explore shared pathogenic pathways
4) To identify gaps and priorities for future blood biomarker research in pulmonary fibrosis

2.2 A systematic review of blood biomarkers in IPF

2.2.1 Methods

2.2.1.1 Eligibility criteria

Blood biomarker studies reporting clinical outcomes in adults with untreated IPF diagnosed according to contemporaneous consensus guidelines[229-231], stratified according to at least one pre-specified biomarker listed in Table 2-1, measured at either baseline and/or change over 3 months, were eligible. Several blood biomarkers have been explored in IPF, and therefore review articles were identified to select biomarkers that have shown promise as prognostic biomarkers in IPF, which enabled search terms to be streamlined. Following the identification of potentially suitable biomarkers, expert opinion (Prof Gisli Jenkins) was sought to ensure the list was inclusive and appropriate. Only prospective studies were included to minimise biases and confounding factors that are typically associated with blood biomarker studies. There were no sample size restrictions, though individual case reports were ineligible.

Pathogenic pathway	**Biomarker**
Epithelial dysfunction	Krebs von den Lungen (KL-6), surfactant protein A (SP-A), surfactant protein D (SP-D), matrix metalloproteinase-1 (MMP-1), cancer antigen 125 (CA-125), carbohydrate antigen (CA19-9), vascular endothelial growth factor (VEGF), insulin like growth factor binding protein 2 (IGFBP2)
ECM modelling	Collagen synthesis peptides (CSP), neoepitopes, lysyl oxidase like 2 (LOXL2), periostin, osteopontin
Immune dysregulation	C-C motif chemokine ligand 18 (CCL-18), chemokine ligand 13 (CXCL13), interleukin-8 (IL-8), heat shock protein (HSP70), chitinase-3-like-protein 1 (YKL40), intracellular adhesion molecule 1 (ICAM-1)

Table 2-1 – Blood biomarkers eligible for inclusion in systematic review according to pathogenic pathways

2.2.1.2 Search strategy and study selection

Electronic databases including MEDLINE (1946 to latest), Embase (1974 to latest), Google Scholar, the Cochrane Register of Controlled Trials and ClinicalTrials.gov were searched on 12th November 2020 using keywords and controlled vocabulary terms for "idiopathic pulmonary fibrosis" and "biomarkers". Further search terms for each of the pre-specified biomarkers were included and are available in the appendix. Prognostic search filters were applied to further refine search criteria[232]. Pre-print servers including medRxiv, bioRxiv and Wellcome Open Research were searched to identify unpublished studies, ensuring the review was inclusive as possible. Reference lists of retrieved articles were searched to identify further studies.

2.2.1.3 Data extraction

Data were extracted from study publications in duplicate to minimise the risk of error, and included study design details, participant demographics (age, sex, smoking status) and outcome data (mortality, FVC change at 12 months and disease progression). Biomarker values alongside their standard deviation at baseline, and three-months (where available) were retrieved in individuals with and without the event (mortality and disease progression). Summary estimates reporting the association between biomarkers and outcomes, alongside details of covariates adjusted for, were extracted where available.

2.2.1.4 Risk of bias assessment

The Quality in Prognosis Studies (QUIPS) has been recommended by the Cochrane Prognosis Methods Group for assessing the risk of bias in prognostic factor studies. The QUIPs tool assesses validity and risk of bias across six domains: participation, attrition, prognostic factor, outcome, confounding and statistical analysis[233]. Each domain contains multiple items that are judged separately before an overall judgement based on a three-grade scale (high moderate or low) is applied. Studies were eligible for inclusion regardless of their risk of bias rating. The overall quality of evidence for each outcome was rated using GRADE (Grading of Recommendations, Assessment, Development and Evaluations) [234].

2.2.1.5 Analysis

Overall mortality was selected as the primary outcome as this is of most relevance to patients. Since death from IPF typically occurs too infrequently in clinical studies, surrogate endpoints for predicting survival have emerged, such as an FVC decline greater than 10%. Therefore, secondary endpoints for the systematic review included change in FVC over 12 months and disease progression defined as FVC relative decline ≥ 10% or death at 12 months. All eligible studies were included in the data synthesis and summary tables. In studies reporting outcomes in multiple cohorts, each cohort was treated individually.

2.2.2 Results

Electronic database searches identified 4930 articles with a further 69 articles identified through searches of preprint servers (Figure 2-1). Articles from all sources were combined, duplicates removed, and titles screened for suitability. Following further screening of abstracts, and review of full texts, 23 studies published worldwide, evaluating a total of 15 blood biomarkers in 2901 participants were shortlisted for inclusion (Table 2-2). All included studies were published between 2007 and 2020. Due to heterogeneity in study design, differences in endpoints and reporting of data using biomarker thresholds, summary estimates were unable to be pooled. Therefore, the findings of this review have been described narratively and individual study results presented in tables found in appendix 10.3. A visual summary table is included at the end of this chapter (Table 2-4).

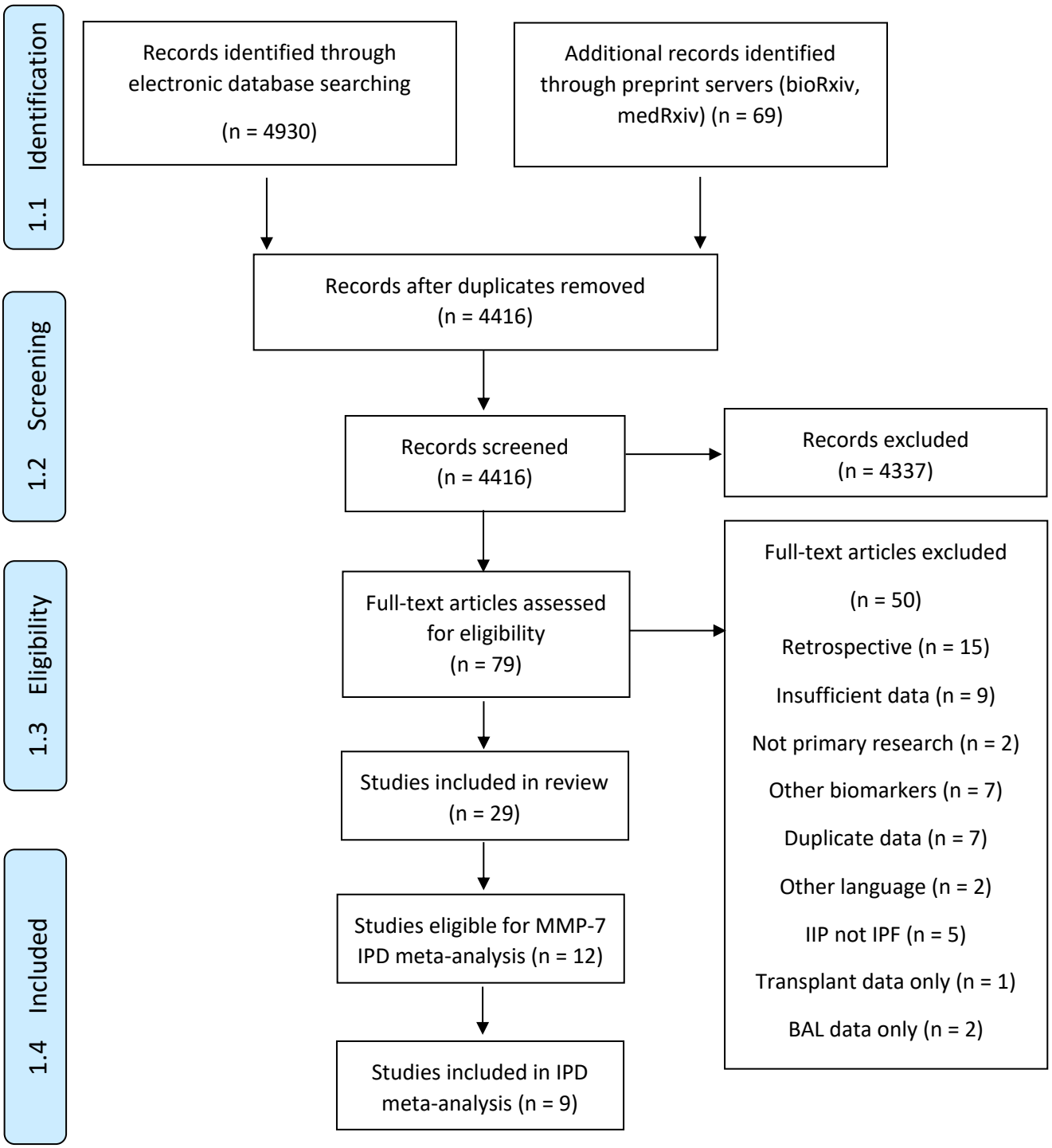


Figure 2-1 - Flow diagram illustrates systematic search and screening strategy, including numbers of studies meeting eligibility criteria and numbers excluded.

Author and year of publication	Country of study	IPF Sample size	Study follow up, months	Age (years)	Sex – male (%)	Baseline FVC % predicted	Baseline DL_{CO} % predicted	Relevant Biomarkers evaluated	Relevant outcomes reported
Bauer, 2017[235]	multi-national	211 (BUILD-3[236])	NR	63.1 (8.9)	64	75.7 (10.7)	47.7 (10.7)	collagen synthesis peptides	Disease progression (FVC≥10% decline, DL_{CO} ≥ 15%, acute exacerbation or death) up to end of study, change in FVC at 4 months
Chien, 2014[237]	USA multi-national	69 (ARTEMIS[238])	24	66.2 (7)	75	69.8 (12.1)	42.1 (11.1)	LOXL2	Overall mortality, lung function decline at 24 months (FVC≥10% with DL_{CO} ≥ 5%, or DL_{CO} ≥ 15% with FVC ≥ 5%), disease progression (mortality, hospitalisation, or lung function decline)
	USA multi-national	104 (GAP[239])		66.7 (8.9)	70	66.1 (17.7)	47.8 (18)		
Collard, 2010[240]	South Korea single centre	47 (AE-IPF)	NR	66 (8)	77	75 (18)	64 (20)	KL-6, SP-D	Overall mortality, acute exacerbation
		20 (without AE-IPF)		63 (7	80	84 (19)	74 (22)		
Doubkova, 2016[241]	Czech Republic single centre	18	NR	68.5 (49-79) [a]	56	68 (median)	52 (median)	SP-A, SP-D	Overall mortality, change in FVC
Gui, 2020[242]	China single centre	126	60	NR	75.4	70.1 (17)	50.5 (12.6)	KL-6, CXCL13	Overall mortality, change in FVC over 12 months
Hamai, 2016[243]	Japan single centre	65	31 (26.6-35.4) [b]	69.3 (8.6)	77	75.6 (21.9)	47.1 (15.8)	SP-A, SP-D, CCL-18, KL-6	5-year mortality
Hoyer, 2020[244]	Denmark multi-centre	184	36	NR	NR	NR	NR	PRO-C3, PRO-C6	Overall mortality, disease progression (FVC decline >10% and/or DL_{CO} decline >15% at any time)
Jiang, 2018[245]	China single centre	20 (85 ILD)	12	53.5 (10.5)	59	71.1 (17.7)	49.4 (24.3)	KL-6	Disease progression (FVC decline ≥ 10% or DL_{CO} decline ≥ 15%, or death) at 12 months
Jenkins, 2015[216]	UK multi-centre	55 (Discovery)	26 (1.6-35.2) [a]	68.5 (9.5)	78	75.9 (23.5)	44.4 (18.3)	ECM-neoepitopes	Overall mortality, disease progression at 12 months (all-cause mortality or >10% FVC decline)
		134 (Validation)	21.2 (0.8-36.2) [a]	70.7 (7.7)	79	78.1 (17.2)	42.1 (13.5)		
Kennedy, 2015[246]	Ireland single centre	13	6	72.6 (10.7)	77	83.3 (26.9)	39.1 (16.1)	SP-D	Change in FVC at 6 months
Kinder, 2009 [247]	USA single centre	82	36 (16-72) [b]	62 (10)	62	64 (18)	54 (16)	SP-A, SP-D	Death or transplantation at 1 year
Maher, 2017[124]	UK multi-centre	106 (Discovery)	36	70.8 (8.3)	78	79 (18.9)	43.3 (14.8)	SP-D, CA125, CA19-9, IGFBP-2, IL-8, ICAM-1	Overall mortality, disease progression at 12 months (all-cause mortality or FVC decline ≥ 10%)
		206 (Validation		72.5 (7.7)	76	81.4 (19.2)	49 (16.9)	SP-D, CA125, CA19-9	
Naik, 2012[248]	USA multi-centre	54 (COMET[83])	18.5	64.3 (8.2)	72	68.5 (15.8)	40. 8 (14.3)	Periostin	Disease progression at 48 weeks (death, acute exacerbation, transplantation, relative FVC decline ≥ 10% or DL_{CO} > 15%)

Neighbors, 2018[249]	multi-national	221 CAPACITY [53]	12	66.9 (7.4)	72	73.4 (13.4)	46.5 (9.4)	CCL-18, CXCL13, YKL-40, Periostin	At 12 months: Disease progression (FVC ≥10% absolute decline or death), change in FVC, death
		244 ASCEND [176]		67.7 (7.2)	77	68.3 (10.9)	43.9 (11.9)		
Ohshimo, 2014[250]	Germany single centre	64 (without AE-IPF)	36 (25.2)	70 (8)	73	68 (15)	44 (14)	KL-6, CCL-18	Acute exacerbation
		13 (with AE-IPF)		67 (5)	85	54 (17)	43 (10)		
Ohta, 2017[251]	Japan multi-centre	60	6.2 (5.8-8.5) [a]	69.2 (8.1)	92	85.8 (20.1)	59.7 (21.8)	Monomeric Periostin, Periostin, KL-6, SP-D	Change in FVC at 6-12 months
Okamoto, 2011[252]	Japan multi-centre	37	NR	66.3 (8.6)	84	80.2 (20)	NR	Periostin	Overall months
Organ, 2019[253]	UK multi-centre	145	34.5 (median)	71.7 (7.7)	81	79.8 (20.4)	48.2 (17.9)	ECM-neoepitopes, collagen synthesis peptides	Overall mortality, disease progression at 12 months (all-cause mortality or >10% FVC decline)
Papiris, 2018[254]	Greece single centre	23 (stable)	12	71 (69-74) [b]	82	72 (60-93) [b]	56 (38-65) [b]	IL-8	Overall mortality at 12 months
		18 (exacerbated)		68.5 (67-78) [b]	61	60 (44-64) [b]	35 (30-36) [b]		
Prasse, 2009[255]	Germany and Italy	72	24	67.2 (8.6)	NR	NR	NR	CCL-18	Overall mortality, change in FVC at 6 months, disease progression at 24 months (>10% FVC decline or death)
Raghu, 2018[256]	multi-national	154	12	67.9 (8.4)	64	71.5 (19.6)	40.9 (15.9)	SP-A, SP-D, CCL-18, KL-6, ICAM-1, Periostin, YKL-40	Disease progression at 52 weeks (FVC decrease ≥10% predicted or DL_{CO} decrease > 15% or lung transplantation or death)
Richards, 2012[257]	USA single centre	140 (Derivation)	22 (19)	67.2 (8.3)	72	62 (19.6)	44.8 (17.1)	IL-8, ICAM-1	Overall mortality, disease progression (FVC relative decline ≥ 10% within any 1 year of follow up)
		101 (Validation	17 (16)	68 (8.7)	66	60.8 (17)	45.4 (19)		
Vuga, 2014[258]	USA single centre	95	> 24	69 (9.7)	74	66 (19.5)	50 (19.5)	CXCL13	Overall mortality

Table 2-2 – Methodological characteristics of all included non-MMP7 studies with baseline participant characteristics and outcome data.

Age, baseline FVC and baseline DLCO reported as mean (standard deviation) unless otherwise stated. NR, not reported; AE-IPF, acute exacerbation of IPF; a = median and range; [b] = median and IQR; # = Post-hoc analysis (Clynick et al 2020) of Navaratnam et al, 2014. Original study did not report biomarker data

2.2.2.1 *Risk of bias assessment*

Risk of bias assessment identified several possible biases in the included studies (Table 2-3). In most studies, the study population were defined using clear inclusion/exclusion criteria, and outcomes were measured objectively and consistently across all study participants. Whilst biomarkers were measured using the sample matrices (plasma or serum), details of assay platforms were frequently missing. The association between outcomes and blood biomarkers measurements can be confounded by demographic variables such as age and gender, as well as other factors such as smoking status and lung function [259]. In many studies, confounders were either not measured, or there was inconsistent adjustment in analyses. A further bias was the use of data-dependent biomarkers thresholds to present results, and these thresholds were inconsistent across studies, preventing pooled analyses.

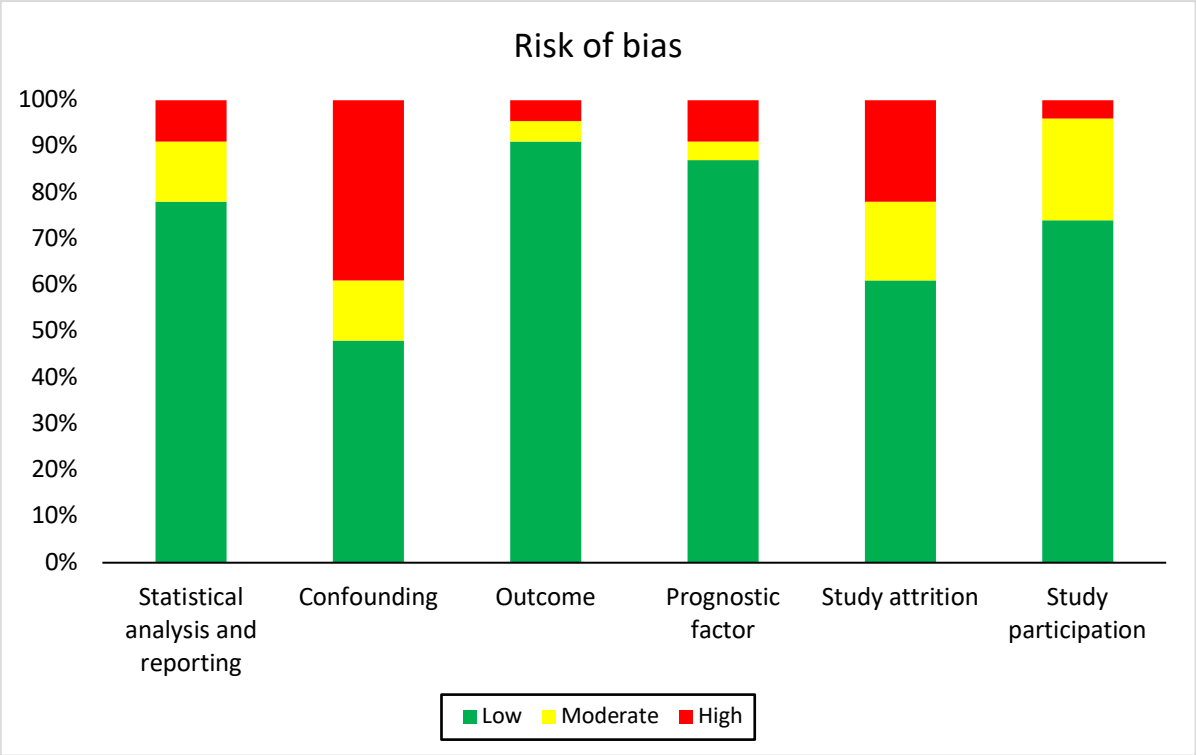


Figure 2-2 - Risk of bias for included studies in systematic review.

The risk of bias across studies was rated as low, moderate, or high risk in six categories using the QUIPs tool

Study	Study participation	Study attrition	Prognostic factor	Outcome	Confounding	Statistical analysis
Bauer, 2017	Low	Low	Moderate	Low	High	Low
Chien, 2014	Low	Low	Low	Low	Moderate	Low
Collard, 2010	Low	Low	Low	Low	High	Low
Doubkova, 2016	Moderate	High	High	High	High	High
Gui, 2020	Low	Low	Low	Moderate	High	Low
Hamai, 2016	Moderate	Moderate	Low	Low	Low	Low
Hoyer, 2020	High	High	High	Low	High	High
Jiang, 2018	Low	Low	Low	Low	High	Low
Jenkins, 2015	Low	Moderate	Low	Low	Low	Low
Kennedy, 2015	Moderate	Low	Low	Low	High	Moderate
Kinder, 2009	Low	Low	Low	Low	Low	Low
Maher, 2017	Low	Moderate	Low	Low	Low	Low
Naik, 2012	Low	Low	Low	Low	Low	Low
Neighbors, 2018	Low	Low	Low	Low	Low	Low
Ohshimo, 2014	Low	Low	Low	Low	Low	Low
Ohta, 2017	Low	High	Low	Low	High	Low
Okamoto, 2011	Low	High	Low	Low	Low	Moderate
Organ, 2019	Low	Moderate	Low	Low	Low	Low
Papiris, 2018	Low	Low	Low	Low	High	Moderate
Prasse, 2009	Moderate	Low	Low	Low	Low	Low
Raghu, 2018	Low	Low	Low	Low	Moderate	Low
Richards, 2012	Low	Low	Low	Low	Moderate	Low
Vuga, 2014	Moderate	High	Low	High	Low	Low

Table 2-3 - Risk of bias assessment for included studies. The risk of bias across studies was rated as low, moderate, or high risk in six categories using the QUIPs tool.

2.2.2.2 Baseline biomarkers predicting mortality

Of the epithelial biomarkers, CA19-9 and CA-125 were strongly associated with mortality, with a three-fold increased risk reported in 206 participants in the PROFILE study[124]. The remaining epithelial biomarkers were associated with contrasting and inconclusive estimates; SP-A and SP-D in separate studies of 82 and 206 participants respectively, and KL-6 in two studies totalling 191 participants were associated with increased mortality,[242 247 260 124]but no association with death was demonstrated in multiple other studies evaluating these biomarkers.[240 241 247 260]

Biomarkers of ECM modelling were evaluated in numerous studies. LOXL2 levels greater than 700pg/mL were associated with mortality in 104 participants, whilst levels greater than 800pg/mL were not associated with mortality in 69 participants.[237] No association with mortality was observed for periostin.[249 252] ECM neoepitopes and collagen synthesis peptides were investigated in two separate publications from the PROFILE study.[216 253] Baseline concentrations of neoepitopes C1M and C3A in 134 participants, the ratio of P1NP:C1M in 145 participants, and PRO-C3 levels in 184 participants were associated with mortality. [33 34]

Biomarkers representing immune dysfunction were examined in several studies with conflicting findings. CCL-18 was associated with mortality in a two-year follow up of 72 participants,[255] with similar associations observed for one-year mortality in the test and replication cohort of 123 and 237 participants, respectively.[249] In a further study of 62 participants followed for five years, CCL-18 was unable to predict death.[260] CXCL13 and its relationship with mortality was explored in three studies totalling 581 participants, which concluded that increased levels were associated with mortality. However, effect sizes varied and included unadjusted estimates, confidence intervals were wide, and follow up time ranged from one to five years.[242 249 258] An inconsistent association was observed between IL-8 and mortality, with no association in a test cohort of 140 participants, but a weak association was observed in a validation cohort of 101 participants.[257] A similar estimate was observed in 41 individuals with acute exacerbations.[254] In the only study of ICAM-1, baseline values were strongly associated with mortality in both cohorts of a study totalling

241 participants.[257] No association with mortality was observed for YKL-40 in a single study.[249]

2.2.2.3 Change in biomarkers predicting mortality

Three publications reporting from the PROFILE cohort evaluated the relationship between longitudinal biomarker measurement and mortality.[216 253 124]In both the discovery and validation cohorts totalling 312 participants, rise in CA-125 over three-months doubled the risk of death, and in the discovery cohort alone, change in IGFBP-2 over three months weakly predicted death. Change in SP-D, CA19-9, IL-8, and ICAM-1 were not predictive of mortality. Seven neoepitopes and their change over three months predicted mortality, all of which are degraded by matrix metalloproteinases (BGM, C1M, C3M, C5M, C6M, CRPM and C3A). A validation cohort of 145 participants from PROFILE demonstrated replication of C1M, C3M, C6M and CRPM, but the rate of change of collagen synthesis peptides was not associated with mortality.[253]

2.2.2.4 Baseline biomarkers predicting disease progression

Biomarkers of epithelial dysfunction were not consistently predictive of disease progression. SP-A levels were lower in those with worsening lung function,[261] but unable to predict disease progression.[256] A significant association of elevated SP-D levels and disease progression was replicated,[124] with elevated levels reported in acute exacerbations.[240] In another study, SP-D negatively correlated with FVC change over six months.[262] Further studies reported no association with disease progression or FVC change alone.[256 261 263] KL-6

was unable to predict disease progression,[245 256] though KL-6 was associated with an increased risk of exacerbation.[240 264] KL-6 was correlated with FVC change over 12 months in a study of 126 participants,[242] but not in more restricted studies of 26 and 60 participants each.[252 263] CA19-9 in both cohorts of the PROFILE study, and CA-125 in the validation arm alone were associated with disease progression.[11]

Biomarkers characteristic of ECM remodelling were similarly inconclusive for disease progression. Periostin negatively correlated with FVC change over 6-12 months,[252 263] and was associated with disease progression in two further cohorts.[248 249] However, findings were not replicated in the validation cohort, nor supported by a separate study.[256] In the only study of LOXL2, baseline biomarkers predicted disease progression in both cohorts.[265] In the PROFILE study, five neoepitopes (C3M, C6M, CRPM, C3A, VICM) were elevated in progressive disease.[124 216]

Immune dysfunction biomarkers were not consistently predictive of disease progression. CCL-18 was the most studied biomarker, with inconsistent findings observed for baseline levels and disease progression in two studies.[255 256] In another study, an initial association with disease progression was not validated in a larger cohort.[249] No association with exacerbation was reported.[45] Two studies explored change in FVC and observed elevated baseline CCL-18 levels were associated with increased FVC change at timepoints ranging from 6-12 months.[249 255] CXCL13 was unable to predict disease progression,[249] but baseline levels correlated with FVC change at one year in two studies.[242 249] IL-8 levels were elevated in progressors in the discovery cohort of the PROFILE study,[20] and though IL-8 predicted

progression in the derivation cohort of another study, findings were not replicated.[257] ICAM-1 values in the discovery cohort of the PROFILE study,[20] and levels above empirically defined thresholds,[257] predicted a greater risk of progression, which was not supported by a further study.[256] YKL-40 did not predict disease progression in the included studies.[249 256]

2.2.2.5 Change in biomarkers predicting disease progression

In the PROFILE study, participants with progressive disease had rising concentrations of CA-125 over 3 months compared to those with stable disease, but no relationship was replicated with SP-D or CA19-9.[124] In a restricted study of 20 participants, increase in KL-6 levels between follow up visits were associated with disease progression.[245]

2.2.3 Conclusion

There were insufficient data for meta-analysis of biomarkers due to differences in study designs and inconsistent outcome reporting. Several biomarkers were associated with mortality in single studies, but replication of findings was weak. There is currently insufficient replication of biomarkers to implement into clinical testing, but this study provides pilot data for further investigation in other fibrotic ILDs.

Biomarker	Mortality	Change in biomarkers predicting mortality	Disease progression	Change in biomarkers predicting disease progression	FVC change	Change in biomarkers predicting FVC change
SP-A		-		-		-
SP-D						-
KL-6		-				-
CA-125					-	-
CA19-9					-	-
LOXL2		-		-	-	-
Periostin		-		-		-
CCL-18		-		-		-
CXCL-13		-		-		-
IL-8				-	-	-
YKL-40		-		-		-
ICAM-1				-	-	-
IGFBP-2	-		-	-	-	-

Table 2-4 - Summary of study results.

Each dot represents a study (or individual cohort in studies with more than one cohort). Green dots represent studies showing an association between the biomarker and outcome, and red dots represent studies where no association was found. Larger circles represent studies with a sample > 100 participants, and smaller circles represent studies with sample sizes smaller than 100 participants. Outcomes where no studies were found for the listed biomarker are represented with a dash (-)

2.3 An individual participant data meta-analysis of MMP7

Following initial searches and data extraction, it became apparent there were sufficient studies of MMP-7 to enable meta-analysis, though results were reported inconsistently across studies, limiting the ability to pool summary estimates. Therefore, individual participant data (IPD) were sought to explore the association between MMP-7 and clinical outcomes in IPF. IPD meta-analyses enable pooling of outcome data and are regarded by Cochrane as the gold standard for collating evidence. They enable standardisation of analyses, consistent adjustment for potential confounding factors and subgroup analyses stratified by participant characteristics, offering unique and robust insights.

2.3.1 Methods

2.3.1.1 Search strategy and risk of bias assessment

An identical search strategy and risk of bias assessment to that described above was applied to identify and appraise studies of MMP-7.

2.3.1.2 Individual participant data (IPD)

Once eligible studies were identified, corresponding authors were contacted using encrypted electronic mail communication, with at least three reminders, each four weeks apart (appendix). Data-sharing portals such as Vivli, Yoda and Clinical Study Data Request were utilised to request data from sponsored clinical studies[266-268]. Data requested included

MMP-7 measurements at baseline and three-months, and details of assays. Data were also sought for participant demographics (age, sex, smoking status), lung function at baseline and twelve months and survival status including time to death.

2.3.1.3 Statistical analysis

In studies reporting outcomes in multiple cohorts, each cohort was treated individually. Overall mortality was selected as the primary outcome and hazard ratios (HR) for MMP-7 levels were estimated. Studies with a duration of follow up greater than three years were censored for survival analyses. Three years was chosen as this correlates with the median survival in IPF. Secondary outcomes included change in percent predicted FVC from baseline at 12 months, and disease progression defined as FVC relative decline ≥ 10% or death at 12 months. Odds ratios (OR) were estimated to predict the likelihood of disease progression.

IPD meta-analyses can be conducted using either a one-stage or two-stage approach. In the two-stage approach, data from studies are analysed separately producing an effect estimate and confidence interval which are then aggregated using standard meta-analysis methodology. For example, if the outcome is binary such as disease progression, maximum likelihood estimation could be used to fit the following logistic regression model in each trial separately[269]:

$$\ln\left(\frac{E(\gamma_{ij})}{1-E\left(\gamma_{ij}\right)}\right)=\ln\left(\frac{p_{ij}}{1-p_{ij}}\right)=a_i+\ \theta_i\,\chi_{ij}$$

where γ_{ij} is 1 or 0 for participants with or without the outcome of interest, respectively; p_{ij} is the probability of participant *j* experiencing the event; a_i is the intercept; and θ_i denotes the treatment effect (log OR). Confounding factors can be adjusted by including in the equation alongside χij.

In the second stage, the effect estimates are combined across trials using either a fixed or random effects model. A fixed-effect model assumes there is a single true underlying effect that is shared by all included studies, and any difference in observed effects are due to sampling error. The pooled estimate from across all the studies, θ is[269]:

$$\theta = \frac{\Sigma w_i \, \theta\hat{\imath}}{\Sigma w_i}$$

where w_i = the weight given to each study, and $\theta\hat{\imath}$ is the effect estimate from each study. The most common method to estimate θ is the inverse variance method, which provides a weighted average, where the weight of each trial is defined as[269]:

$$w_i = \frac{1}{var\,(\theta_i)}$$

A random effects model assumes the true effect size varies across studies, and the summary effect is an estimate of the distribution's mean. A random effects model is recommended If there is heterogeneity across studies, or when results will be generalised beyond included studies. To obtain meta-analysis using a random effects model, an inverse variance is also used, but the weights of each trial are now adjusted to incorporate an estimate of $\mathfrak{r}^2$ which describes the variance of the real effect size between the studies[269].

$$w_i = \frac{1}{var\,(\theta_i) + \mathfrak{r}^2}$$

In the alternative one-stage approach, data from all trials are analysed simultaneously in a single step using a model that accounts for clustering of participants within studies. The two-stage approach is frequently chosen because it uses standard, well documented methods, and several simulation studies have demonstrated both approaches give similar results[269]. For the quantitative analysis of MMP-7, a two-stage IPD meta-analysis with random effects was applied, as there was substantial heterogeneity in study designs and a two-stage approach enabled pooling of dataset available across separate servers and portals. Demographic factors such as age and gender, can often be a source of confounding in biomarker studies, as can disease severity. Therefore, all estimates were adjusted for confounders identified *a priori* including age, gender, smoking history, and disease severity measured with baseline lung function.

There were differences in units of MMP-7 measurement and assays were inconsistent across studies. To standardise baseline MMP-7 values, and enable meta-analysis, z scores specific to each study were calculated and analysed as exposure variables. The z score, measured in standard deviation units, describes the relationship between an individual value and the mean of the group of values. A z-score of 0 suggests the datapoint's score is identical to the mean, whereas a score of 1.0 would indicate a datapoint is one standard deviation greater than the mean and a negative score indicates the datapoint is below the mean. The z score can be calculated using the following equation:

$$z = \frac{\chi - \mu}{\sigma}$$

where χ = datapoint; μ = the group mean; and σ = the group standard deviation.

MMP-7 change over three-months was calculated where available, using the relative percentage change from baseline. All three-month analyses were additionally adjusted for baseline MMP-7 values alongside the covariates listed above. Participants with missing three-month MMP-7 measurements were excluded using listwise deletion.

Once studies are pooled, there can be several sources of heterogeneity which are important to explore. Poor overlap of confidence intervals from individual studies generally indicates the presence of statistical heterogeneity, and more formally, the I^2 statistic can be calculated to quantify the proportion of variance in study estimates attributable to heterogeneity, rather than sampling error. However, the I^2 statistic can be unreliable when there are smaller numbers of studies, or modest sample sizes, and therefore caution must be applied in interpretation during such circumstances[270]. Importantly, the I^2 test also has significant power to detect smaller amounts of heterogeneity that may be clinically unimportant. The I^2 can be calculated using the following equation:

$$I^2 = \left(\frac{Q - df}{Q}\right) x\ 100\%$$

where Q is the chi-squared statistic; df is the degrees of freedom

The interpretation of I^2 thresholds can be interpreted, as suggested by Cochrane, according to the following:

- 0% to 40%: might not be important
- 30% to 60%: moderate heterogeneity

- 50% to 90%: severe heterogeneity
- 75% to 100%: considerable heterogeneity

The I^2 should be interpreted alongside p values and in the context of the direction and magnitude of effect. For all analyses perform in the meta-analysis, the I^2 and corresponding p values were calculated to identify statistical heterogeneity.

Meta-regression allows prediction of outcome variables according to methodological or clinical factors as covariates, to establish whether those covariates are responsible for inter-study heterogeneity[271]. Meta-regression was performed where there were at least ten studies to explore variability in estimates according to the following categorical variables: design of study (cohort vs. randomised study), number of centres (single vs. multi-centre), assay methods (ELISA vs. non-ELISA), blood samples used for analysis (plasma vs. serum) and manuscript publication status (peer reviewed vs. non-peer reviewed).

Publication bias occurs when studies with non-significant results are less likely to be published than those studies that report a significant effort. Publication bias was assessed using visual inspection of funnel plots for asymmetry and application of Egger's test where sufficient studies were included. Egger's test uses linear regression to assess the relation between the intervention effect estimates and the standard errors weighted by their inverse variance[272 273]. Availability bias was assessed by comparing study characteristics and study results in included studies to those of excluded studies where IPD could not be

retrieved. The presence of availability bias can influence the results of a meta-analysis towards an inaccurate treatment effect.

All statistical analyses were performed using Stata 16 (Statacorp, Texas US), using the *ipdmetan* command. This command enables data to be combined from datasets available through separate servers and portals, whilst also enabling adjustment for a consistent set of confounders.

2.3.2 Results

12 studies exploring outcomes in relation to MMP-7 were shortlisted for inclusion, with IPD available from nine of these studies (75%) reporting outcomes in eleven cohorts totalling 1664 participants. Reasons for study exclusion were no response from corresponding author (n=2) and original consent during data collection did not allow data sharing (n=1).

Author and year of publication	Included in IPD MA	Country of study	IPF Sample size	Study follow up, months (median, IQR)	Age (years)	Sex – male (%)	Baseline FVC % predicted	Baseline DL_{CO} % predicted
Bauer, 2017[235]	No	multi-national	211 (BUILD-3[236])	NR	63.1 (8.9)	64	75.7 (10.7)	47.7 (10.7)
Hamai, 2016[243]	Yes	Japan single centre	65	28 (16-45)	69.3 (8.6)	77	75.6 (21.9)	47.1 (15.8)
Maher, 2017[124]	Yes	UK multi-centre	106 (Discovery)	15 (15-15)	70.8 (8.3)	78	79 (18.9)	43.3 (14.8)
	Yes		200 (Validation)	15 (15-15)	72.5 (7.7)	76	81.4 (19.2)	49 (16.9)
Navaratnam, 2014/Clynick, 2020[76 274]	Yes	UK multi-centre	205	42 (20-60)	73.2 (8.7)	74	84.7 (18.7)	43.7 (15.8)
Neighbors, 2018[249]	Yes	multi-national	221 CAPACITY [53]	18 (17-21)	66.9 (7.4)	72	73.4 (13.4)	46.5 (9.4)
	Yes		244 ASCEND[275]	12 (11-12)	67.7 (7.2)	77	68.3 (10.9)	43.9 (11.9)
Oldham, 2019	Yes	USA multi-centre	199	19 (8-32)	71.5 (8.9)	74	68.5 (19.1)	48.5 (20.4)
Peljto, 2013[276]	No	multi-national	438 (INSPIRE[277])	19 (14-25)	66.6 (7.5)	74	72.2 (12.4)	47.3 (8.9)
Raghu, 2018[256]	Yes	multi-national	154	12 (12-12)	67.9 (8.4)	64	71.5 (19.6)	40.9 (15.9)
Richards, 2012[257]	No	USA single centre	140 (Derivation)	22 (19) [b]	67.2 (8.3)	72	62 (19.6)	44.8 (17.1)
	Yes		97 (Validation)	42 (14-60)	68 (8.7)	66	60.8 (17)	45.4 (19)
Rosas, 2018[278]	Yes	USA multi-centre	58	11 (11-12)	67.6 (7.3)	81	71.1 (15.6)	41.5 (13.9)
Sokai, 2015[279]	No	Japan single centre	57	15 (0.4-61) [a]	69.4 (8.5)	90	84.2 (21.3)	43.7 (14.2)
Tzouvelekis , 2017[280]	Yes	USA single centre	97	17 (8-17)	70 (8)	79	70.2 (16.5)	47.2 (16.9)

Table 2-5 - Methodological characteristics of MMP-7 included studies with baseline participant characteristics and outcome data.

Age, baseline FVC and baseline DLCO reported as mean (standard deviation) unless otherwise stated. Study follow up time reported in median (IQR) unless otherwise stated. [a] = median and range, [b] = mean (SD)

2.3.2.1 *Risk of bias assessment*

Risk of bias assessment identified similar limitations and risk of biases to those identified in non-MMP7 studies. Participants were described clearly, and biomarkers were measured using the same laboratory technique for all participants. Important confounding factors were available from all studies.

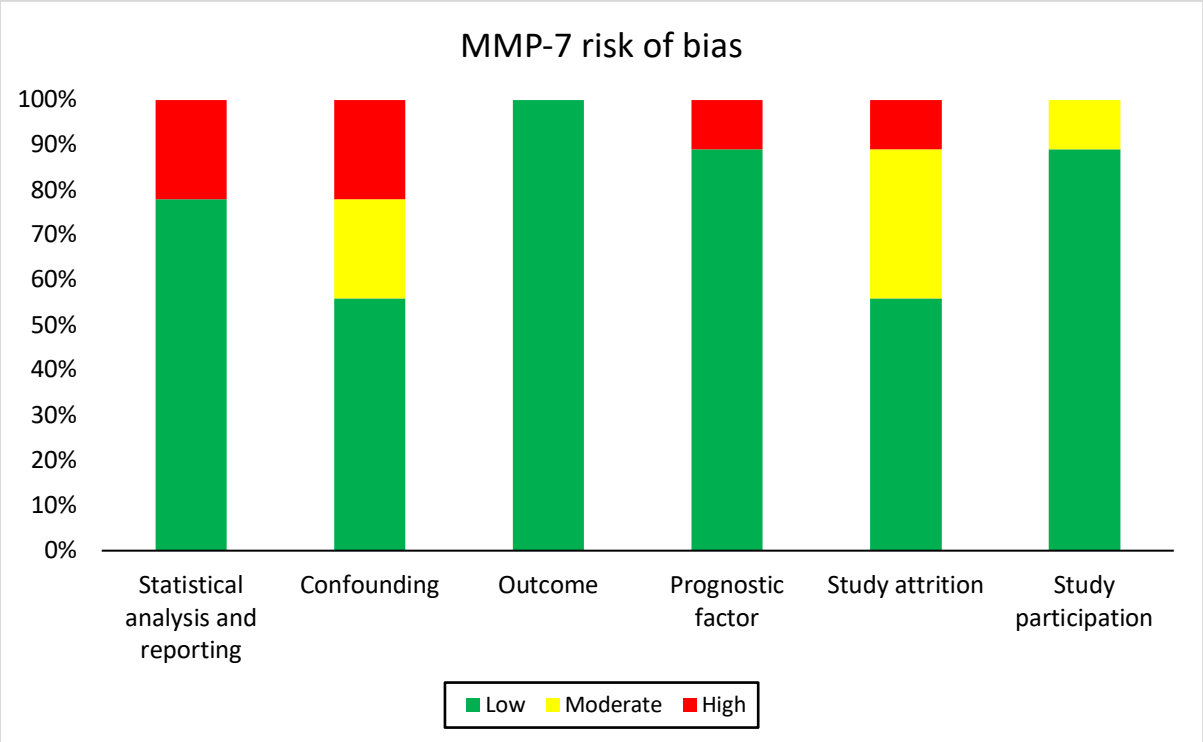


Table 2-6 - Risk of bias for included MMP-7 studies.

The risk of bias across studies was rated as low, moderate, or high risk in six categories using the QUIPs tool

2.3.2.2 *Baseline MMP-7 and mortality*

Data to enable survival analysis was available from eight studies totalling 1492 participants. Two further studies where IPD was unavailable reported mortality outcomes. In IPD meta-analysis, after adjustment for age, sex, smoking status, and baseline FVC, MMP-7 measured

at baseline was associated with an increased risk of overall mortality, with each standard deviation increase associated with a 23% increased risk of mortality (aHR 1.23, 95%CI 1.03;1.48, I^2=64.3%). Severe heterogeneity in estimates were noted but none of the covariates assessed were able to explain the variability.

Summary estimates examining the association between baseline MMP-7 and 12-month mortality were inconclusive (aHR 1.33, 95%CI 0.99;1.78; I^2=59.6%) with moderate heterogeneity noted. In the two studies where IPD was unavailable, contrasting results were reported. A threshold of 5.7ng/mL doubled the risk of death (aHR 2.18 95%CI 1.1;4.32) in 438 participants over a median follow up of 19 months[281], and a further study of 57 participants reported a relationship between mortality and baseline MMP-7[282].

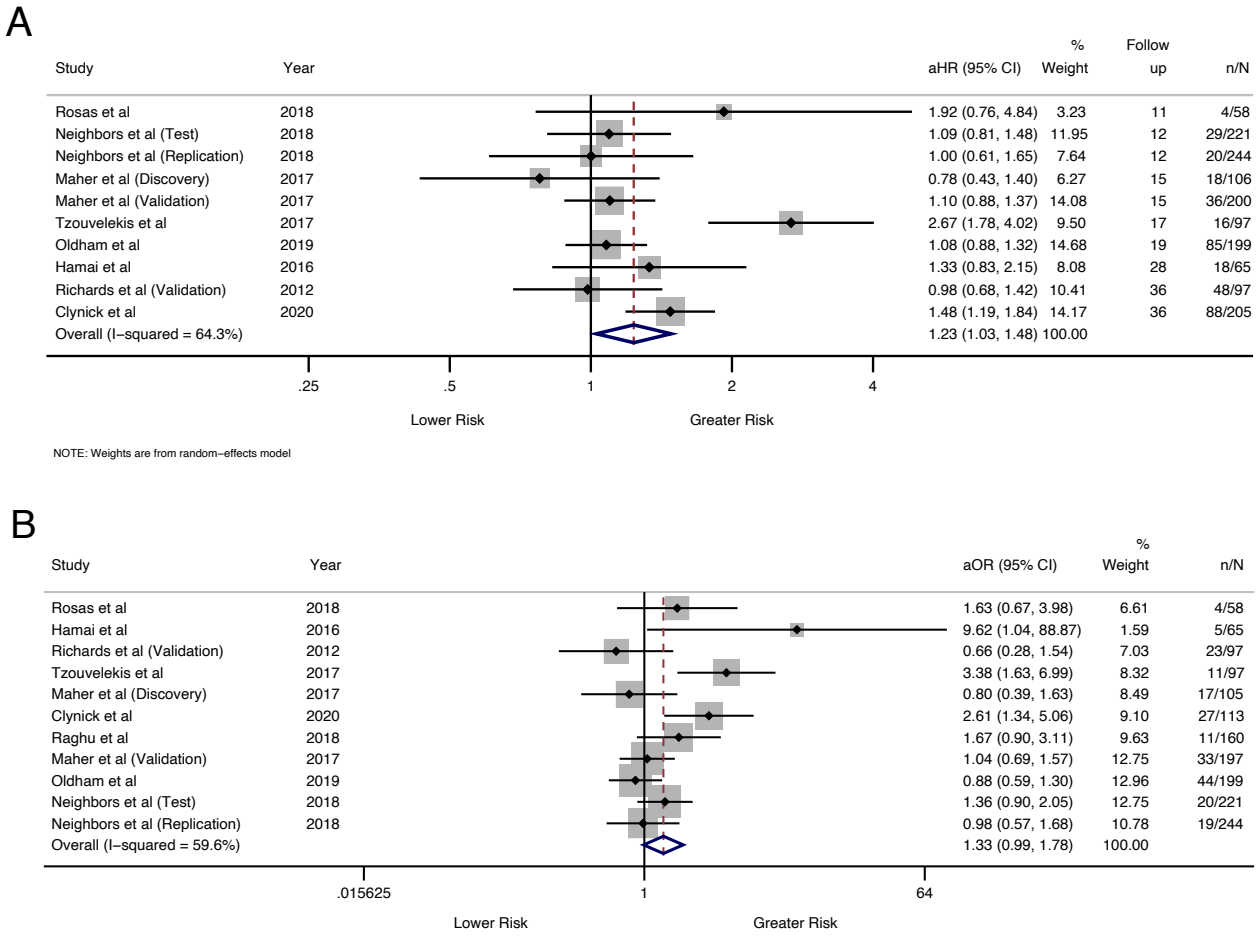


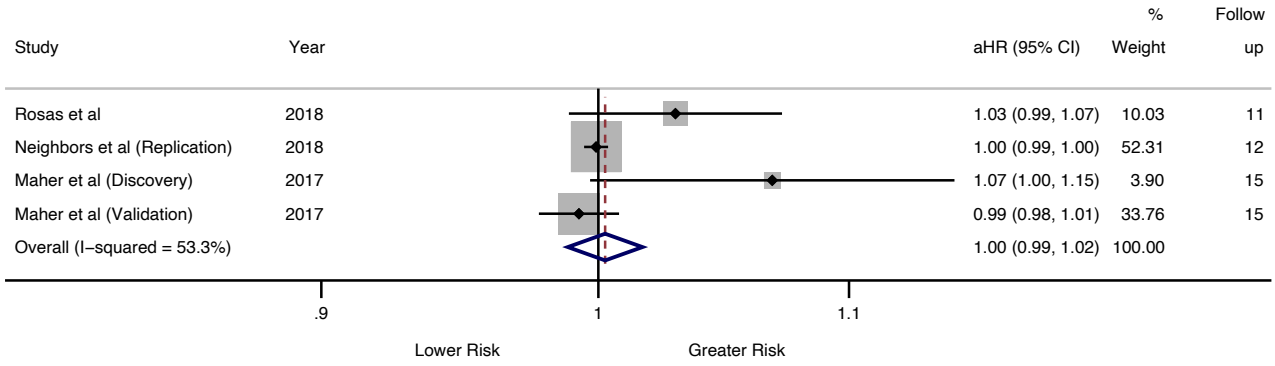
Figure 2-3 – Mortality forest plot for baseline MMP-7

A – Overall mortality. B: Mortality at 12 months. Effect sizes with 95% confidence intervals per standard deviation increase in baseline MMP-7. Study follow up time shown in months. n denotes the number of deaths, and N represents the total number of participants included per study.

2.3.2.3 Change in MMP-7 and mortality

MMP-7 change over three-months and its relationship with mortality was explored in three studies with 498 participants. Following adjustment for age, sex, smoking, baseline FVC and baseline MMP-7, no association was found with either overall mortality (aHR 1.00, 95%CI 0.99;1.02, I^2=53.3%) or twelve-month mortality (aOR 1.00, 95%CI 0.99;1.01, I^2=37.4%).

A.



B.

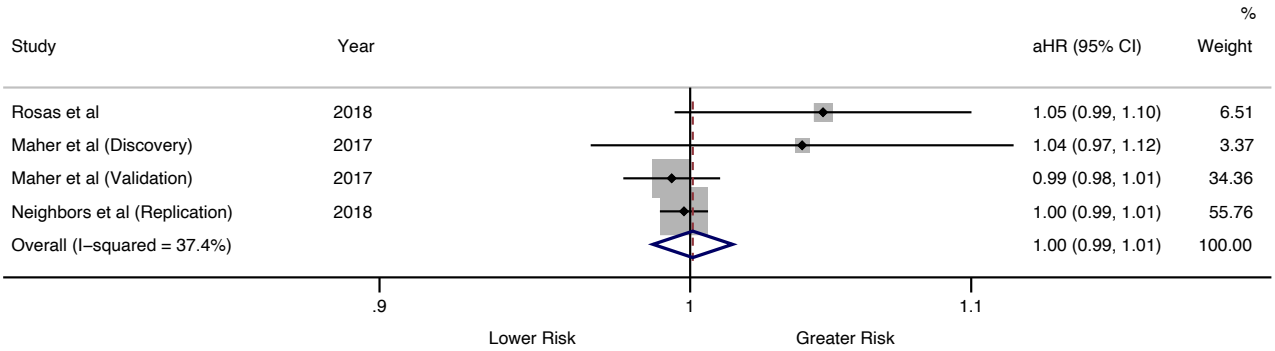


Figure 2-4 - Three month change in MMP-7 and mortality forest plot

A. Pooled hazard ratios with 95% confidence intervals per percent relative increase in MMP-7 from baseline to three-months for A. Overall mortality and B. 12-month mortality. Study follow up time shown in months. n denotes the number of deaths, and N represents the total number of participants included per study

2.3.2.4 Baseline MMP-7 and disease progression

Eight studies with 1338 participants were included in IPD meta-analysis exploring the relationship between baseline MMP-7 and disease progression as defined by an FVC relative decline ≥ 10% or death at 12 months. Following adjustment for age, sex, smoking status, and baseline FVC, there was a 27% increased likelihood of disease progression for each standard deviation increase in baseline MMP-7 (aOR 1.27, 95%CI 1.11;1.46, I^2=5.9%). Statistical heterogeneity was low, but meta-regression identified assay techniques (ELISA vs.

other) to be a source of variation. Subgroup analyses were performed according to MMP-7 measurement assay, and in analyses restricted to studies utilising ELISA techniques, the pooled odds ratio of disease progression was estimated as 1.56 per SD increase (95%CI 1.26;1.82, I^2=0%). Two studies could not be included in meta-analysis due to the unavailability of IPD. In the first study including 211 participants with a median follow up of 19 months, baseline MMP-7 measurements above 3.8ng/mL were associated with an increased risk of disease progression (aHR 2.2 95%CI 1.4;3.7)[235]. The other study including 57 participants found no association between MMP-7 and disease progression.

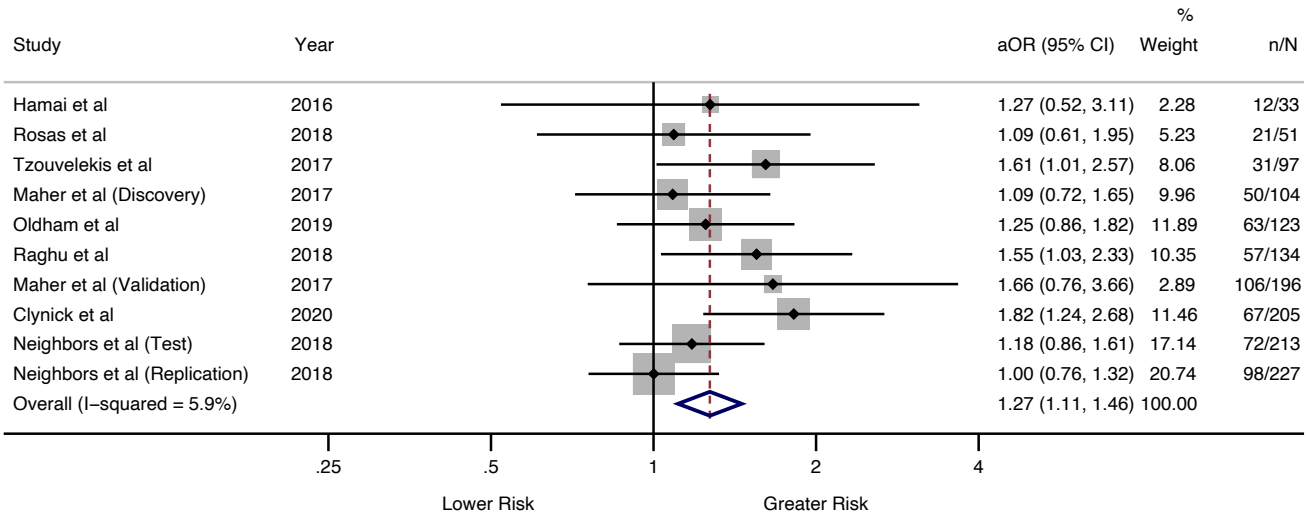


Figure 2-5 - Disease progression forest plot.

Pooled odds ratios with 95% confidence intervals for risk of disease progression, per standard deviation increase in baseline MMP-7. n denotes the number of progressors, and N represents the total number of participants included in the analysis per study.

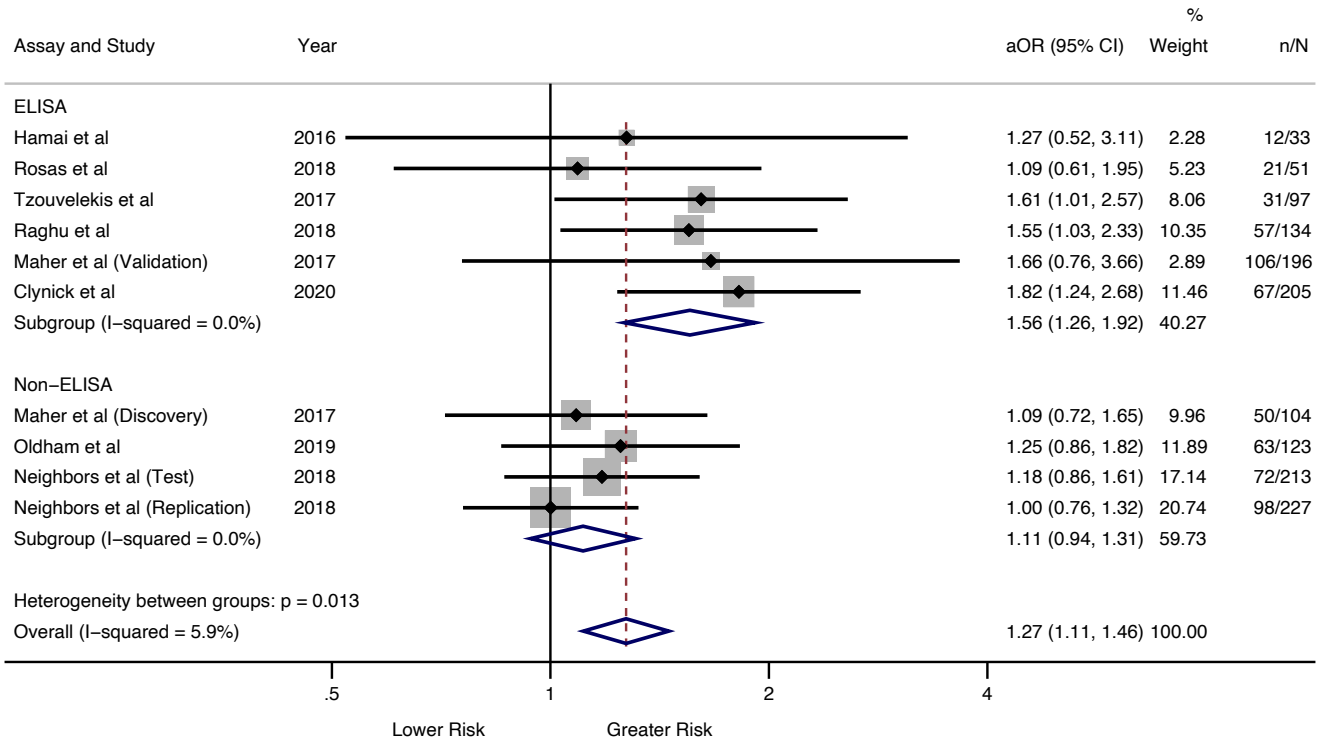


Figure 2-6 – Disease progression forest plot separated by ELISA and non-ELISA measurements.

Pooled odds ratios with 95% confidence intervals for risk of disease progression, per standard deviation increase in baseline MMP-7. n denotes the number of progressors, and N represents the total number of participants included in the analysis per study.

2.3.2.5 Baseline MMP-7 and change in FVC at 12 months

The association between baseline MMP-7 and FVC change over 12 months was examined in six studies with 891 participants. Meta-analysis demonstrated there was a -0.85% relative change in 12-month FVC percent predicted (95%CI -1.65; -0.05, I^2=0%) per standard deviation increase in baseline MMP-7 after adjustment for age, sex, smoking status, and baseline FVC.

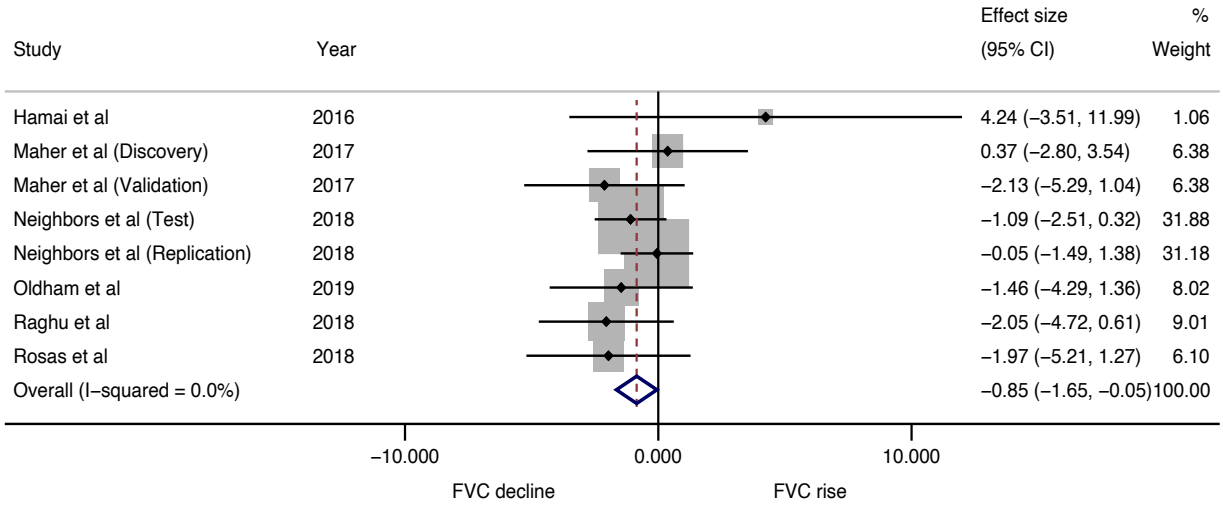


Figure 2-7 - Relative change in FVC% percent predicted forest plot.

Pooled effect size with 95% confidence intervals for FVC% percent predicted relative change at 12 months, per standard deviation increase in baseline MMP-7.

2.3.2.6 Change in MMP-7 over three months predicting disease progression

IPD to explore the relationship between change in MMP-7 over three months and disease progression were available from three studies with 481 participants. No association with disease progression was found (aOR 1.00 per percent increase, 95%CI 0.99;1.01, I^2=22.5%), after adjustment for confounding factors. There were insufficient studies to perform meta-regression to identify sources of variation. Notably, in a study not included in meta-analysis, a two-fold rise in MMP-7 over four months doubled the risk of disease progression[235].

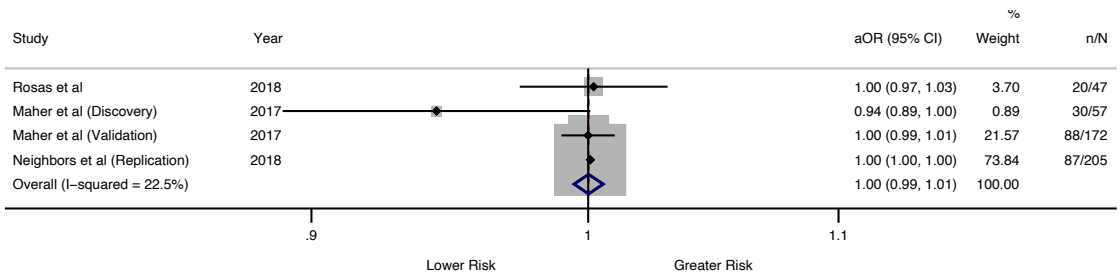


Figure 2-8 – Three month change in MMP-7 and disease progression forest plot.

Pooled odds ratios with 95% confidence intervals for risk of disease progression, per percent relative increase in baseline MMP-7 to three months. n denotes the number of progressors, and N represents the total number of participants included in the analysis per study

2.3.2.7 Change in MMP-7 over 3-months and 12-month FVC change

In four studies, change in MMP-7 over three-months was not associated with 12-month FVC change (effect size 0.01% increase per percent MMP-7 increase, 95%CI -0.07;0.08, I^2=60.8%), after adjustment for age, sex, smoking status, baseline FVC, and baseline MMP-7.

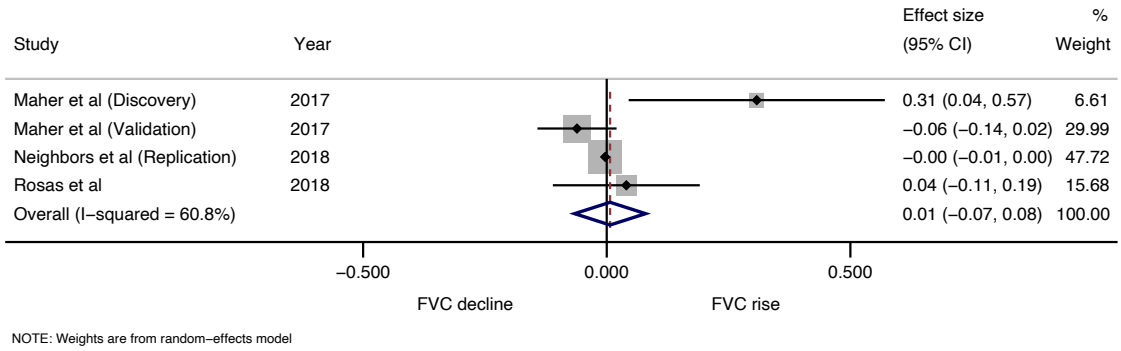


Figure 2-9 - Three month change in MMP-7 and 12m FVC relative change forest plot

Pooled effect size with 95% confidence intervals for relative change in FVC at 12 months, per percent relative increase in baseline MMP-7 to three months.

2.3.2.8 Publication bias

Publication bias was assessed using visual inspection of funnel plots and Egger's test where at least ten studies were included in meta-analysis. For each of the outcomes assessed there was no statistical evidence of publication bias.

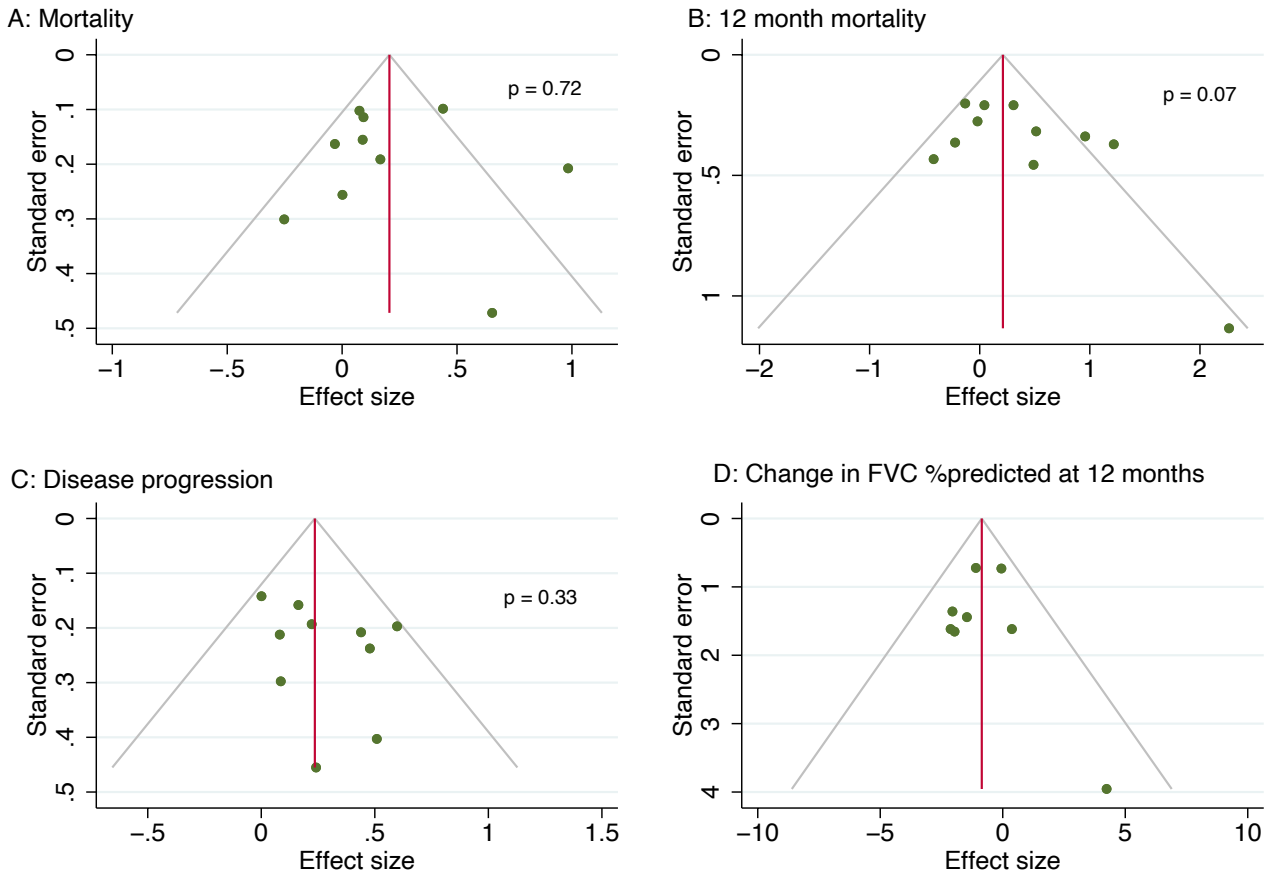


Figure 2-10 - Funnel plots for outcomes evaluated in baseline MMP-7 IPD meta-analysis.

A: overall mortality, B: 12-month mortality, C: Disease progression, D: Change in percent predicted FVC at 12 months. Publication bias assessed using Egger's test for outcomes with at least ten studies, and p values presented next to funnel pot.

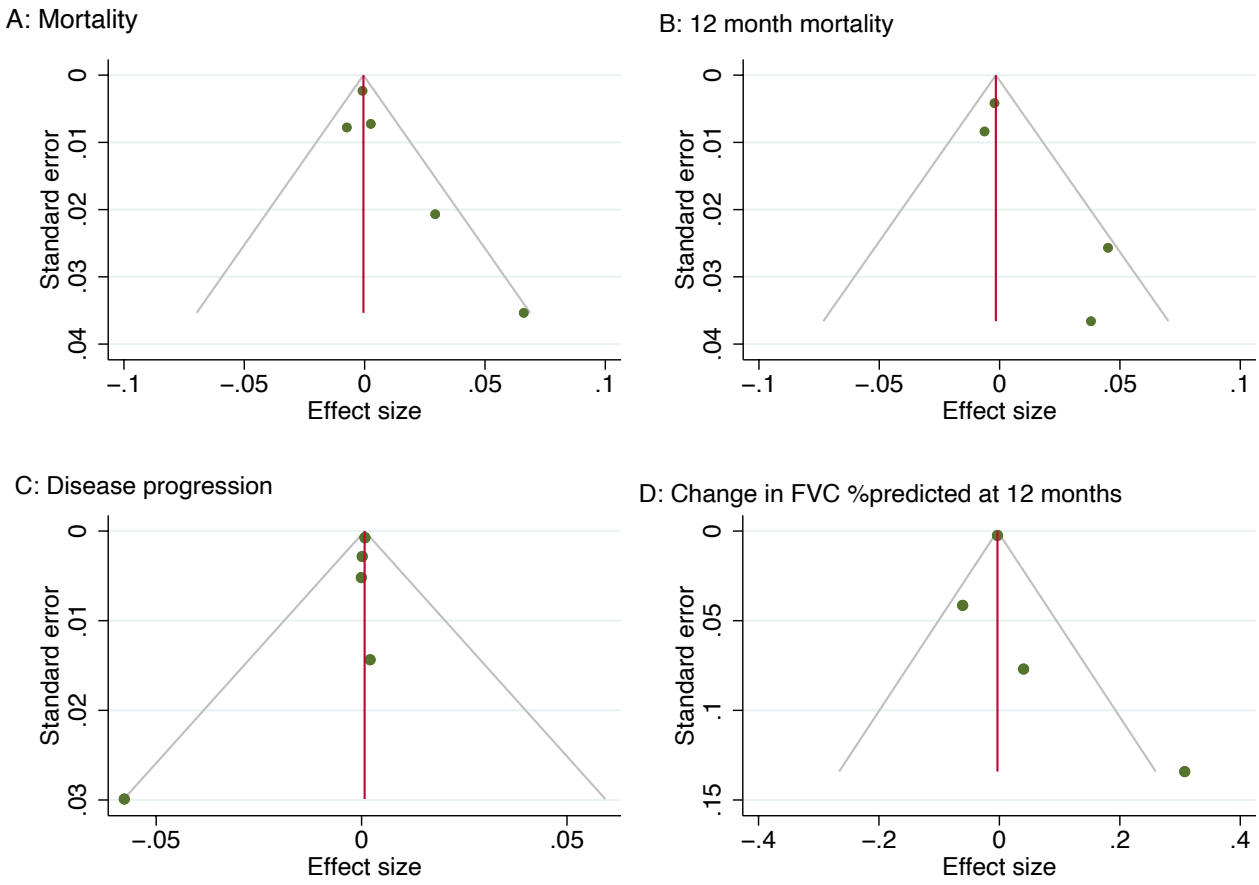


Figure 2-11 - Funnel plots for outcomes evaluated for three-month change in MMP-7 IPD meta-analysis.

A: overall mortality, B: 12-month mortality, C: Disease progression, D: Change in percent predicted FVC at 12 months.

2.3.2.9 GRADE

GRADE was used to rate the confidence in each of the outcomes assessed. Findings for the relationship between baseline MMP-7 and overall mortality are rated with moderate certainty. IPD enabled standardisation of exposure and outcome variables, and consistent adjustment for covariates. Follow up time in all studies were sufficiently long to enable death to occur. The direction of effect was similar in the majority of studies, although differences in the magnitude of effect were seen. There was substantial heterogeneity leading to a downgrading of the certainty of evidence to moderate. Estimates for three-

month MMP-7 change and mortality are similarly rated with moderate certainty due to substantial heterogeneity, but also wide confidence intervals in individual studies suggesting summary estimates were imprecise.

Disease progression outcomes are rated with high certainty for both baseline and three-month change in MMP-7. Definitions of disease progression were standardised across the studies, the risk of bias in studies was low, narrow confidence intervals in individual studies did not suggest imprecision, no significant heterogeneity was noted, and there was no evidence of publication bias. Change in FVC at 12-month estimates are rated with high certainty for baseline MMP-7 measurements and moderate certainty for three-month change in MMP-7. The downgrading of the latter was primarily due to significant statistical heterogeneity in the pooled estimate.

2.3.3 Conclusion

This is the first meta-analysis to utilise robust IPD methodology to explore the association between MMP-7 and clinical outcomes in IPF. This review demonstrates baseline MMP-7 measurements, but not three-month change in MMP-7, accurately predict mortality and disease progression in IPF after adjustment for important covariates. Further study should focus on exploring the prognostic role of MMP-7 in fibrotic ILDs other than IPF.

2.4 Discussion

2.4.1 MMP-7

Meta-analysis was only possible for studies evaluating MMP-7 as a prognostic biomarker, and therefore IPD was sought for this biomarker specifically, representing the first study to adopt such methodology in IPF blood biomarker studies. The key findings from this review demonstrate MMP-7 levels measured at baseline predict all-cause mortality and disease progression and correlate with FVC change over 12 months. With each standard deviation increase in baseline MMP-7 measurements, overall mortality risk increased by 23% and there was a 27% increased likelihood of disease progression. MMP-7 levels did not change significantly over three-months, and although the number of included studies were limited, there did not appear to be a relationship between longitudinal MMP-7 change and clinical outcomes. However, of the three studies where IPD was not available, one study did suggest that a rise in MMP-7 over three-months was associated with an additionally increased risk of disease progression beyond that of participants with high but stable MMP-7[235]. These findings require further study but suggest when MMP-7 does indeed rise over three-months, it may suggest a particularly poor prognosis. Heterogeneity was noted in estimates for multiple outcomes, with meta-regression indicating measurement assays (ELISA vs. other) were a significant source of variability. GRADE is used to assess the certainty of findings, with mortality estimates rated as moderate certainty and disease progression and change in FVC estimates with high certainty.

An understanding of the role of metalloproteinases (MMPs) in IPF is crucial to understanding the implication of these data. MMPs are zinc-containing endopeptidases that

degrade all components of the extracellular matrix (ECM), but are also understood to be pivotal in regulating other processes including growth factors and proteins related to inflammation and repair[283]. 23 MMP genes in total have been identified in humans and can be classified by their in-vitro substrate specificity into seven groups. Although MMPs are highly expressed in fibrotic lungs, their dominant cellular expression (epithelial cells, fibroblast, macrophage, or fibrocyte), and their activity (profibrotic or antifibrotic) can vary.

MMP-7 also known as matrilysin, is a profibrotic metalloproteinase secreted by exocrine and dysfunctional mucosal epithelial cells in numerous organs including the skin, lungs, liver and intestines[284]. The primary responsibility of MMP-7 includes degrading extracellular matrix components including collagen, fibronectin, gelatins, and proteoglycans, activating other bioactive substrates such as cytokines and chemokines, and inducing epithelial-mesenchymal transition[215]. Thus, MMP-7 plays a pivotal role in the regulation of wound healing, aging, bone growth, and signalling pathways that are involved in cell growth, inflammation, and angiogenesis[285]. Under normal physiological conditions, MMP-7 expression is tightly regulated, but activity increases in response to wound damage to enable repair and remodelling. In the lungs specifically, MMP-7 is localised in the activated alveolar and bronchiolar epithelial cells[286], with activity uncontrollably increased in IPF, resulting in activation of numerous profibrotic mediators including TGF-β, extracellular matrix remodelling and irreversible tissue damage. Though the role MMP-7 is likely to be pleiotropic[287], increased expression in IPF would be consistent with increased disease activity and fibrogenesis, supporting its role as a potential prognostic biomarker in IPF.

Limited retrospective studies evaluating the role of MMP-7 in other lung fibrotic diseases have reported similar findings. In a cohort of mixed ILD, baseline MMP-7 were associated with reduced survival in HP and unclassifiable ILD, but not in individuals with CTD-ILD, suggesting MMP-7 may represent a biomarker of progressive lung fibrosis irrespective of aetiology[288]. This was supported in a more recent study of SSc-ILD where baseline MMP-7 levels were associated with poorer baseline lung function and an increased risk of death or lung transplant[289]. Studies in non-pulmonary clinical conditions characterised by fibrosis such as chronic kidney disease and liver cirrhosis have similarly observed elevated blood MMP-7 levels, that are associated with increased fibrosis, suggesting MMP-7 may help identify a common final fibrotic pathway shared across organs and fibrotic diseases[290 291].

These findings, supported by evidence from animal studies where MMP-7 knockout mice were protected from pulmonary fibrosis following administration of bleomycin suggest that MMP-7 may be a potential therapeutic target[292]. Notably, in a small open-label trial of seven patients with IPF, the daily use of doxycycline, which is understood to be a non-specific MMP inhibitor, was associated with significantly reduced disease progression[293]. However, the pleiotropic properties of MMP may make direct systemic inhibition a problematic therapeutic strategy for IPF. Previous studies of broad-spectrum metalloproteinase inhibitors in various cancers have yielded unsatisfactory results, with poor efficacy and dose-limiting musculoskeletal pain a problematic side effect[287]. Thus, the development of inhibitors with greater specificity for individual MMPs coupled with selective lung targeting might hold more potential for the management of fibrotic lung diseases.

2.4.2 Other serum biomarkers

Systematic review of the remaining 15 blood biomarkers indicated several associations between baseline biomarkers and outcomes including mortality and disease progression, though replication of effects were weak. Several biomarkers including CCL-18, CXCL-13 and ICAM-1 and KL-6 were replicated as predictors of mortality, and SP-D, KL-6, CA19-9, LOXL2, periostin, CCL-18, IL-8, and ICAM-1 as predictors of disease progression. These biomarkers represent various pathogenic pathways, supporting the role of a complex interplay between epithelial cell dysfunction, matrix turnover and immune dysregulation in the pathogenesis of IPF. In the limited number of studies that assessed dynamic short-term changes in biomarker concentrations over three-months, no biomarker was replicated as a predictor of mortality or disease progression, other than CA19-9 in the PROFILE study. Further study is warranted, but these findings suggest blood biomarker concentrations may not change longitudinally, or alternatively a duration of three-months may be too short to track biomarker change in relation to disease progression and mortality.

2.4.3 Limitations of included studies

This review highlights limitations in included studies, with significant heterogeneity in methodology and analyses, resulting in inconsistent findings. Though the review focussed on prospective studies, many of the included studies did not include prespecified statistical power calculations, and offered insights based on relatively modest sample sizes. It was unclear whether non-significant biomarkers findings were due to a lack of association, or insufficient power to detect an effect. Numerous studies utilised biomarkers thresholds that were chosen based on the available data to maximise effect sizes. Thresholds were not

uniform across studies limiting the possibility of combining summary estimates. Furthermore, summary estimates were unadjusted or only partially adjusted in many studies. Bioanalytic methods to quantify biomarkers varied across studies, with few reporting details of sample collection, processing and storage, and details of quantification assays including their measure of precision. Inter and intra-individual biological variability are known to confound biomarker studies and further research should focus on standardising blood biomarker studies in IPF. For MMP-7 specifically, analysis of IPD overcame some of these limitations by analysing biomarker levels as continuous variables converted to z-scores to minimise assay variability, supported the standardisation of outcomes, and enabled estimates to be adjusted for a consistent of confounders.

2.4.4 Limitations of review

The findings of this study should be contextualised alongside its limitations. IPF diagnostic criteria have evolved over the past three decades, and therefore earlier studies in the formerly known cryptogenic fibrosing alveolitis (CFA) were ineligible for inclusion. Similarly, studies reporting outcomes in mixed populations of fibrotic ILDs, rather than IPF alone were excluded, and therefore findings should not be extrapolated to non-IPF ILD without further study. Applying strict inclusion criteria, to only include studies evaluating blood biomarkers in individuals with untreated and well-characterised IPF diagnosed according to international consensus guidelines, increases the robustness and generalisability of the study's findings to this group. The findings of this review will be evaluated separately in well-defined participants with non-IPF fibrotic ILD as part of the INJUSTIS study (described in Chapter 5). Furthermore, the exclusion of treated individuals with IPF limits the opportunity

to explore the prognostic and theranostic value of MMP-7 in those receiving anti-fibrotic therapy. Further limitations include the exclusion of two articles unavailable in the English language, which could not be translated to assess their suitability. Whilst the search criteria included preprint servers, there are likely to be unpublished negative biomarker studies that are unavailable, and the exclusion of such studies has the potential to bias the findings of this review towards a positive association between biomarkers and clinical outcomes. Publication bias was not detected in MMP-7 meta-analysis, but some outcomes included fewer than ten studies, limiting the power of the Egger's test to detect the presence of publication bias, and therefore the findings should be interpreted with caution. IPD could not be obtained for three out of twelve suitable studies raising the possibility of availability bias, but a comparison of methodological and participant characteristics, alongside summary results did not reveal obvious differences compared with the included studies. Nonetheless, narrative findings from these studies were included. Lastly, there was significant statistical heterogeneity in some of the outcomes which could not be explained by the factors assessed.

2.4.5 Future direction

This review identifies numerous priorities for further blood biomarker research in IPF. Rigorously designed longitudinal studies with published protocols of planned methodology and analysis plans are necessary, and sample size calculations including the use of discovery and validation cohorts to replicate findings, should underpin all further blood biomarker research. Biomarker assays should be standardised to enable study and results comparisons. Furthermore, biomarkers representing various pathogenic pathways should be combined in

future studies to increase our understanding of IPF pathogenesis and assess whether combinations of biomarkers increase the specificity and sensitivity for predicting disease outcomes. MMP-7 change following initiation of anti-fibrotic therapy may represent a biomarker of treatment response and predict an earlier response to pharmacotherapy than more conventional methods. Further research should examine the relationship between anti-fibrotic therapy and MMP-7. Moreover, the potential role of MMP-7 as a therapeutic target requires greater understanding and this should be prioritised for future research. The utility of blood biomarkers showing potential in IPF should ultimately be explored in well-defined individuals with non-IPF fibrotic ILD, where there are likely to be mechanistic similarities and common fibrotic pathways. From a clinical perspective, MMP-7 should be considered for implementation as a prognostic tool, especially when lung physiology tests are contraindicated or unavailable.

2.5 Summary

This review summarises the evidence for several blood biomarkers representing various pathogenic pathways that may have prognostic potential in IPF. The application of robust methodology to synthesise IPD from studies evaluating MMP-7 demonstrates baseline MMP-7, but not three-month change in MMP-7 predicts overall mortality and disease progression in untreated IPF irrespective of other factors such as age, sex, and lung function. The evidence for the clinical adoption of other biomarkers is currently insufficient, though several biomarkers show promise, and further well-designed studies are warranted. As further studies become available, quantitative synthesis using an IPD approach should be strongly considered to produce more reliable results.

Chapter 3 Evidence synthesis of blood biomarker directed intervention for severe acute respiratory syndrome, a contemporary example

3.1 Introduction

The clinical manifestations of SARS-CoV-2 range from asymptomatic disease to respiratory failure and death. Although the pathology is poorly understood, SARS-CoV-2 infection is thought to trigger a dysregulated host immune response associated with the release of multiple cytokines and chemokines, referred to as the "cytokine storm syndrome" (CSS)[294]. Interleukin-1 (IL-1) and Interleukin-6 (IL-6) are the among the most important pro-inflammatory cytokines released during the cytokine storm, activating numerous other cytokines and stimulating several downstream pathways[295], leading to acute lung injury. Identifying individuals likely to develop cytokine storm syndrome and thus acute lung injury remains elusive and has led to considerable interest around IL-1 and IL-6 as potential prognostic biomarkers and renewed interest in therapies targeting blood cytokines.

Severe COVID-19 shares several parallels with IPF, and the role of interleukins have been well described in IPF pathogenesis[296]. IL-1 is understood to regulate inflammation and fibrosis by stimulating fibroblasts to synthesise collagen and help induce EMT [297]. Elevated serum concentrations of IL-6 have been reported in individuals with acute exacerbations of IPF[298], and appears to predict disease progression in SSc-ILD[299]. The use of tocilizumab which is known to be an IL-6 inhibitor has been demonstrated to slow the rate of decline in pulmonary function in SSc-ILD[300]. These findings suggest both IL-1 and IL-6 may be shared therapeutic targets in COVID-19 and ILD, whilst also carrying potential as prognostic

biomarkers. Other than improving short-term outcomes in COVID-19, inhibiting IL-1 and IL-6 may prevent post-COVID-19 fibrosis, with further research urgently warranted.

In this chapter I summarise the evidence for managing severe COVID-19 with monoclonal antibodies that target IL-1 (anakinra) and IL-6 (tocilizumab, sarilumab, siltuximab). The key aim is to evaluate the role of interleukin-targeted therapies, to ascertain whether these blood biomarkers hold potential as therapeutic targets. Cytokine-suppression and the utilisation of a blood biomarker-guided approach to managing COVID-19 is likely to aid our understanding of precision medicine, whilst also informing further research in pulmonary fibrosis. Importantly, future research will determine the influence of interleukin inhibitors on the development of post-COVID-19 fibrosis. This study was performed during the peak of the COVID-19 pandemic before the approval of IL-6 inhibitors for the treatment of COVID-19. The study protocol can be found on PROSPERO (registration number: CRD42020176375), and the key findings from this chapter have been published as a manuscript "Systematic review and meta-analysis of anakinra, sarilumab, siltuximab and tocilizumab for COVID-19" in *Thorax*[301].

3.1.1 Aims of study

1) To critically appraise the role of a blood biomarker driven therapeutic strategy
2) To assess the role of blood interleukins as prognostic biomarkers in COVID-19
3) To assess the effectiveness of interleukin inhibitors for managing severe COVID-19
4) To use findings to inform research around blood biomarker guided therapies in pulmonary fibrosis

3.2 Methods

3.2.1 Eligibility criteria

Original studies evaluating the use of IL-1 and IL-6 inhibitors in suspected or confirmed COVID-19, specifically anakinra (IL-1 inhibitor) and tocilizumab, sarilumab and siltuximab (IL-6 inhibitors) were eligible for inclusion. Studies were restricted to those exploring outcomes in adults only. All prospective studies were included, and no minimal study sample size was specified. Due to the associated risk of bias, case reports and single-arm retrospective studies were ineligible for inclusion. No restrictions on language or year of publication were applied.

3.2.2 Search strategy and study selection

Electronic database searches including MEDLINE (1946 to latest) and EMBASE (1974 to latest) were searched on 7th January 2021. Pre-print servers including bioRxiv and medRxiv were searched to identify unpublished studies. Search parameters included keywords and alternate terms for COVID-19, interleukin inhibitors, and the specific agents under investigation. Two reviewers carried out the searches independently, followed by screening of titles and abstracts, before full text review

3.2.3 Data extraction

Data were extracted from included studies using a data-extraction proforma and verified by a second reviewer. Extracted information comprised study details including study design, country of study, sample size and duration of follow up; participant demographics;

intervention characteristics including name of agent, administered dose and route); clinical outcomes including duration of hospital stay, requirement and duration of invasive and non-invasive ventilation, duration of oxygen therapy and survival outcomes; treatment characteristics including adverse events. Where reported, ordinal outcomes were extracted at timepoints closest to day 15 following therapeutic intervention.

3.2.4 Risk of bias assessment

Due to heterogeneity in study designs, several tools available through the National Institute of Health were applied to assess the risk of bias[302]. All tools graded the overall quality as either good, fair, or poor. The Cochrane risk-of-bias tool (RoB2) was applied specifically to randomised studies[303]. Studies were eligible for inclusion regardless of their risk of bias rating. The overall quality of evidence for each outcome was rated using GRADE (Grading of Recommendations, Assessment, Development and Evaluations)[234].

3.2.5 Analysis

Two primary endpoints were selected based on their clinical usefulness. The first was duration of hospitalisation, which was extracted and differences in the duration of hospital stays between participants in the intervention and placebo arms calculated and pooled. Studies only reporting the median and interquartile range of duration of hospitalisation were converted to mean and standard deviation estimates using the Box-Cox method[304]. The other primary endpoint was severity on an ordinal scale at day 15 following intervention. The ordinal scale was adapted to a four-point scale: i) death; ii) advanced ventilatory support with either invasive mechanical ventilation (IMV) or Extra Corporal

Membrane Oxygenation (ECMO); iii) hospitalised but not requiring advanced ventilatory support; iv) discharged. The number of participants meeting each outcome were pooled using rank-based Wilcoxon Mann Whitney tests, to provide a generalised odds ratio (GenOR). The GenOR estimates the likelihood of a better outcome between randomly selected paired observations representing two ordinal categorical variables, in this case intervention and placebo[305].

Overall mortality and mortality at 28 days were chosen as key secondary endpoints, as they have obvious clinical relevance. Hazard ratios where available, and proportions of individuals alive or dead were extracted from studies to enable calculation of unadjusted relative risk ratios. Where data were reported in figures without tabular format, values were extracted using a digital plot analyser[306]. Treatment related adverse events were extracted.

Quantitative synthesis was performed using random effects meta-analysis and data were presented in forest plots, stratified by retrospective or prospective study design. For all analyses performed, the I^2 statistic was estimated to detect the presence of statistical heterogeneity and meta-regression was applied to explore variability. Factors assessed in meta-regression included study design (whether single-centre or multi-centre), the inclusion of non-peer reviewed manuscripts, studies where participants received concomitant steroids, the route of drug administration, and outcome measurement day. Publication bias was assessed using funnel plot analysis and Egger's test where there were sufficient studies. Prospective studies without a control arm were included in the narrative summary, but not in quantitative analysis.

3.3 Results

A total of 2585 studies were retrieved following electronic database search, and 576 studies were identified through preprint servers. Following removal of duplicates, title and abstract screening and full text review, 71 articles were shortlisted for inclusion. Most studies were in individuals who received tocilizumab (n=58), with anakinra evaluated in six studies, sarilumab in four studies, and siltuximab in one study. A single study investigated both anakinra and tocilizumab, and another study investigated both sarilumab and tocilizumab (Figure 3-1).

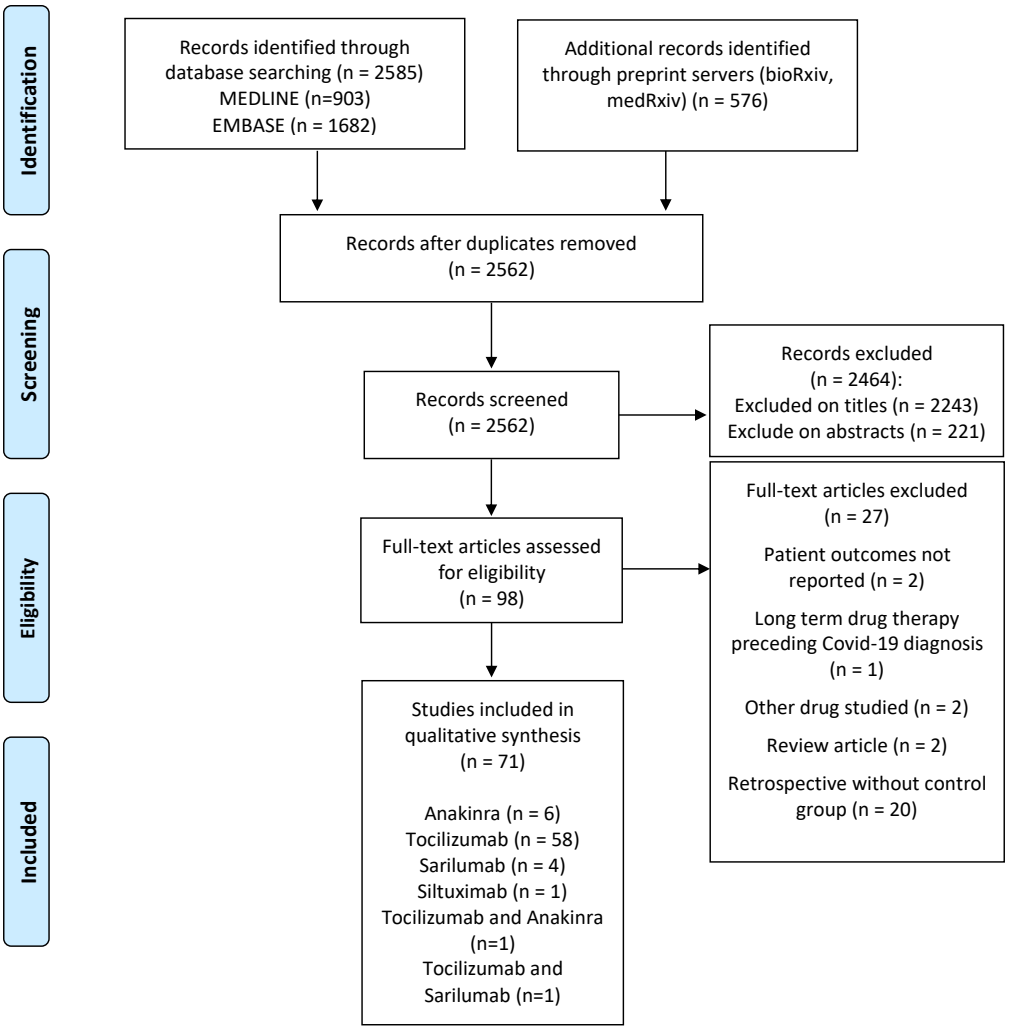


Figure 3-1 - Flow diagram illustrates number of studies meeting eligibility criteria and numbers excluded.

Most studies (62/71) were published in peer-reviewed journals, and a further nine studies were available through preprint servers only. Of the 71 studies, 29 were prospective and 42 were retrospective studies with control arms. Of the prospective studies, 17 studies had a control arm, and six of these were randomised trials (Figure 3-2).

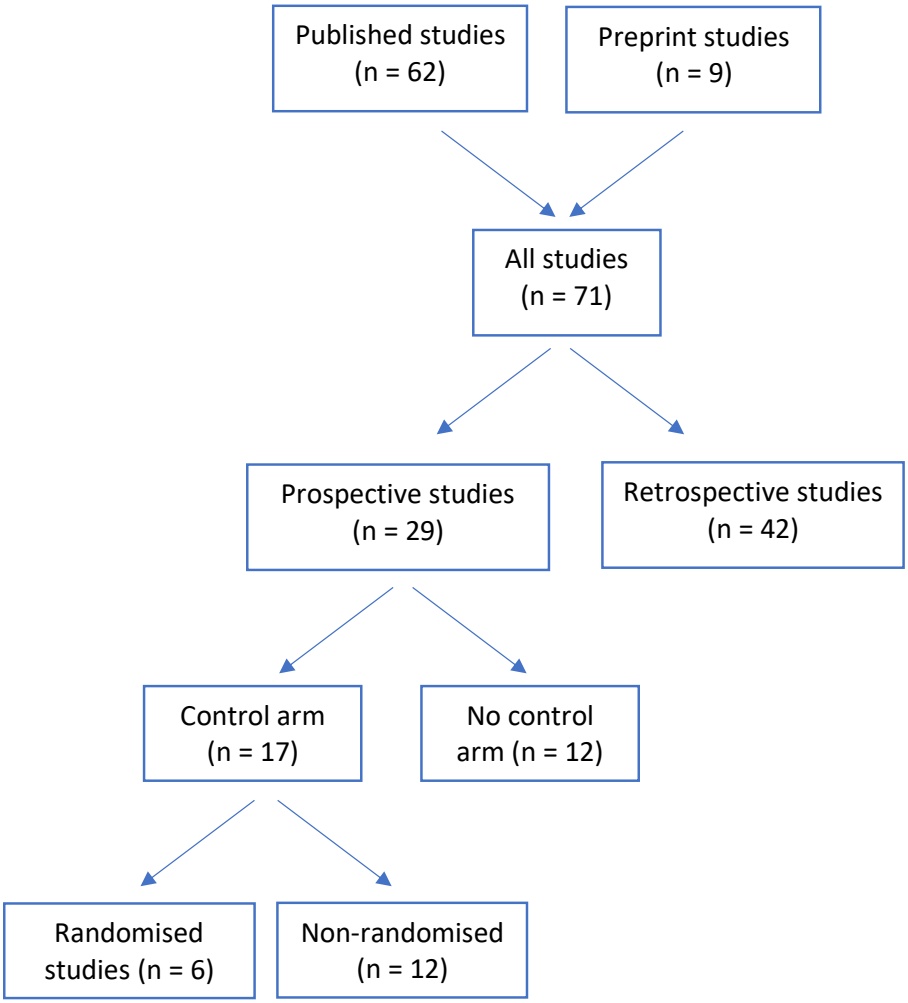


Figure 3-2 - Included studies stratified by study design

A total of 22,058 participants were included in the systematic review of which 7328 (33%) received intervention and the remaining 14730 (67%) were assigned to standard care alone. Study characteristics of included studies are included in Table 3-1, and detailed individual study results are provided in the appendix.

Author, year	Drug	N, Tx/ Control	Study country	Centre	Study design	Author, year	Drug	N, Tx/ Control	Study country	Centre	Study design	Author, year	Drug	N,Tx/ Control	Study country	Centre	Study design
Balkhair [307]	A	45/24	Oman	SC	Prospective with control	Roumier [308]	T	49/47	France	SC	Prospective with control	Kimmig [309]	T	54/57	USA	SC	Retrospective
Huet [310]	A	52/44	France	SC	Prospective with control	Salama [311]	T	249/ 128	USA	MC	Double blind RCT	Klopfenstein [312]	T	20/25	France	SC	Retrospective
Kooistra [313]	A	21/39	Netherlands	MC	Prospective with control	Salvarani [314]	T	60/63	Italy	MC	Open label RCT	Lewis [315]	T	497/497	USA	MC	Retrospective
*Kyriazopoulou [316]	A	130/130	Greece	MC	Prospective	*Sanchez-Montalva [317]	T	82/0	Spain	SC	Prospective	Martinez-Sanz [318]	T	260/969	Spain	MC	Retrospective
Cauchois [319]	A	12/10	France	MC	Retrospective	Sciascia [320]	T	63/0	Italy	MC	Prospective	Narain [321]	T	73/3076	USA	MC	Retrospective
Cavalli [322]	A	29/16	Italy	SC	Retrospective	Stone [323]	T	161/ 82	USA	MC	Double blind RCT	Nasa [324]	T	22/63	India	MC	Retrospective
Narain [321]	A	57/3076	USA	MC	Retrospective	Strohbehn [325]	T	32/41	USA	SC	Phase 2 open label	Patel [326]	T	60/1505	USA	SC	Retrospective
Benucci [327]	Sa	8/0	Italy	SC	Prospective	Toniati [328]	T	100/0	Italy	SC	Prospective	* Petrak[329]	T	81/37	USA	MC	Retrospective
Della-Torre [330]	Sa	28/28	Italy	SC	Prospective with control	Biran [331]	T	210/ 420	USA	MC	Retrospective	Pettit [332]	T	42/41	USA	SC	Retrospective
* Gordon, 2021 [333]	Sa	45/397	UK	MC	Adaptive RCT	Canziani [334]	T	64/64	Italy	MC	Retrospective	Potere [335]	T	74/74	Italy	SC	Retrospective
Gremese [336]	Sa	53/0	Italy	SC	Prospective	Capra [337]	T	62/23	Italy	SC	Retrospective	*Ramaswamy [338]	T	10/10	USA	MC	Retrospective
Sinha [339]	Sa	255/0	USA	SC	Prospective	Chillmuri [275]	T	83/ 685	USA	SC	Retrospective	Rodriguez-Bano [340]	T	21/65	Spain	MC	Retrospective
*Gritti [341]	Si	30/30	Italy	SC	Prospective with control	De Rossi [342]	T	90/68	Italy	SC	Retrospective	Rojas-Marte[343]	T	88/344	USA	SC	Retrospective
Albertini [344]	T	22/22	France	SC	Prospective with control	Eimer [345]	T	22/22	Sweden	SC	Retrospective	Roomi [346]	T	96/97	USA	SC	Retrospective
Antony [347]	T	80/0	USA	MC	Prospective	Fisher [348]	T	45/70	USA	SC	Retrospective	Rosas, J.[349]	T	20/17	Spain	SC	Retrospective
Campins [350]	T	58/0	Spain	SC	Prospective	Galvan Roman [351]	T	58/88	Spain	SC	Retrospective	Rossi [352]	T	84/84	France	SC	Retrospective

*Carvalho [353]	T	29/24	Brazil	SC	Prospective with control	*Garcia [354]	T	77/94	Spain	SC	Retrospective	Rossotti [355]	T	74/148	Italy	SC	Retrospective
Dastan [356]	T	42/0	Iran	SC	Prospective	Gokhale [357]	T	70/91	India	SC	Retrospective	Ruiz-Antoran[358]	T	268/238	Spain	MC	Retrospective
* Gordon [333]	T	350/397	UK	MC	Adaptive RCT	Guaraldi [359]	T	179/ 365	Italy	MC	Retrospective	Somers [360]	T	78/76	USA	SC	Retrospective
Hermine [361]	T	63/67	France	MC	Open-label RCT	Guisado-Vasco [362]	T	132/ 475	Spain	SC	Retrospective	Tian [363]	T	65/130	China	MC	Retrospective
Malekzadeh [364]	T	126/0	Iran	MC	Prospective	Gupta [365]	T	433/ 3492	USA	MC	Retrospective	Tsai [366]	T	66/66	USA	SC	Retrospective
Mikulska [367]	T	29/66	Italy	SC	Prospective with control	Hill [368]	T	43/45	USA	SC	Retrospective	*Wadud[369]	T	84/84	USA	SC	Retrospective
Morena [370]	T	51/0	Italy	SC	Prospective	Holt [371]	T	24/30	USA	SC	Retrospective	Zheng [372]	T	92/89	China	SC	Retrospective
Perrone [373]	T	708/481	Italy	MC	Single arm open label	Ip [374]	T	134/413	USA	MC	Retrospective						
*Rosas [375]	T	294/144	USA	MC	Double blind RCT	Kewan [376]	T	28/23	USA	SC	Retrospective						

Table 3-1 - Included studies with study characteristics.

*Sample size for treatment (Tx) and control group (control) shown. * non peer-reviewed preprint study; #, study investigating both anakinra and tocilizumab; A, anakinra; Sa, sarilumab; Si, siltuximab; T, tocilizumab; SC, single-centre; MC, multi-centre. All studies published in 2020 unless otherwise stated.*

3.3.1 Risk of bias assessment

Several biases and limitations were identified using risk of bias assessment tools. In most studies the study population was clearly defined using specific inclusion and exclusion criteria, and in studies with a control arm, participants were typically selected from the same population. Details of interventions were lacking in several studies, with doses and drug regimens not clearly reported. Endpoints were inconsistent and included ordinal scales, mortality, or duration of hospitalisation. Sample size justifications were rarely provided in the included studies, and details of statistical analyses were variably reported. In the majority of studies, participants were administered concomitant therapies including corticosteroids, antivirals, and antibodies, limiting the ability to discern whether the intervention under investigation was related to the outcome (Figure 3-3). Following a formal risk of bias assessment, 23 (32%) studies were rated as good, 37 (52%) fair and 11 (15%) poor. Publication bias, assessed by observation of funnel plots and Egger's test, was not present for any of the outcomes assessed.

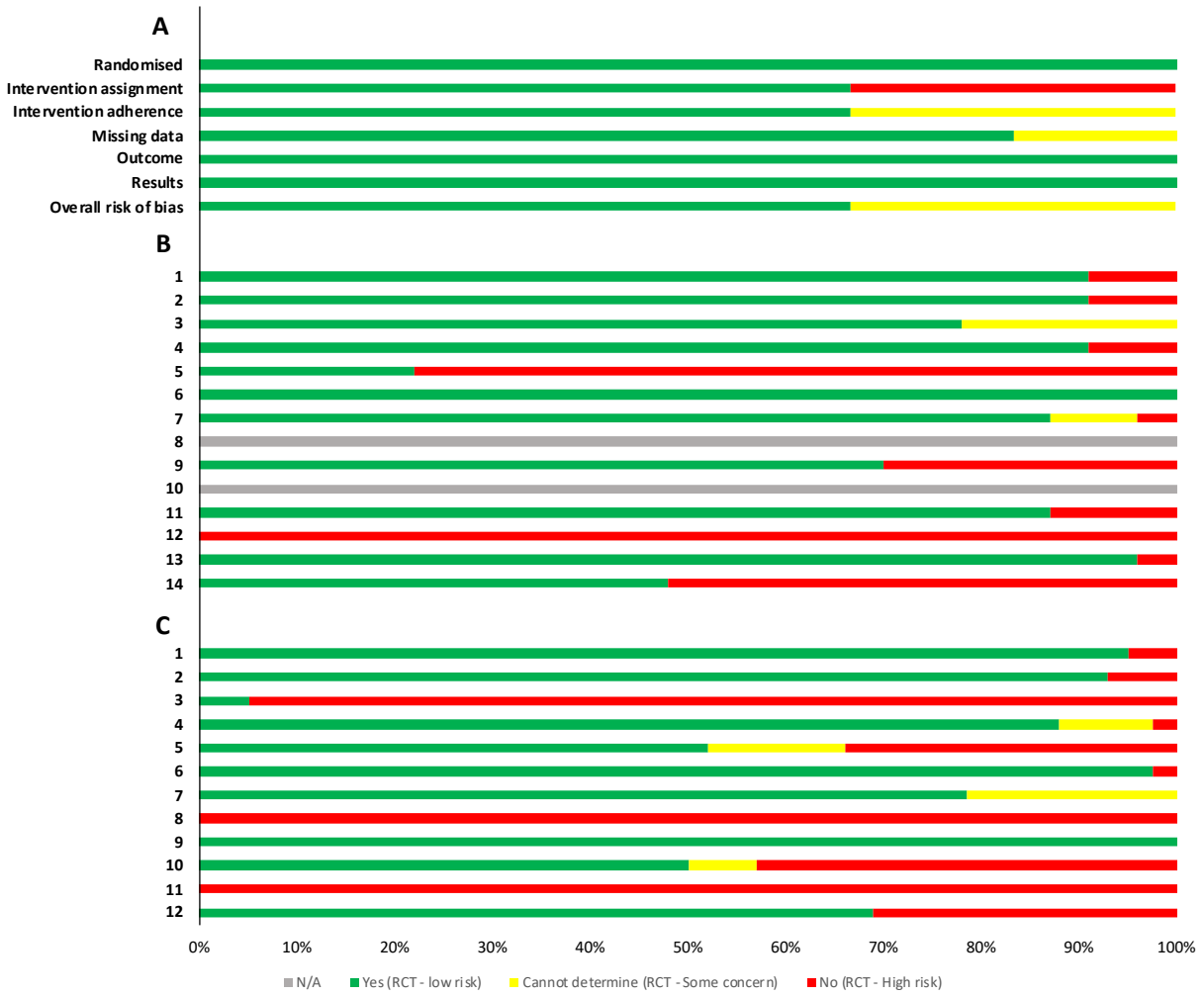


Figure 3-3 - Summary of risk of bias assessment

A *- Randomised clinical trials assessed using Cochrane risk of bias 2 tool (n=6). Risk of bias was assessed in six categories and scored as either low risk of bias, some concern, or high risk of bias, before an overall risk of bias was given to each study.* ***B*** *- Non-randomised prospective studies (n=23). Questions numbered in the first column. 1. Was the research question or objective in this paper clearly stated? 2. Was the study population clearly specified and defined? 3. Was the participation rate of eligible persons at least 50%? 4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants? 5. Was a sample size justification, power description, or variance and effect estimates provided? 6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured? 7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed? 8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)? 9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? 10. Was the exposure(s) assessed more than once over time? 11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? 12. Were the outcome assessors blinded to the exposure status of participants? 13. Was loss to follow-up after baseline 20% or less? 14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?* **C -** *Summary of risk of bias assessment for retrospective studies (n=42). Questions numbered in first column. 1. Was the research question or objective in this paper clearly stated and appropriate? 2. Was the study population clearly specified and defined? 3. Did the authors include a sample size justification? 4. Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)? 5. Were the definitions, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants? 6. Were the cases clearly defined and differentiated from controls? 7. If less than 100 percent of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible? 8. Was there use of concurrent controls? 9. Were the investigators able to confirm that the exposure/risk occurred prior to the development of the condition or event that defined a participant as a case? 10. Were the measures of exposure/risk clearly defined, valid, reliable, and implemented consistently (including the same period) across all study participants? 11. Were the assessors of exposure/risk blinded to the case or control status of participants? 12. Were key potential confounding variables measured and adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis?*

3.3.2 Tocilizumab

Twenty prospective studies, of which eight had a control arm, and a further 40 retrospective studies of tocilizumab were identified, reporting outcomes from a total of 20,972 patients, of whom 6563 (31%) received tocilizumab. Inclusion criteria varied across the studies, frequently necessitating respiratory failure and laboratory evidence of hyperinflammation typically defined as an elevated CRP, with fewer studies measuring IL-6. Dosages of tocilizumab were not entirely consistent with intravenous 8mg/kg or 400mg the most studied route and dose.

12 studies with 1782 patients provided ordinal outcome data adapted to a four-point scale. The median time for reporting outcomes following intervention was 14 days (IQR 14-28). In the REMAP-CAP RCT, tocilizumab was associated with clinical improvement at day 14 (aOR 1.83 95%CI 1.40;2.41)[333], whilst in a separate RCT, tocilizumab did not alter ordinal severity outcomes (HR 1.06 95%CI 0.80;1.41)[323]. Neither of these RCTs were included in meta-analysis due to differences in statistical methodologies and clinical endpoints. In meta-analysis of the remaining prospective studies (Figure 3-4), tocilizumab was not associated with better outcomes on an ordinal scale (GenOR 1.09 95% CI 0.99;1.19, I^2 = 84.3%). In retrospective studies, tocilizumab was associated with better outcomes indicating a 34% greater chance of less-severe outcomes compared with control patients (GenOR 1.34 95% CI 1.10;1.64, I^2 = 98%). These results should be interpreted with caution due to severe heterogeneity which could not be explained by variability in the factors assessed.

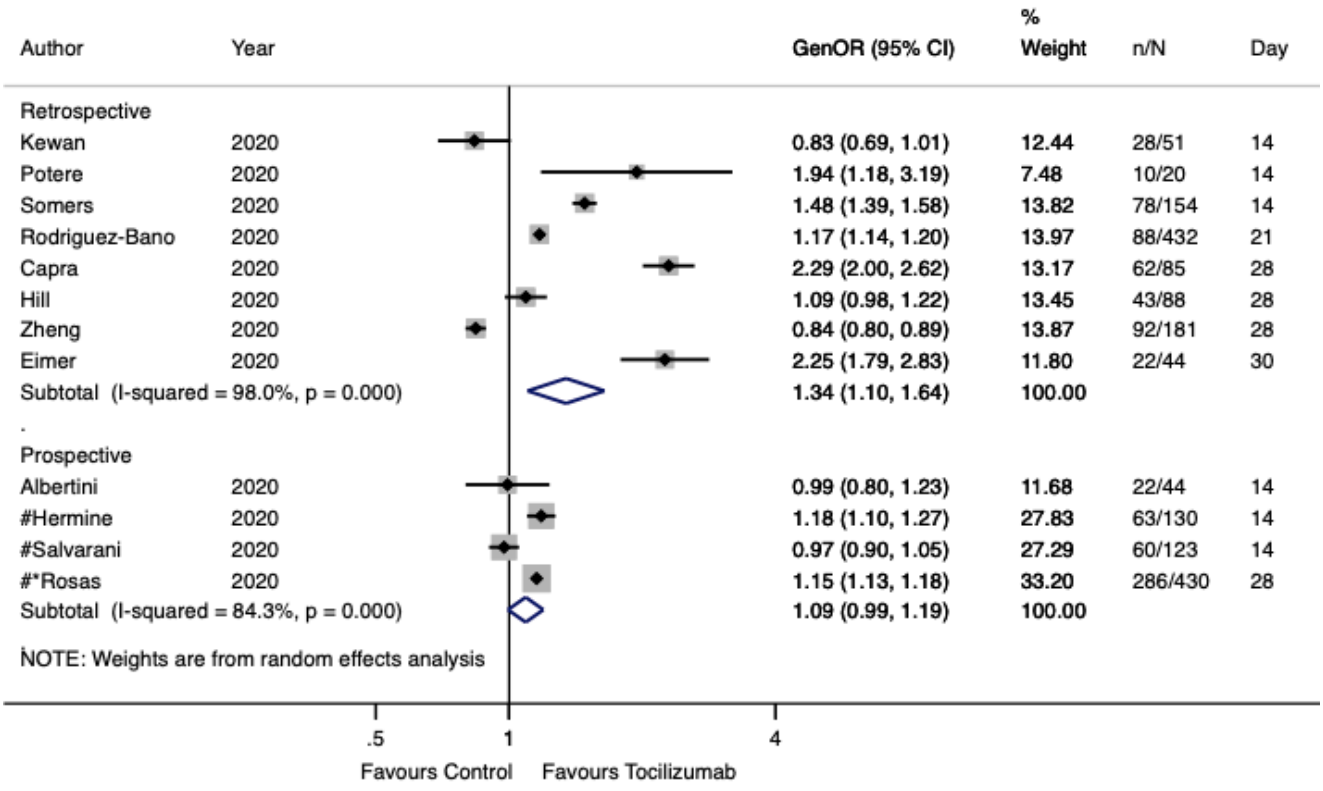


Figure 3-4 -Forest plot demonstrating comparing tocilizumab with placebo for ordinal outcomes using generalised odds ratios (OR)

*Generalised OR shown for each study with 95% confidence interval and day at which ordinal outcome recorded. Sample sizes given for patients receiving intervention (n) alongside total included (N) in study. Summary estimates presented separately for prospective and retrospective studies. * non peer-reviewed preprint studies; # randomised controlled trials*

The duration of hospitalisation was reported for a total of 1553 survivors in two RCTs and nine retrospective studies. Meta-analysis of retrospective studies showed no difference in the mean duration of hospital stay compared with controls who received standard of care alone (0.36 days 95% CI -0.07;0.80, I^2 = 93.8%), with variability in route of administration (intravenous or subcutaneous) associated with heterogeneity (R^2 = 81.64%, p<0.001).

The risk ratio (RR) for unadjusted mortality data was available for 15,085 patients across 42 studies, which included six RCTs. Tocilizumab was associated with a 17% lower unadjusted risk of mortality compared with the control arm in prospective studies (RR 0.83 95%CI

0.72;0.96, I^2 = 0.0%), which did not reach statistical significance in RCTs alone (RR 0.85 95%CI 0.71;1.01 I^2 = 0.0%) (Figure 3-5). In meta-analysis of retrospective studies, tocilizumab was associated with a 24% lower risk of mortality (RR 0.76 95%CI 0.64;0.92, I^2 = 80.3%) (Figure 3-6), although there was substantial heterogeneity which could not be explained by variability in the factors assessed. The combined case fatality rate (CFR) across all studies was 21.2% (1118/5284) in the intervention arm and 31.1% (3049/9801) in the control arm (p<0.001).

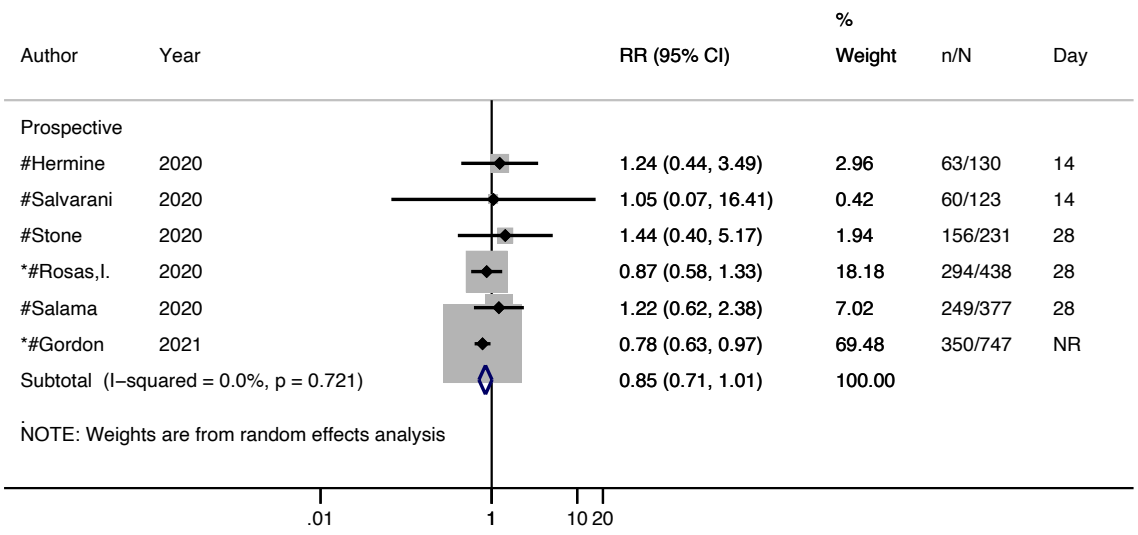


Figure 3-5 – Forest plot showing mortality risk ratios for tocilizumab RCTs alone.

*Risk ratios with associated 95% confidence interval and day of censorship presented for each study. Sample sizes given for patients receiving intervention (n) and total included in study (N). Summary estimates presented separately for prospective and retrospective studies. * non peer-reviewed preprint studies NR, not reported*

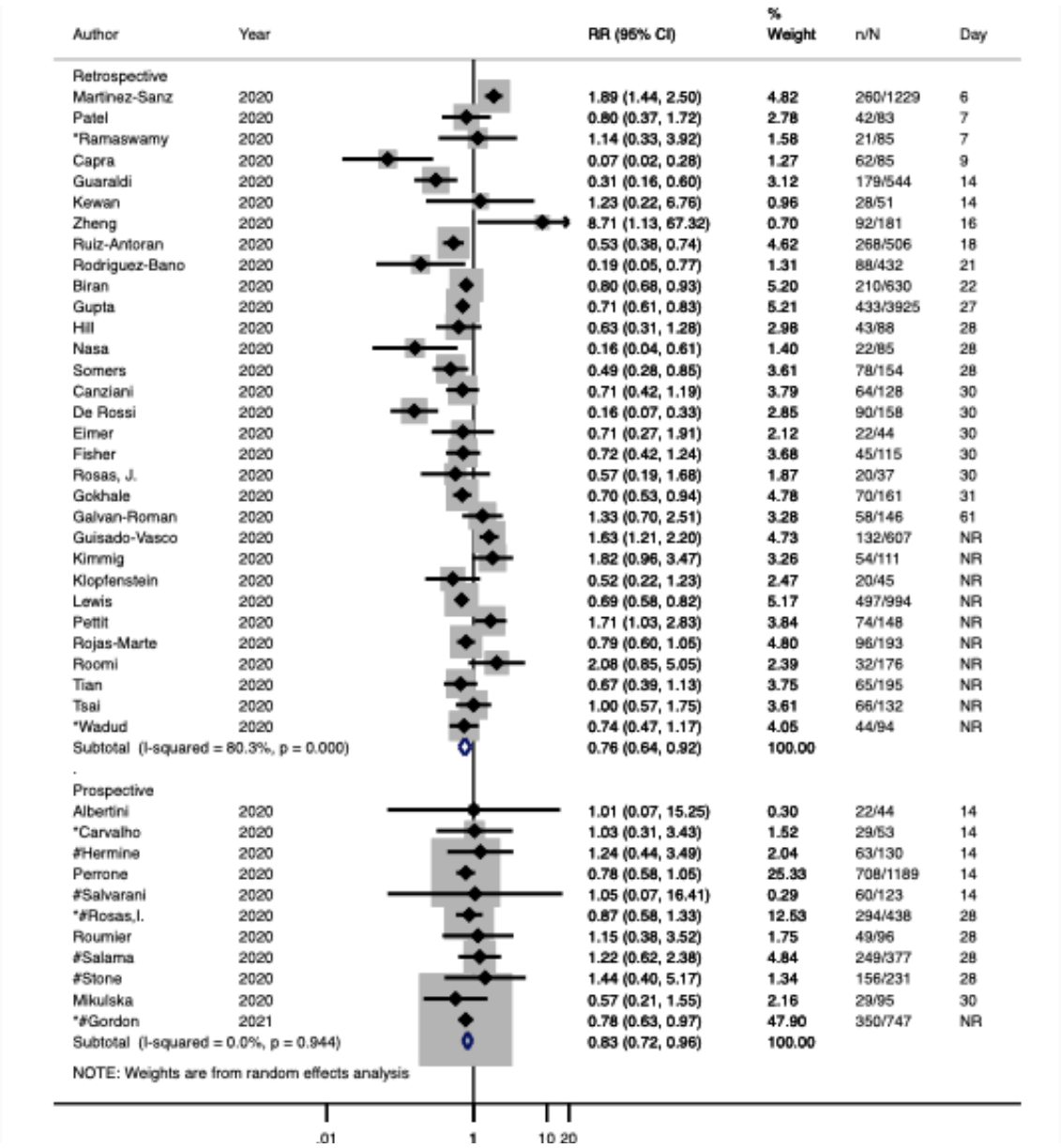


Figure 3-6 - Forest plot showing mortality risk ratios for all tocilizumab studies

*Risk ratios with associated 95% confidence interval and day of censorship presented for each study. Sample sizes given for patients receiving intervention (n) and total included in study (N). Summary estimates presented separately for prospective and retrospective studies. * non peer-reviewed preprint studies; # randomised controlled trials; NR, not reported*

Adjusted hazard ratios for overall mortality were reported in 22 studies totalling 13,702 patients, at a median follow up time of 28 days (IQR 14-30). In meta-analysis of prospective studies, an emerging survival benefit was demonstrated, but the estimate was inconclusive (HR 0.70 95%CI 0.44;1.10, I^2 = 0%) (Figure 3-7). In the remaining retrospective studies,

tocilizumab was associated with a 48% lower risk of adjusted mortality with substantial heterogeneity (HR 0.52 95%CI 0.41;0.66, I^2 =76.6%).

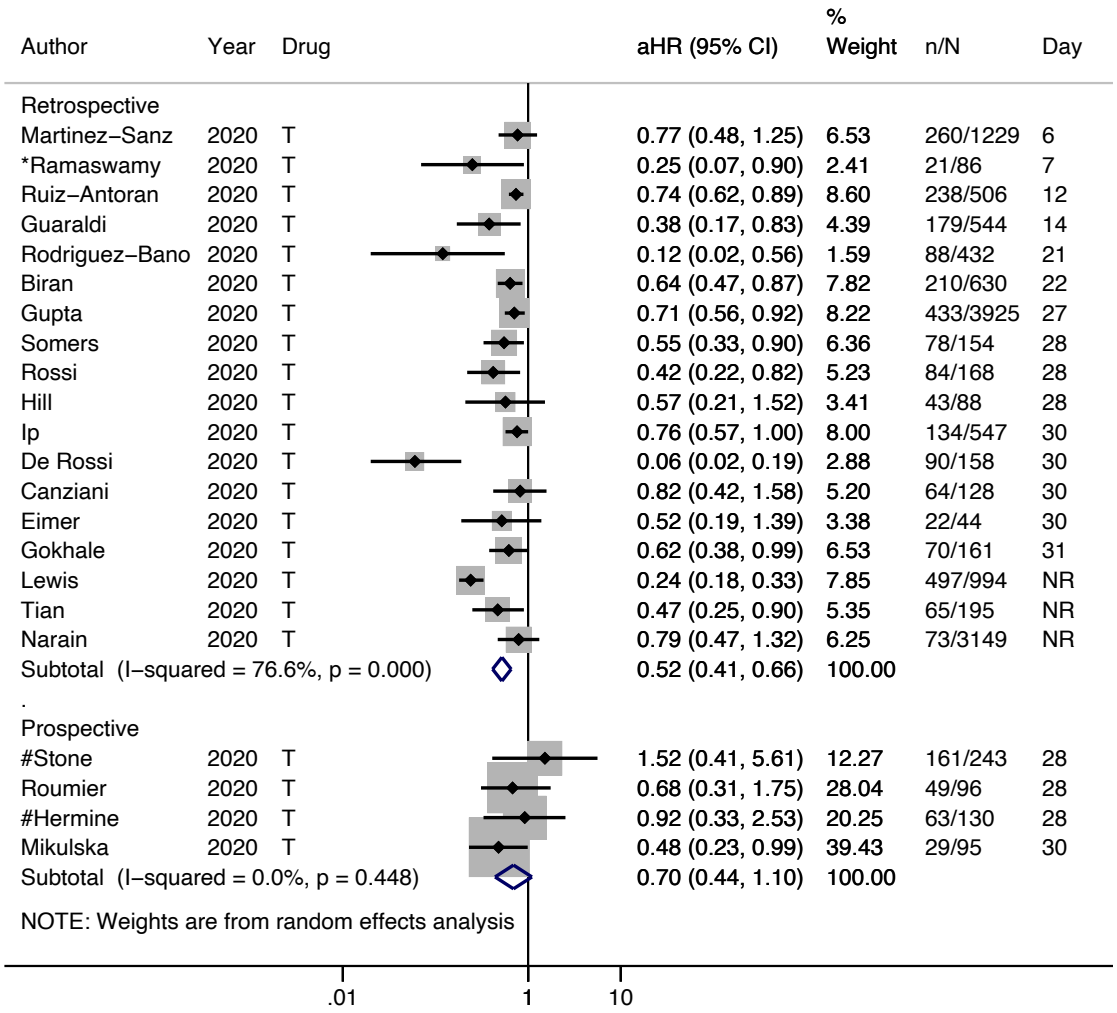


Figure 3-7 - Forest plot showing adjusted hazard ratios for tocilizumab studies

*Adjusted HRs with associated 95% confidence interval and day of censorship presented for each study. Sample sizes given for patients receiving intervention (n) and total included (N) in study. Summary estimates presented separately for prospective and retrospective studies. * non peer-reviewed preprint studies; # randomised controlled trials; NR, not reported*

3.3.3 Anakinra

Seven studies, of which four were prospective and three were retrospective, evaluated outcomes in 346 patients who received anakinra and 3339 controls. Ordinal outcome data were limited with small studies demonstrating a possible association between anakinra and improved clinical outcomes[307 319 322]. A significant association with mortality was observed in

a prospective study (aHR 0.49 95%CI 0.26;0.91)[316], but not in a retrospective study of 57 patients (aHR 0.79 95%CI 0.44;1.42)[321]. In pooled risk ratios from three prospective studies, there was no association with mortality (RR 0.70 95%CI 0.31;1.58, I^2 = 32.8%) (Figure 3-8).

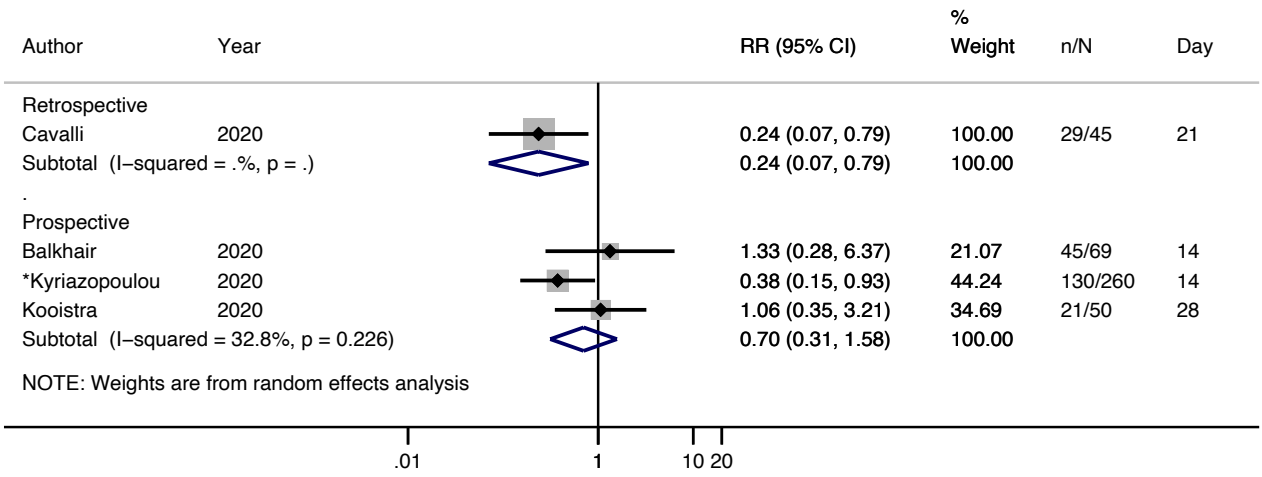


Figure 3-8 - Forest plot showing mortality risk ratios for all anakinra studies

*Risk ratios with associated 95% confidence interval and day of censorship presented for each study. Sample sizes given for patients receiving intervention (n) and total included in study (N). Summary estimates presented separately for prospective and retrospective studies. * non peer-reviewed preprint studies*

3.3.4 Sarilumab

Five prospective studies exploring outcomes in 389 participants were identified. In an RCT, sarilumab was associated with increased survival (aOR 2.01 95%CI 1.18;4.71), reduced duration of hospitalisation (aHR 1.60 95%CI 1.17;2.40) and improved ordinal outcomes at day 14 (aOR 1.86 95%CI 1.22;2.91)[333]. In a further prospective non-randomised study of 28 participants[330], sarilumab did not impact mortality (aHR 0.36 95%CI 0.08;1.68) or the duration of hospitalisation (mean difference 0.02 95%CI -0.51;0.54). The combined CFR across all included studies was 11% (43/389) in patients receiving sarilumab, whilst in the only study with available control mortality data the CFR was 35.8% (142/397).

3.3.5 Siltuximab

A single prospective study of 60 patients was identified [341], with a lower risk of mortality in patients who received siltuximab (aHR 0.46 95%CI 0.22;0.97).

3.3.6 Treatment related adverse events

Treatment related adverse events were reported in most studies (70%). Though secondary bacterial infections and deranged liver enzymes were reported in patients who received interleukin inhibitors, the frequency of events was consistent with comparator groups who received standard of care.

3.4 Discussion

3.4.1 Summary of findings

This systematic review evaluated the role of specific interleukin inhibitors for the management of COVID-19. Although severe inter-study heterogeneity was observed, tocilizumab had beneficial effects when an adapted four-point ordinal scale was applied, though confidence intervals in pooled prospective studies were not conclusive. For this reason, the certainty of findings for this outcome are rated as moderate using GRADE. A survival benefit with Tocilizumab was observed that was consistent across retrospective and prospective studies, with pooled analysis of unadjusted risk ratios demonstrating a 17% reduced risk of mortality in prospective studies. The certainty of findings related to overall mortality are rated as high. Tocilizumab did not alter the mean duration of hospitalisation, with low certainty of findings.

There was significant heterogeneity in study designs that evaluated outcomes in non-tocilizumab studies leading to insufficient data to enable quantitate synthesis. In the only study that reported adjusted mortality estimates, anakinra lowered the risk of death, however when unadjusted risk ratios were pooled across several non-randomised studies, a mortality benefit was not observed. In the only study for sarilumab, intervention improved hospital outcomes and reduced the duration of hospitalisation. No randomised studies were identified for siltuximab. For all interleukin inhibitors, the frequency of adverse events was similar in treatment and control arms. We did not detect any significant publication bias in the reporting of effects.

This review included a large number of studies. In such cases, systematic reviews offer several advantages as they enable a more precise assessment of the effect size and usefulness of an intervention, which can help inform clinical judgement. Conversely, meta-analysis can be equally helpful when there are few studies (as low as two), providing the studies are sufficiently similar using a measurement of heterogeneity. Combining small numbers of studies should be considered when there is a clear theoretical or logical basis which will enable the attainment of additional information beyond that offered in the original studies[377].

3.4.2 Limitations

There are multiple limitations that must be considered alongside the findings of this study. This review included 71 studies, although only six were randomised trials. Non-randomised trials of interventional agents are associated with several biases that can limit inference. Reassuringly, when analyses were restricted to RCTs, the pooled effect size favoured tocilizumab, although confidence intervals did not reach statistical significance due to limited power. A further limitation relates to the patient selection criteria across the studies which was not entirely consistent. Respiratory failure and hyperinflammation were necessitated in most studies, but hyperinflammation was inconsistently defined using various combinations of IL-6, CRP, and ferritin. Moreover, the severity of respiratory failure ranged from individuals requiring basic respiratory support to advanced ventilatory support. The dosage, route and timing of administration of the therapeutic agent under investigation varied across the studies, and concomitant medications such as hydroxychloroquine and antivirals were frequently prescribed, precluding causal associations of interleukin inhibitors with outcomes. Study outcomes were not uniform, and a combination of clinical, laboratory and radiological

outcomes were reported, rather than a single consistent endpoint. Furthermore, the duration of follow up and timing of reported outcomes varied across the studies.

To mitigate study differences, meta-regression was applied for all analyses according to study differences to identify possible sources of heterogeneity. Though residual heterogeneity could not always be explained, concomitant steroid use, route of drug administration and day outcome measured appeared to contribute within specific outcomes. To maximise the clinical usefulness of the review, four interleukin inhibitors, alongside several endpoints were included. Furthermore, all studies with a control group irrespective of their risk of bias or study design were included but retrospective and prospective studies were analysed and presented separately. Where insufficient data precluded meta-analysis, key study observations were described using qualitative synthesis, ensuring the review was comprehensive. Included studies carried international representation and were performed in various ethnic backgrounds, and thus findings should be generalisable to the global population.

3.4.3 Biomarker guided therapy

One of the fundamental aims of this chapter was to determine whether blood biomarkers offer prognostic potential, using a contemporary and generalisable example of COVID-19. In the included studies, there was considerable missing data and heterogeneity in reporting for IL-1 and IL-6. For IL-6 specifically, several studies identified a cut-off threshold, whereas other studies did not have IL-6 measurements available. Numerous studies utilised serum

CRP concentrations to define hyperinflammation since circulating IL-6 is the primary inducer of CRP from the liver. Intriguingly, where available, serum IL-6 levels were unusually low, particularly when compared with CRP concentrations in the same individuals. An initial upsurge in serum IL-6 levels following treatment with IL-6 inhibitors was observed in few studies, but there was insufficient granularity in the data to assess baseline measurements and detailed trends in IL-6 according to outcomes. This meant the prognostic potential of IL-6 could not be assessed with any degree of confidence. IL-6 measurement and interpretation is not considered to be straightforward, as the cytokine peaks at different times and is influenced by age, exercise, circadian rhythms, concomitant therapies, and other comorbidities such as obesity[295]. Assay measurements can also be influenced by variability related to sampling and processing such as storage time, room temperature and assay sensitivity. An understanding of these factors is crucial for the accurate interpretation of serum IL-6 measurements, and thus for utilising a biomarker-guided prognostic strategy. Understanding disease pathways related to the biomarker of interest may help identify downstream components that are easier to measure with less instability.

Another important aim was to determine whether biomarkers can be targeted therapeutically. There is increasing evidence to suggest there may be a “window of opportunity” with interleukin inhibitors, with benefit observed if administered at the appropriate time after symptom onset[378]. In several individuals included in this review, IL-6 inhibition did not alter outcomes, and a RCT published following the completion of this review demonstrated an increased risk of mortality with tocilizumab[379]. Although the inflammatory effects of IL-6 are well known, this pleiomorphic cytokine has several

important physiological roles in humans including being pivotal in innate and adaptive immunity, regulating the acute-phase response, lipid homeostasis, and neural development[295]. Therefore, any attempts to target this cytokine must be balanced against any possible deleterious consequences, including the risk of further immunosuppression leading to secondary infection. Conversely, if the inflammatory cascade has advanced uncontrollably, administration of cytokine blockade may be too late, and is unlikely to be beneficial. Similar challenges with timing are likely to exist when targeting blood biomarkers in pulmonary fibrosis, particularly in individuals with acute exacerbations or rapidly progressive disease.

The experience and knowledge gained from biomarker studies in COVID-19 should be applied to future studies in other conditions. A detailed understanding of the natural history and pathogenesis of disease, alongside an appreciation of the signalling pathways associated with the biomarker of interest are pivotal before biomarker-targeted therapies can be recommended for clinical use. In that regard, smaller more detailed mechanistic and biomarker-focussed studies are likely to yield more information and help identify individuals who are likely to benefit, rather than large multi-centre interventional RCTs. Moreover, future interventional studies should include detailed biomarker analyses at baseline and following treatment to identify indicators of response to therapy, to ensure the right person receives the right treatment at the right time.

3.5 Summary

This systematic review and meta-analysis of IL-1 and IL-6 inhibitors for the treatment of severe COVID-19 provides evidence for the use of tocilizumab, whereas the evidence for anakinra, siltuximab and sarilumab was insufficient and further studies are justified. The relationship between blood interleukin levels and interleukin inhibitors according to disease outcomes, and the role of interleukins as prognostic biomarkers could not be assessed due to insufficient data. Nonetheless, this chapter includes an appraisal of a biomarker-targeted therapeutic strategy and highlights several considerations for future research that can be applied to pulmonary fibrosis and other diseases.

Chapter 4 Evidence synthesis for short-term change in physiological markers as an endpoint for future trials

4.1 Introduction

There has been recent progress in the management of IPF with the approval of anti-fibrotics. However, current therapies slow disease progression, rather than halt or reverse existing fibrosis, and more effective therapies are urgently needed. Defining the optimal primary endpoint on which to design such clinical trials remains the subject of debate. Mortality, the obvious and ideal endpoint occurs too infrequently in individuals with mild to moderate IPF and thus requires substantial sample sizes with long durations of follow up to capture sufficient events to detect treatment differences. For this reason, surrogate markers for mortality including change in FVC over 12 months have been commonly used as endpoints in IPF clinical trials[53 54]. However, in a condition characterised by poor survival, earlier endpoints are urgently required. The identification of biomarkers measured at earlier timepoints have the potential to transform clinical trials by enabling assessment of a greater number of therapeutic agents in accelerated clinical trials. Physiological measurements including FVC, DL_{CO} and 6MWD have been studied as prognostic biomarkers in IPF. However, studies have been limited by small samples, retrospective designs, and narrowly defined inclusion criteria, often resulting in inconsistent findings.[380 381]. Whilst physiological decline is inevitable, there is considerable heterogeneity in the rate of disease progression,[15] and thus the accurate and early prediction of disease course is essential for appropriately counselling patients and enabling personalised approaches to therapy.

The purpose of this chapter is to explore physiological variables as prognostic markers and as clinical trials endpoints in IPF. Placebo arms from interventional trials offer an invaluable resource to explore the association between commonly measured physiological variables and disease outcomes in anti-fibrotic naïve individuals. In this chapter I combine IPD from trial placebo arms to determine whether short-term changes in physiological variables can predict mortality and disease progression, and thus act as surrogate endpoints over short-term periods to accelerate future IPF clinical trials. Furthermore, I combine lung function data from treatment arms in clinical trials where the endpoint was met (i.e., studies of pirfenidone or nintedanib) to determine whether a treatment-effect could be observed as early as three-months. The study protocol can be found on PROSPERO (CRD42020164935) and the key findings have been published as a manuscript "Three-month FVC change: a trial endpoint for IPF based on individual participant data meta-analysis" in the American Journal of Respiratory and Critical Care Medicine (AJRCCM).

4.1.1 Aims of study

1) To determine the role of physiology (FVC, $DL_{CO,}$ 6MWD) as prognostic markers in IPF by examining the association between baseline and/or short-term change in measurements and clinical outcomes
2) To investigate whether short term change in physiological variables can act as surrogate endpoints in future IPF interventional adaptive trials
3) To investigate whether treatment benefits can be observed at three-months
4) To investigate the association between demographic physiological factors (age, sex, and smoking history) and disease outcomes in IPF

4.2 Methods

4.2.1 Eligibility criteria

Randomised controlled trials (RCT) that included placebo arms reporting disease outcomes in adults with IPF, diagnosed according to contemporaneous guidelines, and corresponding treatment arms from pirfenidone and nintedanib trials were eligible for inclusion. Studies with a sample size fewer than 30 participants, retrospective studies, non-randomised studies, and studies in other fibrotic ILDs other than IPF were excluded.

4.2.2 Physiological markers

Studies reporting lung function (FVC and DL_{CO}) at either baseline and/or change over three-months were eligible for inclusion. Moreover, studies reporting baseline and three-month change in six-minute walk distance were included. Desaturation during six-minute walk test was not explored in this review since the continuous measurement of oxygen has only been recommended more recently[382], and thus would not have been performed in the majority of included studies.

4.2.3 Search strategy

Electronic databases including MEDLINE (1946 to latest), Embase (1974 to latest), Google Scholar, the Cochrane Register of Controlled Trials and ClinicalTrials.gov were searched on 1st December 2020, independently by two reviewers (Fasi Khan and Laura Fabbri). Pre-print servers including medRxiv, bioRxiv and Wellcome Open Research were searched to identify

unpublished studies, ensuring the review was inclusive as possible. Search terms included keywords to identify physiological variables alongside search filters to restrict results to RCTs and can be found in the appendix. Reference lists of included studies were searched to identify further studies.

4.2.4 Data extraction

Once eligible studies were identified, corresponding authors were contacted using encrypted electronic mail communication, with at least three reminders, each four weeks apart (Appendix 10.2). Data-sharing portals such as Vivli, Yoda and Clinical Study Data Request were utilised to request data from sponsored clinical studies, utilising data-sharing agreements[266-268]. Data requested included treatment randomised to, participant demographics (age, sex, and smoking status), baseline and three-month physiology data (FVC, DL_{CO} and 6MWD) where available, 12-month FVC, duration of follow up and mortality status. Studies where IPD was unavailable were excluded, but demographics were tabulated to explore the possibility of ascertainment bias.

4.2.5 Risk of bias

Since there are no widely available tools for assessing the risk of bias specifically in RCT placebo arms, a modified version of the Quality in Prognostic Studies (QUIPS) tool was applied to assess the risk of bias in included studies[383], with each placebo arm considered as an observational cohort. The QUIPS tool has been described in section 2.2.1.4. The modified version assessed the risk of bias across five domains: study participation, study attrition,

prognostic factor measurement, outcome measurement and study confounding. GRADE ratings were applied to assess the overall quality and certainty of evidence for each of the outcomes assessed. [234]

4.2.6 Analysis

All analyses were performed in placebo arms only unless otherwise stated. IPD meta-analysis with random effects in a two-step design, as described in section 2.3.1 was applied to estimate hazard ratios in time-to-event analyses for associations with overall mortality, and odds ratio in logistic regression models for association with disease progression. Disease progression was defined as 10% relative decline in FVC or death within 12 months of baseline. All estimates were adjusted for confounders identified *a priori* including age, sex, smoking history, and baseline FVC. Participants with missing data were excluded using listwise deletion. Estimates for three-month change in physiological variables were additionally adjusted for baseline measurements. FVC percent predicted values were recalculated for all participants where possible using Global Lung Initiative (GLI) equations[384]. GLI reference equations standardise reference values for predicted FVC, which are multi-ethnic and adjust for age, gender, and height, all of which can be sources of variability.

Baseline physiological markers and their association with mortality and disease progression were estimated per 5% decrement in %predicted FVC and DL_{CO}, and per 50m decrement in 6MWD. Demographic factors were estimated per year increase in age and expressed as binary exposures for gender (male vs. female), and smoking status (current or ex-smoker vs.

never smoker). A threshold of 80% predicted FVC was chosen to define baseline subgroups based on typical critical used in anti-fibrotic management[385], and mean difference in absolute FVC (ml) between baseline and 12 months was estimated and pooled across all studies.

In three-month analyses, the association of continuous physiological variable change with outcomes was estimated using 2.5% relative percent change from baseline %predicted FVC and DL_{CO}, and 20m absolute decline in 6MWD. The median relative percentage change over three-months in FVC was estimated, and the mean difference in absolute FVC (ml) between baseline and 12 months stratified by three-month FVC decline (above or below median) was calculated and pooled. Optimum thresholds based on sensitivity and specificity for three-month FVC change in predicting disease progression and mortality were estimated individually for each study with the empirical Liu method and bootstrapping to derive robust confidence intervals[386]. Optimum thresholds were combined to estimate an overall threshold for three-month relative FVC change and used to estimate association of outcome in those with decline greater than threshold compared to those below. The area under the receiver operator characteristics curve (AUROC) at the optimum FVC threshold was estimated for each study and pooled to assess overall discriminative performance.

Statistical heterogeneity was assessed using the I^2 statistic and meta-regression was performed to explore variability according to various study factors: permitted steroid use, IPF diagnosis within 5 years, inclusion of severe cases (FVC≤50% predicted), and studies with sufficient information to enable calculation of GLI reference equations. Publication bias was

assessed using visual inspection of funnel plots for asymmetry and application of Egger's test where sufficient studies were included[273].

The association between three-month FVC change and disease outcomes was estimated in intervention arms specifically from studies where a treatment benefit was observed. To examine a possible treatment effect at three-months, the change in FVC in pooled treatment arms was compared with pooled estimates in corresponding placebo arms using random effects meta-analysis. Meta-regression was applied using treatment as a covariate to determine statistical significance. To enable power calculations for future interventional trials, standardised effect sizes (Hedge's g) were estimated between placebo and treatment arms within each study and weighted into an overall value at both three and twelve months.

4.3 Results

Electronic database search retrieved 271 articles, with a further five articles identified through ClinicalTrials.Gov (Figure 4-1). No articles were identified through search of preprint servers. Following the removal of duplicates, screening of abstracts and titles, and full text review, 23 studies with a total of 2958 participants were identified for inclusion.

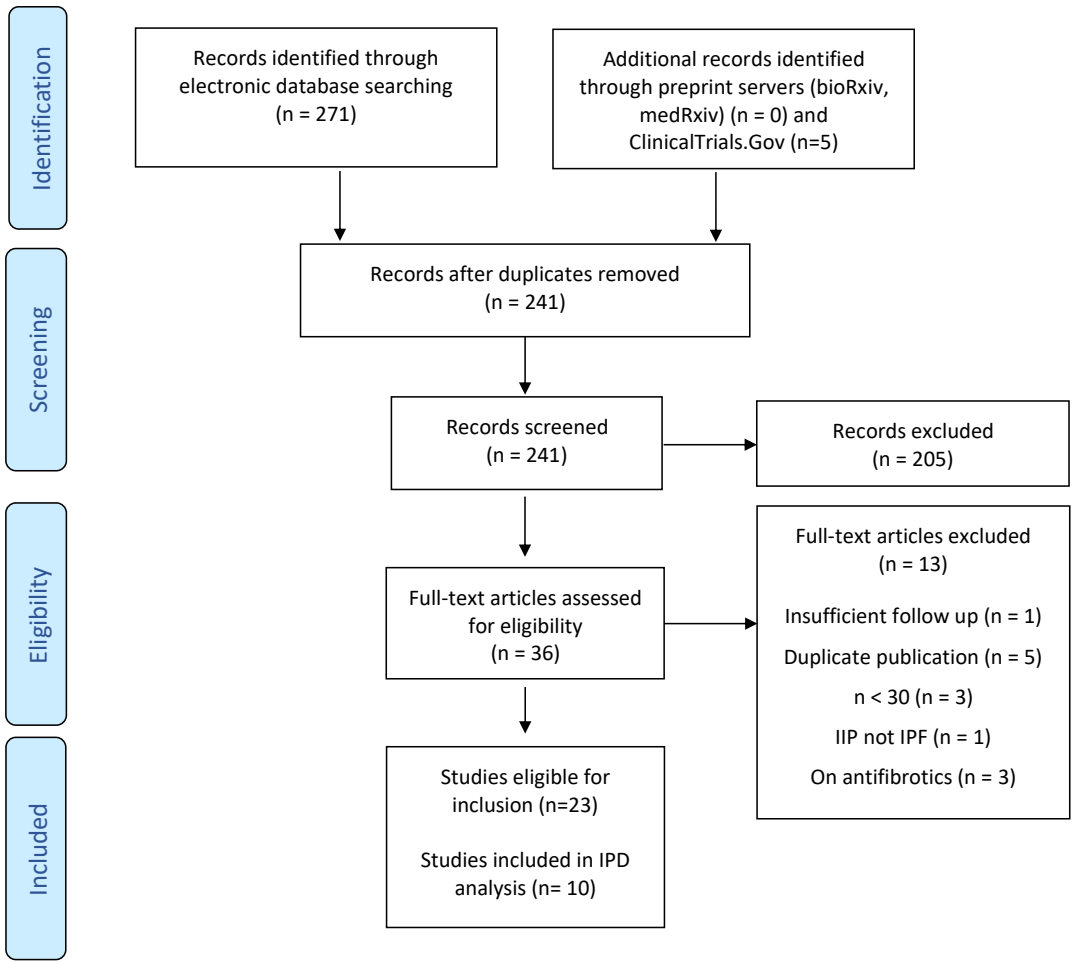


Figure 4-1 – Flow diagram illustrates systematic search and screening strategy, including numbers of studies meeting eligibility criteria and numbers excluded.

Study sponsors and corresponding authors of shortlisted studies were contacted for IPD. Of the 23 studies, IPD were made available from 10 studies that reported outcomes from a total of 1819 participants assessed in 12 placebo cohorts (Table 4-1 and Table 4-2).

Study	Year of publication	Study phase	Centre	Key eligibility criteria	Concomitant therapies
ARTEMIS[238]	2013	Phase 3	multi-centre	Age 40-80	Nil
ASCEND[176]	2014	Phase 3	multi-centre	Age 40-80, diagnosed within last 4 years, FVC 50-90% predicted, DL_{CO} 30-90%, 6MWD ≥ 150m.	Steroids not allowed
BUILD-1[387]	2007	Phase 3	multi-centre	Diagnosed within last 3 years, 6MWD between 150m and 499m, FVC 50-90% predicted, DL_{CO}>30% predicted	Prednisolone up to 15mg OD
BUILD-3[388]	2011	Phase 3	multi-centre	Diagnosed within last 3 years and confirmed by lung biopsy	Prednisolone up to 20mg OD
CAPACITY1[53]	2011	Phase 3	multi-centre	Age 40-80, diagnosed within last 4 years, FVC 50-90% predicted, DL_{CO} > 35% predicted, 6MWD ≥ 150m	Nil
CAPACITY2[53]	2011	Phase 3	multi-centre	Age 40-80, diagnosed within last 4 years, FVC 50-90% predicted, DL_{CO} > 35% predicted, 6MWD ≥ 150m	Nil
IFIGENIA[389]	2005	Phase 3	multi-centre	Age 18-75, FVC ≤80%, DL_{CO} ≤ 80%	Prednisolone and Azathioprine
INPULSIS1[54]	2014	Phase 3	multi-centre	Age ≥ 40, diagnosed within last 5 years, FVC ≥ 50% predicted, DL_{CO} 30-79% predicted	Prednisolone up to 15mg OD
INPULSIS2[54]	2014	Phase 3	multi-centre	Age ≥ 40, diagnosed within last 5 years, FVC ≥ 50% predicted, DL_{CO} 30-79% predicted	Prednisolone up to 15mg OD
MUSIC[390]	2013	Phase 2	multi-centre	Diagnosed within last 3 years, FVC≥50% predicted, DL_{CO} ≥ 30% predicted	Nil
TIPAC[391]	2013	Phase 3	multi-centre	Age > 40, MRC dyspnoea score ≥ 2	Prednisolone, azathioprine, mycophenolate allowed
TOMORROW[54]	2011	Phase 2	multi-centre	Age ≥ 40, diagnosed within last 5 years, FVC ≥ 50% predicted, DL_{CO} 30-79% predicted	Prednisolone up to 15mg OD

Table 4-1- Methodological characteristics of included studies

6MWD, six-minute walk distance, DL_{CO}, gas transfer for carbon monoxide; FVC, forced vital capacity; MRC, medical research council

Study	Placebo sample size	Study follow up, months (median, IQR)	Former or current smoker %	White ethnicity %	Age (years)	Sex – male (%)	Baseline FVC, L	Baseline FVC % predicted	Baseline DL_{CO} % predicted	Baseline 6MWD
ARTEMIS	164	8 (5-13)	67.5	89	66.1 (7.1)	68.1	-	69.9 (13.8) *	45.6 (13.3)	421 (21)
ASCEND	277	12 (11-12)	61	90.6	67.8 (7.3)	76.9	2.67 (0.65)	70.8 (11.2)	44.2 (12.5)	421 (98)
BUILD-1	83	12 (12-12)	-	-	70.2 (9.2)	75	2.72 (0.72)	73.9 (13.6)	49.7 (11.3)	365 (79)
BUILD-3	209	21 (15-24)	67.9	-	63.2 (9.1)	63.6	2.88 (0.82)	73.1 (15.3) *	47.9 (12.7)	-
CAPACITY1	174	17 (17-20)	70.7	96.6	66.3 (7.5)	73.6	2.91 (0.78)	78 (15.5)	46.1 (10.2)	410 (90)
CAPACITY2	173	18 (17-22)	63	98.8	67 (7.8)	71.7	2.86 (0.68)	75.3 (14.2)	47.4 (9.2)	400 (90)
IFIGENIA	75	12 (11-13)	69	100	64.4 (8.6)	74.7	2.36 (0.73)	62.8 (14.2)	44.2 (15.9)	-
INPULSIS1	205	13 (13-13)	75.1	80	66.8 (8.2)	80	2.84 (0.82)	75 (16.2)	47.3 (11.9)	-
INPULSIS2	221	13 (13-13)	67.4	56.2	67 (7.5)	78.3	2.63 (0.8)	72.8 (17)	46.6 (15.4)	-
MUSIC	65	13 (11-17)	60	96.9	63.6 (6.1)	61.5	2.79 (0.82)	74.8 (14.6) *	45.6 (11.2)	-
TIPAC	86	12 (12-12)	76.7	98.8	70.7 (8.6)	65	2.4 (0.75)	71.5 (21)	39.1 (12.8)	331 (118)
TOMORROW	87	19 (14-23)	66.7	77	64.8 (8.5)	73.6	2.76 (0.74)	74.6 (15)	48.1 (13.1)	410 (115)

Table 4-2 - Baseline participant characteristics for placebo arms only.

Baseline FVC % predicted values calculated using standardised global lung initiative (GLI) equations unless marked by asterisk (*). Values for physiological variables reported in mean (standard deviation) unless otherwise stated. 6MWD, six-minute walk distance, DL_{CO}, gas transfer for carbon monoxide; FVC, forced vital capacity; MRC, medical research council; -, data not available

All included RCTs were multi-centre studies published between 2005 and 2014, with most studies, phase 3 in design (10/12). In 75% (8/12) of included cohorts, lung function inclusion criteria specified an FVC ≥ 50% predicted, and a DL_{CO} above either 30% or 35% predicted. In 7 cohorts, concomitant steroid use was permitted, though maximal daily dose was restricted to 15-20mg. The median number of participants in the placebo arm of each study was 169 (IQR 85-207), with a median follow up duration of 13 months (IQR 12-17), and median study age of 66.6 years (IQR 64.4-67.8). The majority of study participants across all studies were male (73.9%). The median baseline %predicted FVC was 73.5% predicted (IQR 70.8-74.8), baseline %predicted DL_{CO} was 46.4% predicted (IQR 44.2-47.4), and 6MWD was 410m (IQR 365-421) (Table 4-2). IPD could not be attained from 13 studies reporting outcomes for 1139 participants. A comparison of study and participant characteristics with included studies reveal no obvious differences (Table 4-3).

Study and year	Reason for exclusion	Study phase	Centre	Placebo sample size	Follow up, months (median)	White ethnicity %	Age (years)	Sex – male (%)	Baseline FVC % predicted	Baseline DL_{CO} % predicted	Baseline 6MWD
Azuma et al, 2005[392]	Data sharing not permitted by original ethics	Phase 2	multi-centre	36	9	-	64.3 (7.6)	94	78.4 (17.2)	57.7 (13.8)	-
Daniels et al, 2010[393]	Data sharing not permitted by original consent	Phase 2	multi-centre	61	22	-	67.8 (52-79) [a]	64	65.6 [b]	39.3 [b]	379
Homma et al, 2012[394]	Data sharing not permitted by original ethics	Phase 3	multi-centre	46	12	-	68.2 (7.7)	76	88.7 (15.5)	64.4 (20.1)	-
King et al, 2009 (INSPIRE)[395]	FDA restrictions on data sharing	Phase 3	multi-centre	275	18	-	65.9 (7.9)	68	73.1 (13.4)	47.3 (9.3)	392.8 (112.9)
Malouf et al, 2011[396]	Data sharing not permitted by original ethics	Phase 2	multi-centre	45	36	-	60 (9)	71	69 (20)	42 (14)	451 (118)
Martinez et al, 2014 (PANTHER)[397]	Data sharing not permitted by original ethics	Phase 3	multi-centre	131	14	96	67.2 (8.2)	75	73.4 (14.3)	46 (12.2)	375 (105)
Noth et al 2012 (ACE-IPF)[398]	Data sharing not permitted by original ethics	Phase 3	multi-centre	73	12	93	66.7 (7.4)	79	58.7 (16.1)	34.6 (13.4)	280.2 (136.2)
Palmer et al, 2018[399]	Offices closed due to COVID-19	Phase 2	multi-centre	47	6	64	69 (49-85) [a]	70	69 (48-96) [a]	45 (12-7'3)[a]	-
Parker et al, 2018[400]	Data not yet submitted to regulatory authorities	Phase 2	multi-centre	59	16	75	67.5 (6.1)	79	70.3 (12.0)	47 (13.8)	391 (112)
Raghu et al, 2004[401]	FDA restrictions on data sharing	Phase 3	multi-centre	168	13	86	63.4 (8.6)	66	64.1 (11.3)	36.8 (10.6)	-
Raghu et al, 2008[402]	Denied by sponsor	Phase 2	multi-centre	41	12	-	65.1 (7.1)	59	63.0 (12.7)	36.9 (10.8)	396.8 (136.8)
Richeldi et al, 2020 (PRAISE)[403]	No response from study personnel	Phase 2	multi-centre	53	12	-	68.4 (7.2)	81	73.1 (11.1)	53.8 (12.2)	-
Taniguchi et al, 2009[404]	Data sharing not permitted by original ethics	Phase 3	multi-centre	104	12	-	64.7 (7.3)	78	79.1 (17.4)	55.2 (18.2)	-

Table 4-3 - Baseline participant characteristics from studies where IPD could not be retrieved

Values for physiological variables reported in mean (standard deviation) unless otherwise stated. [a] = median (range); b = mean only; -, data not available

4.3.1 Risk of bias

Risk of bias assessment identified a low risk of bias for most of the assessed domains (Figure 4-2). Clear and consistent participant criteria were utilised by all studies, and in all studies, except one, study attrition rates were low, with reasons for participant drop out stated. Relevant confounders including age, gender and smoking status were measured in all studies, with data available for most participants. Missing participant outcome data resulted in mortality and disease progression being unable to be defined in 0.05% and 12.9% of all participants respectively. Measurement details for physiological variables and FVC reference equations were unavailable in most studies.

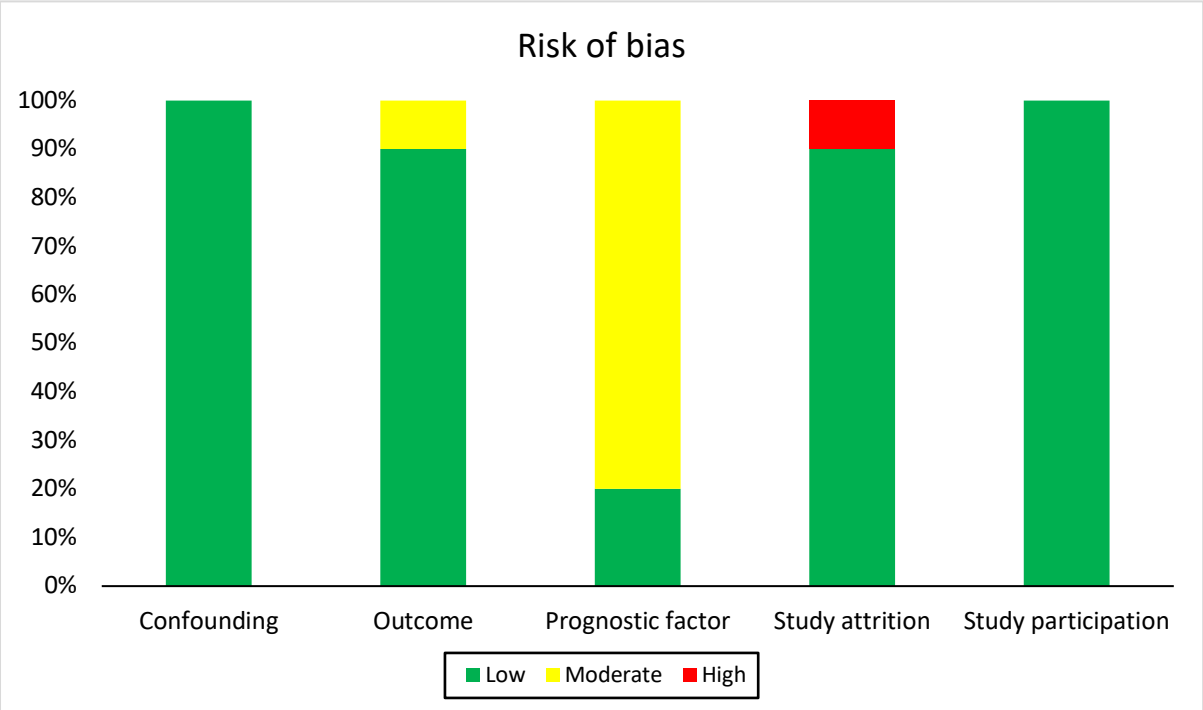


Figure 4-2 -Risk of bias assessment.

The risk of bias across studies was rated as low, moderate, or high risk in five categories using the modified QUIPs tool.

4.3.2 Demographic factors

Baseline characteristics including age, gender and smoking status were available from all placebo arms. Age was associated with increased mortality [adjusted HR (aHR) 1.04 per year increase, 95%CI 1.02;1.06, I^2 = 0.0%], but male gender (aHR 1.16, 95%CI 0.78;1.72, I^2=0.0%) and previous smoking history (aHR 1.34, 95%CI 0.77;2.33, I^2=54.3%) were not (Figure 4-3).

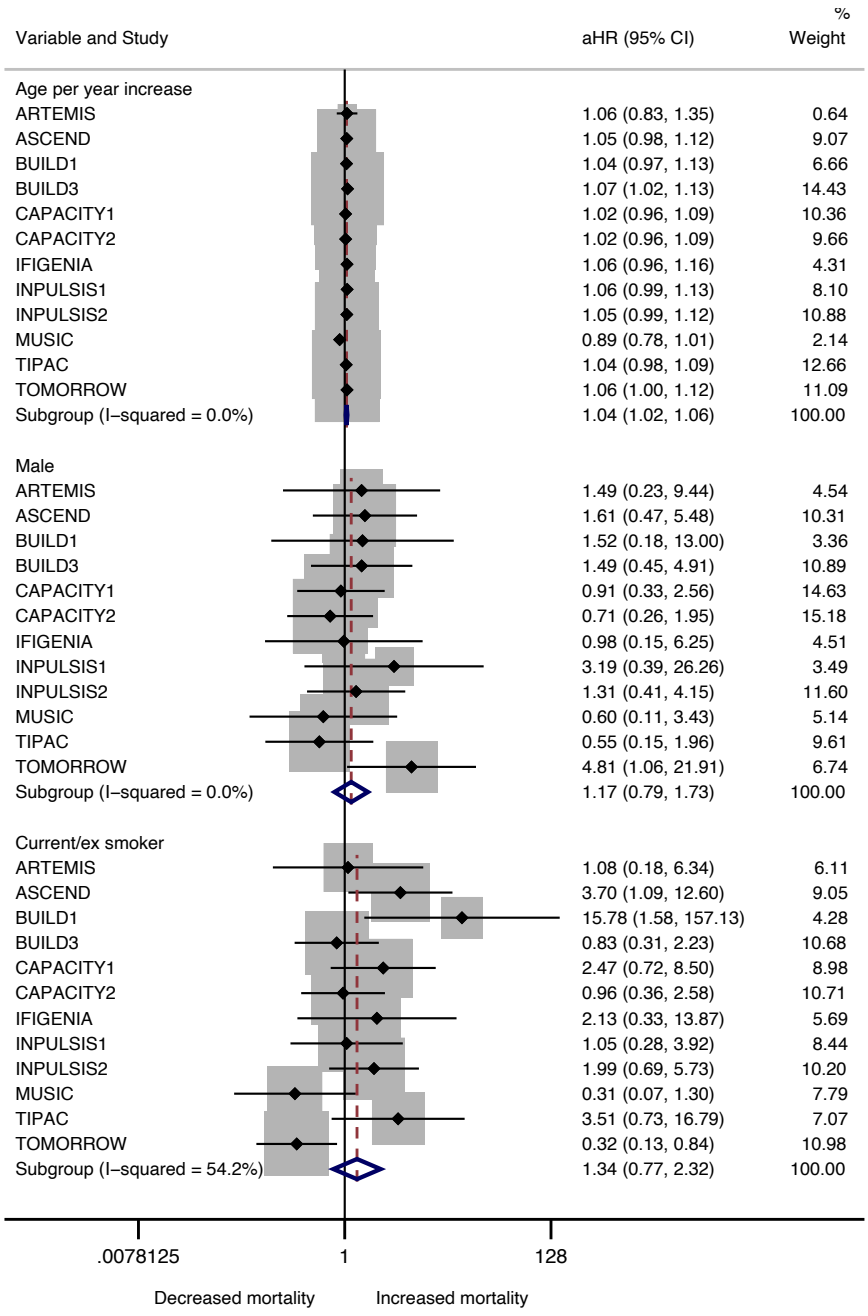


Figure 4-3 - Association of baseline demographic factors with mortality, presented using adjusted hazard ratios. All estimates adjusted for age, sex, smoking status, and baseline FVC.

None of the demographic factors including age (aOR 1.00, 95%CI 0.99;1.02, I^2=4.4%), male gender (aOR 0.93, 95%CI 0.72;1.2, I^2=0.0%) or previous smoking history (aOR 0.97, 95%CI 0.76;123, I^2=0.0%) were associated with increased disease progression (Figure 4-4).

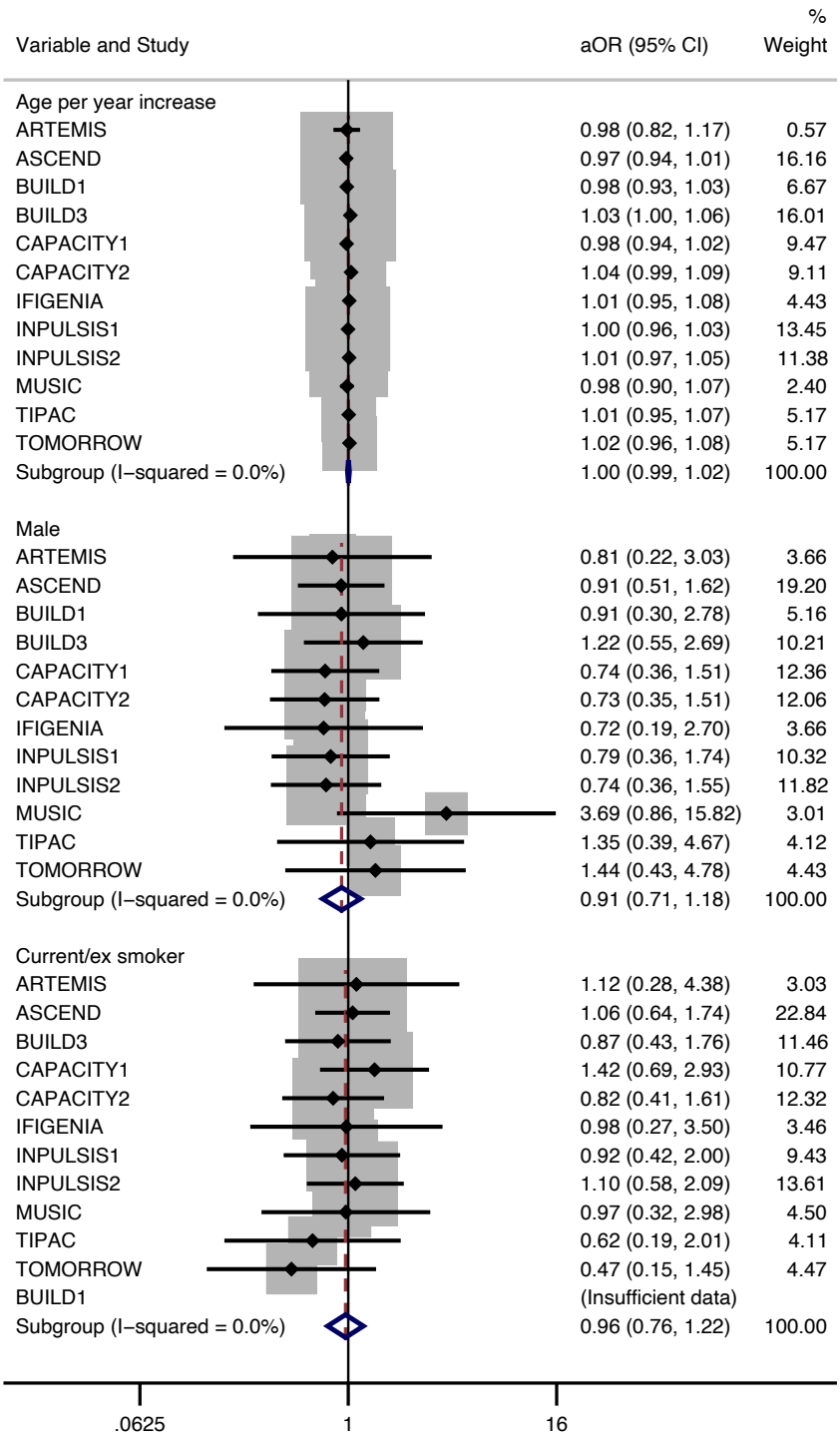


Figure 4-4 - Association of baseline demographic factors with disease progression presented using adjusted odds ratios

All estimates adjusted for age, sex, smoking status and baseline FVC.

4.3.3 Forced Vital Capacity (FVC) – Placebo arms

Baseline FVC measurements were available from all included placebo cohorts. Meta-analysis demonstrated, for every 5% decrement in baseline predicted FVC, there was a 24% increased risk of overall mortality (aHR 1.24, 95%CI 1.17;1.32, I^2=0.0%, 1764 participants) and 12% increased likelihood of disease progression (aOR 1.12, 95%CI 1.07;1.16, I^2=0.0%, 1526 participants) (Figure 4-5).

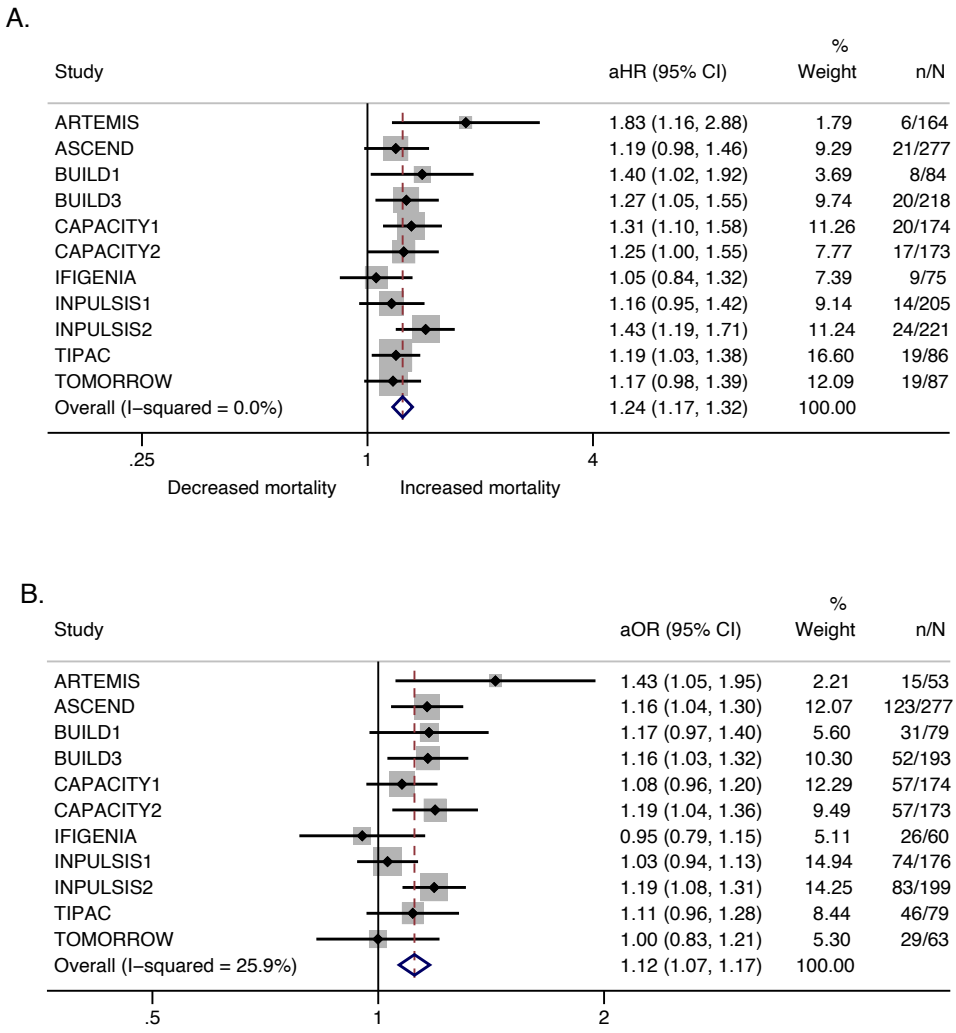


Figure 4-5 – Forest plot for association of outcomes with baseline FVC per 5% decrement

Number of individuals who died (n) alongside total individuals included (N) in the study. All estimates were adjusted for age, sex, and smoking status. A. Overall Mortality presented using adjusted hazard ratios. B. Disease progression presented using adjusted odds ratios.

In all participants, the mean absolute decline in %predicted FVC from baseline to twelve months was 4.86% (95%CI -4.14;5.59, I^2=68%). Mean FVC change was stratified by baseline FVC, and in those with a baseline FVC ≥ 80% predicted, 12-month FVC change was -201ml (95%CI -237; -164, I^2=49.9%) compared with -163ml (95%CI -201; -125, I^2=74.4%) in individuals with a baseline FVC below 80% predicted (p=0.627) (Figure 4-6). In participants with lower baseline FVC, 117 participants died before they reached 12 months of follow up and therefore could not be included in the analysis of mean FVC change. In comparison, of those with a baseline FVC greater than 80% predicted, 16 participants died.

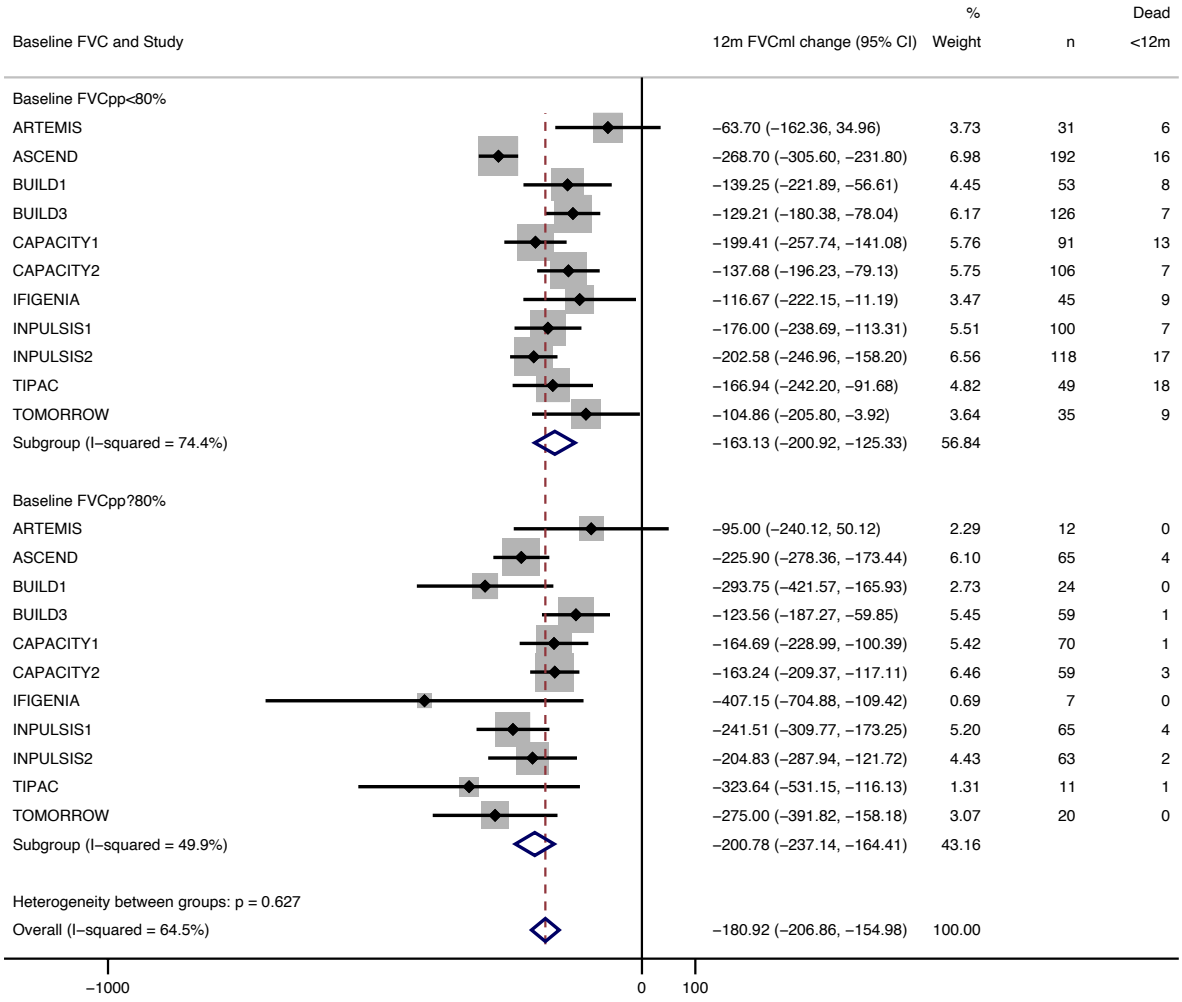


Figure 4-6 - Forest plot for 12m FVC (ml) change stratified by baseline FVC.

Baseline FVC was stratified by a threshold of 80% predicted and absolute FVC (ml) change at 12m was pooled using random-effects meta-analysis and compared across both groups.

Three-month FVC measurements were available from all 12 placebo cohorts. Meta-analysis demonstrated for every 2.5% relative FVC decline over three-months, there was a 15% increased risk of overall mortality (aHR 1.15 per 2.5% relative FVC decline, 95%CI 1.06;1.24, I^2 = 59.4%, 1729 participants) and 30% increased likelihood of disease progression (aOR 1.30 per 2.5% relative FVC decline; 95%CI 1.19;1.41, I^2=66.1%, 1551 participants) (Figure 4-7). Meta-regression was performed, and none of the factors assessed explained the variability in mortality estimates, but concomitant steroid was a source of heterogeneity for disease progression estimates (R^2=31.65%; p=0.036) (Figure 4-25).

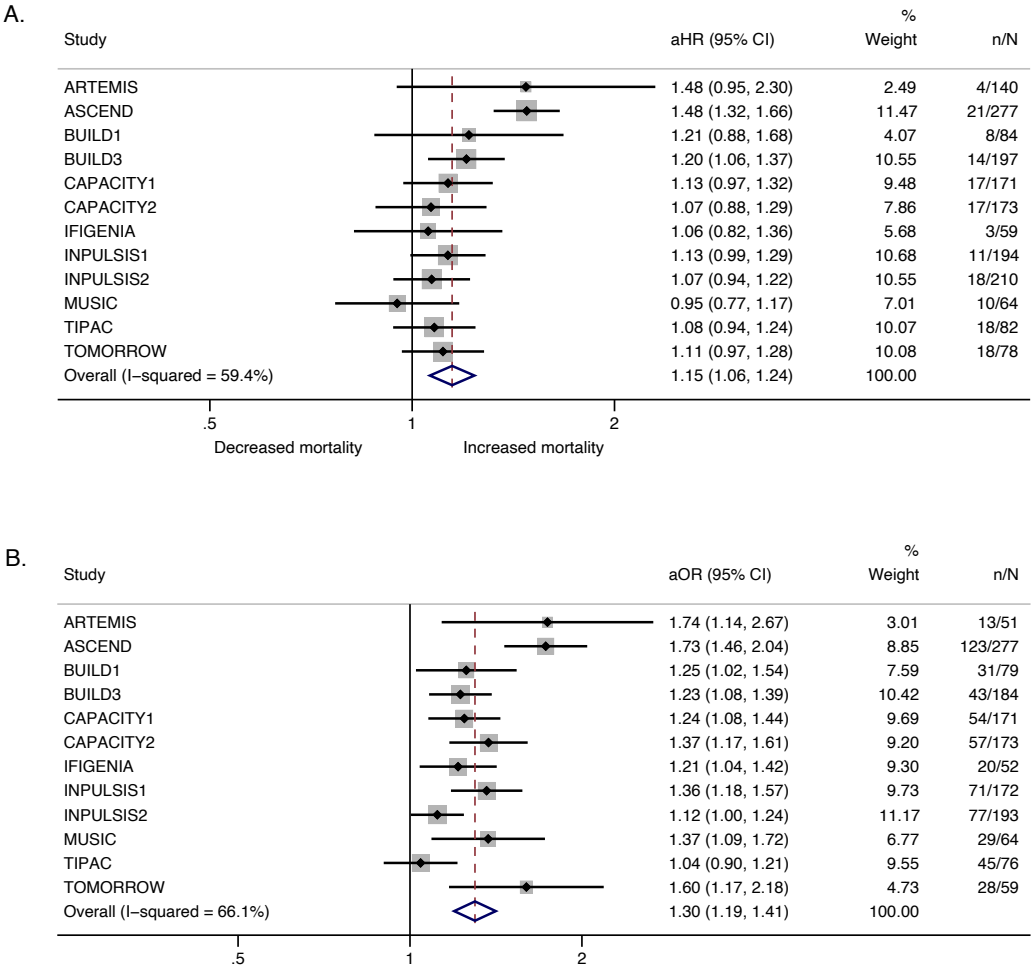


Figure 4-7 - Forest plot of change in FVC (continuous) and outcomes, per 2.5% relative FVC decline over 3 months

Number of individuals who died (n) alongside total individuals included (N) in the study. All estimates were adjusted for age, sex, smoking status, and baseline FVC. A. Overall Mortality. B. Disease progression.

The median relative decline in FVC over three-months across participants in all studies was 2.3%. An FVC relative decline greater than 2.3% was associated with an estimated pooled mean FVC difference of -280ml (95%CI -309; -251, I^2=43.7%) at 12months, compared with an estimated mean difference of -87ml (95%CI -127; -48, $I^{2=}$76.1%) in those participants with a lower three-month decline (p<0.001) (Figure 4-8). A greater proportion of participants with an FVC relative decline > 2.3% over three-months died before their 12-month FVC (9.5% vs 4.9%) and therefore could not be included in the analysis.

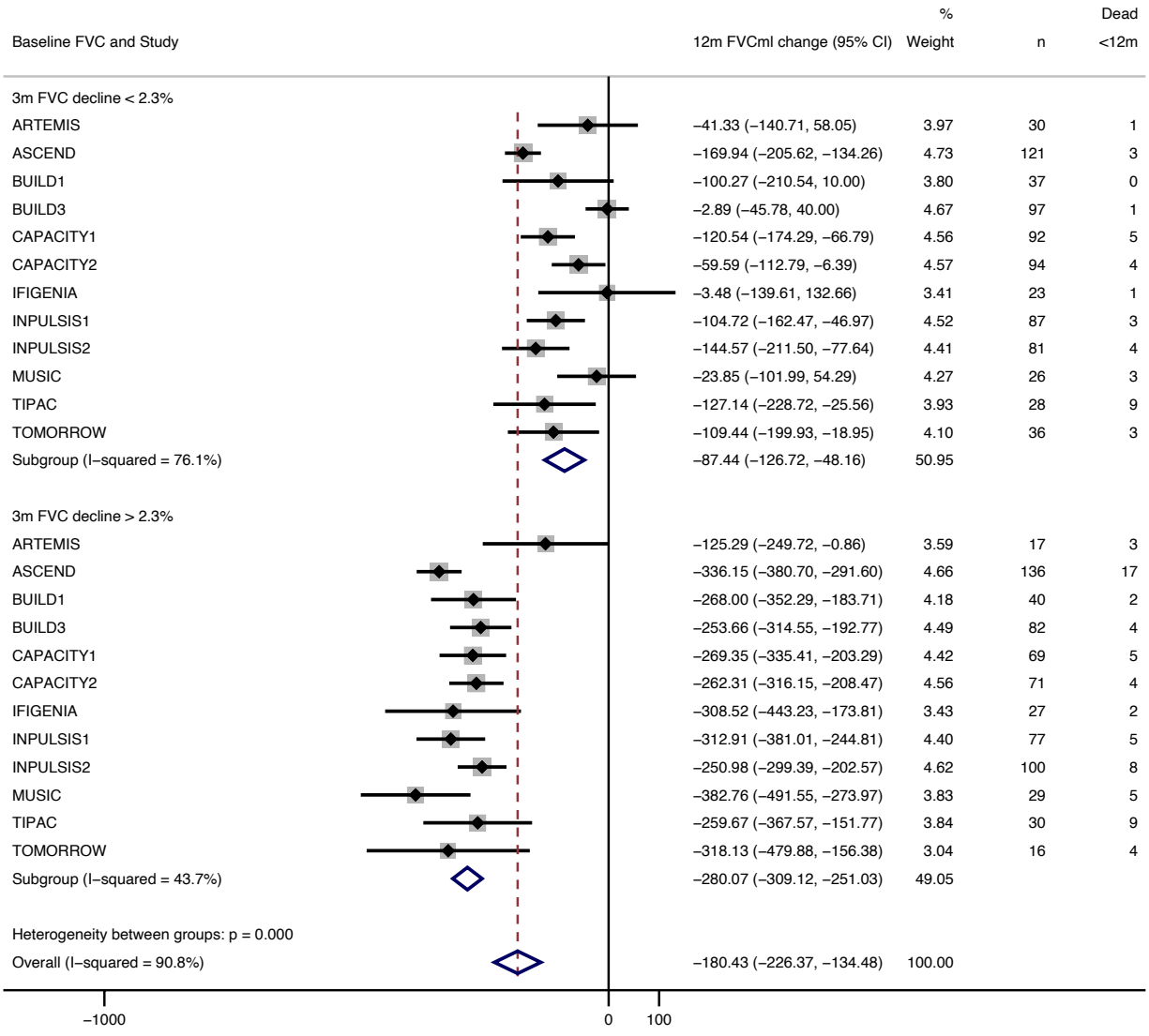


Figure 4-8 - Forest plot for 12m FVC (ml) change stratified by 3m FVC change.

The median FVC change for all participants was calculated (2.3%) and mean FVC (ml) change at 12m was calculated in participants with a greater than 3m threshold change compared with a change below the threshold

Optimal thresholds for three-month FVC relative change in determining death or disease progression were estimated for each study and combined. A threshold of 5.7% (95%CI 4.31;7.04, I^2=0.0%) relative FVC change over three-months had the greatest sensitivity and specificity for predicting overall mortality (Figure 4-9). An FVC relative decline greater than 5.7% over three-months was associated with significantly increased mortality compared with individuals who had an FVC relative decline < 5.7% over three-months (aHR 2.62, 95%CI 1.73;3.96, I^2=25.2%) (Figure 4-10). AUROC for each of the studies for a threshold of 5.7% were estimated and combined for a pooled AUROC of 0.60 (95%CI 0.55;0.64, I^2=21.4%). The pooled AUROC for predicting mortality using an FVC change of 10% at 12 months was 0.69 (95%CI 0.59;0.79, I^2=0.0%) (Figure 4-12).

Optimal thresholds for determining disease progression were estimated at 3% (95%CI 2.10;3.93, I^2=31.3%) (Figure 4-9). An FVC relative change greater than 3% over three-months was associated with a significantly increased likelihood of disease progression compared with an FVC change below 3% (OR 3.64, 95%CI 2.47;5.39, I^2=58.5%) (Figure 4-11). The pooled AUROC for a threshold of 3% for predicting disease progression was estimated as 0.65 (95%CI 0.61;0.70, I^2=65.6%), with studies permitting steroid use a source of heterogeneity (R^2=34.74; p=0.041) (Figure 4-12).

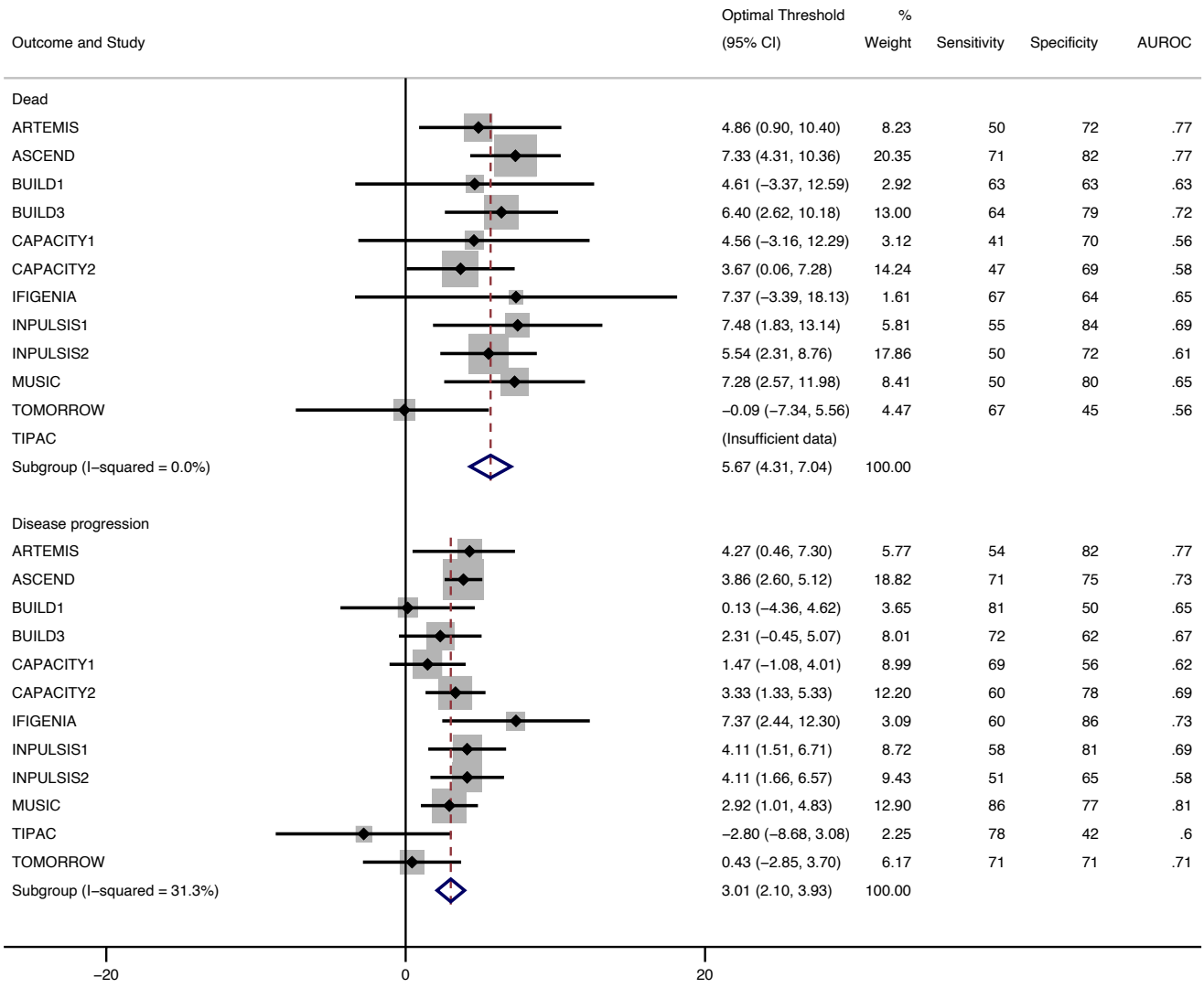


Figure 4-9 - Forest plot of pooled optimal FVC 3-month thresholds for determining death and disease progression.

Optimal thresholds and 95% confidence intervals for 3-month relative FVC decline in predicting death or disease progression were calculated and pooled to create an overall optimal threshold.

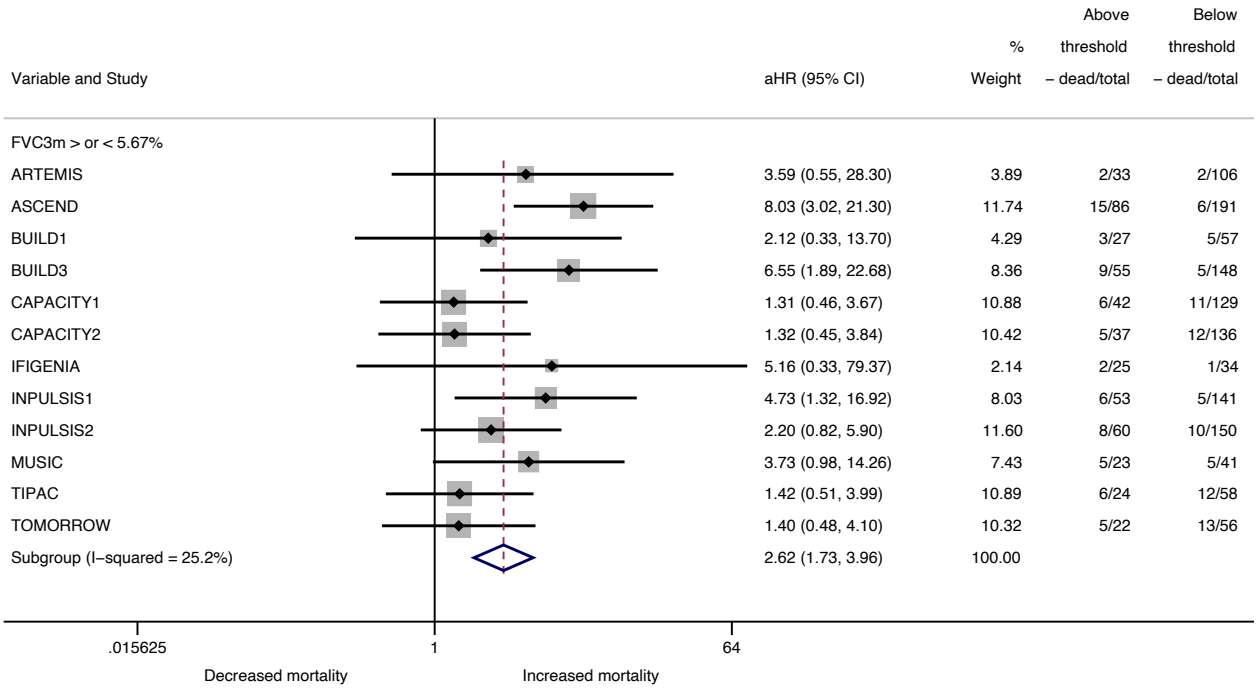


Figure 4-10 - Forest plot for 3-month FVC empirical mortality threshold (5.7%) applied to all studies.

An optimum threshold for 3-month relative FVC change in predicting mortality was calculated and pooled. The pooled threshold (5.7%) was applied to all studies to estimate the risk of overall mortality in individuals with an FVC decline greater than 5.7% predicted over three-months compared with individuals who had an FVC decline less than 5.7% predicted.

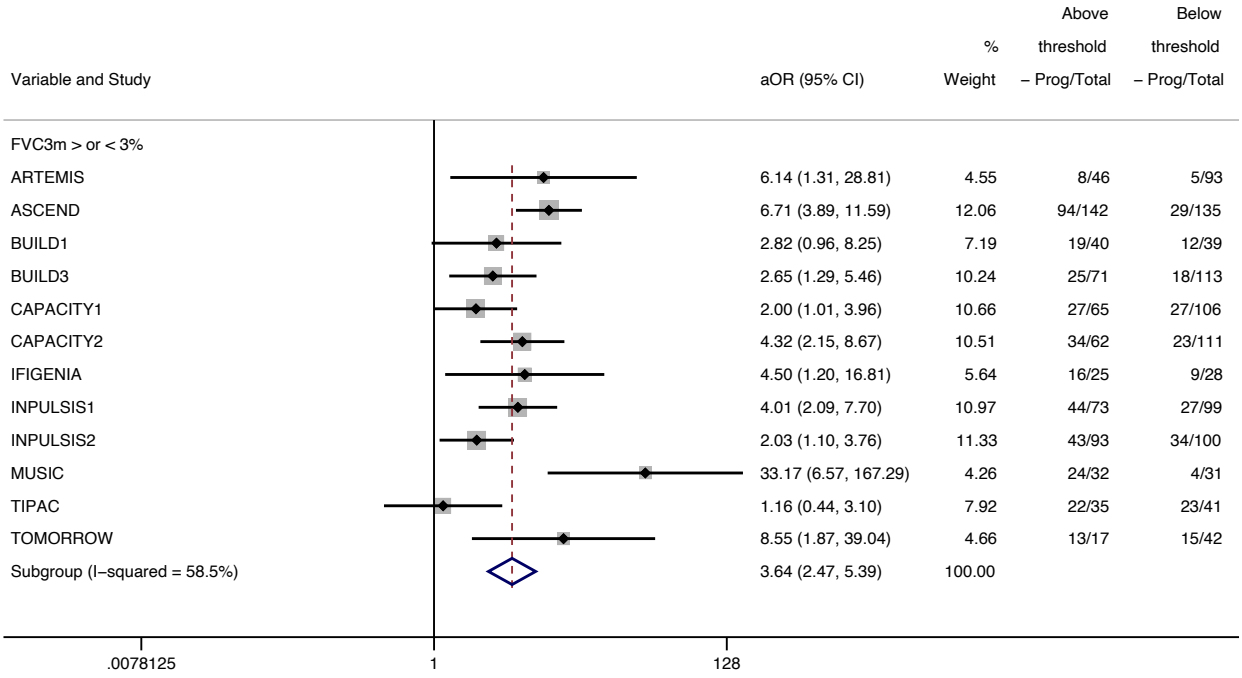


Figure 4-11 - Forest plot for 3-month FVC empirical disease progression threshold (3%) applied to all studies.

An optimum threshold for 3-month relative FVC change in predicting disease progression was calculated and pooled. The pooled threshold (3%) was applied to all studies to estimate the likelihood of disease progression with an FVC decline greater than 3% predicted over three-months compared with individuals who had an FVC decline less than 3% predicted.

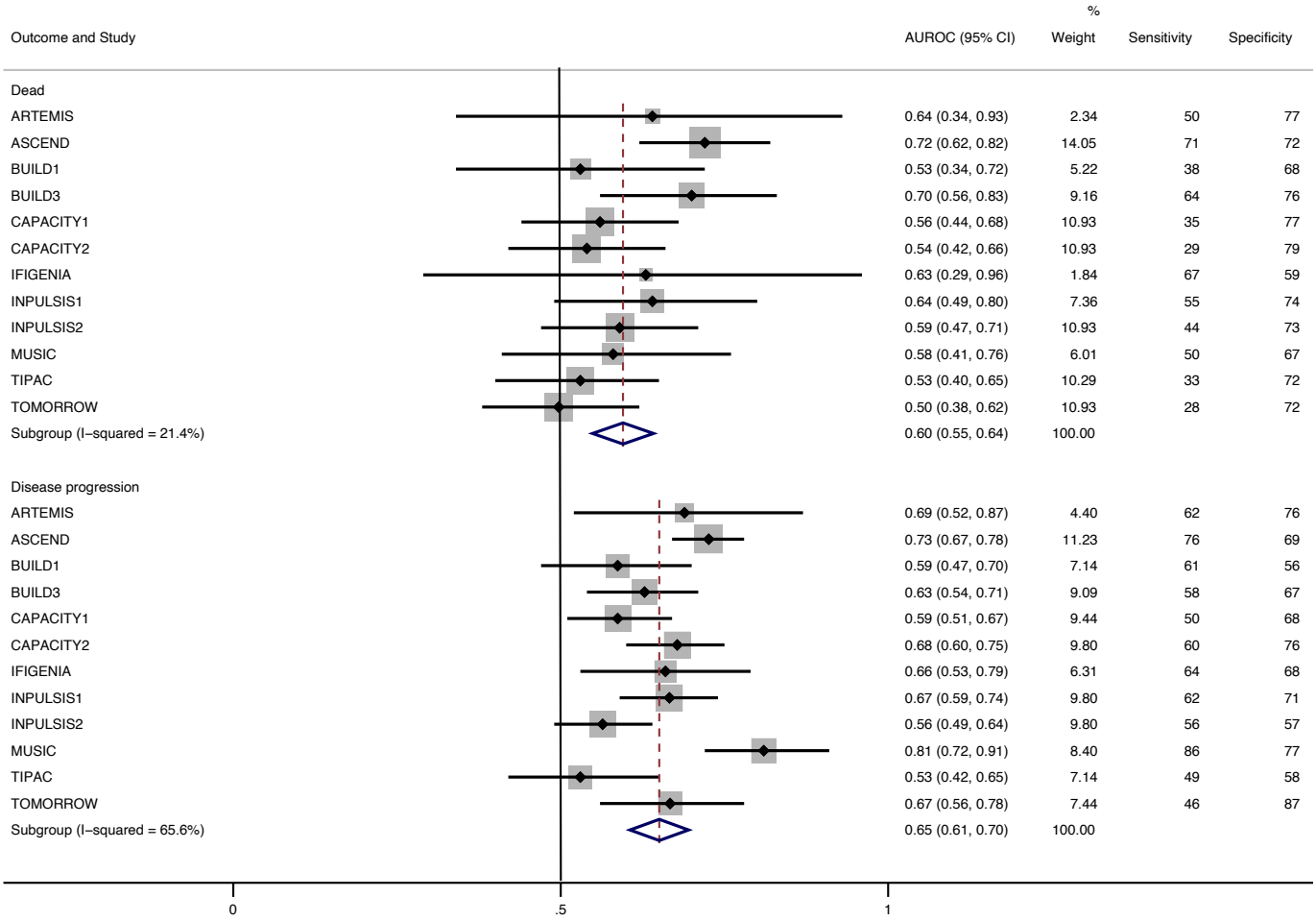


Figure 4-12 - Forest plot of AUROC for overall optimal FVC threshold (5.7% for mortality and 3% for disease progression).

The overall optimal threshold was applied to each study to calculate the AUROC, sensitivity and specificity for predicting outcomes.

4.3.4 FVC in treatment arms

To explore whether short-term FVC change was associated with disease outcomes in individuals receiving anti-fibrotics, I sought treatment arm IPD from 1684 individuals in all six studies evaluating the use of pirfenidone and nintedanib (ASCEND, CAPACITY1, CAPACITY2, INPULSIS1, INPULSIS2, TOMORROW). Baseline characteristics were well matched with corresponding placebo arms (Table 4-4).

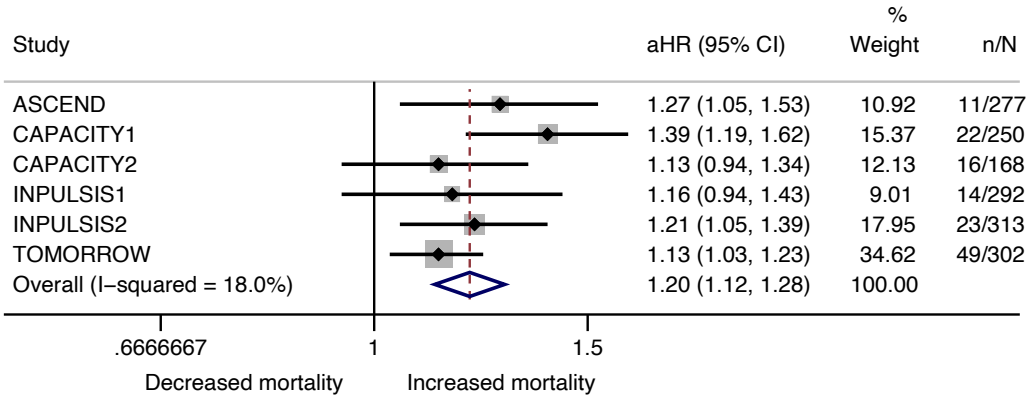
Study	Sample size	Study follow up,	Smoking history %	White, %	Age (years)	Sex – male (%)	Baseline FVC, L	Baseline FVC % predicted	Baseline DL_{CO} % predicted	Baseline 6MWD
ASCEND	278	12 (11-12)	66.2	91.8	68.4 (6.7)	79.9	2.64 (0.66)	69.9 (11.5)	43.7 (10.5)	415 (98)
CAPACITY1	251	18 (17-20)	69	96	66.6 (8.1)	70.1	2.88 (0.75)	76.9 (14.8)	46.7 (9.1)	412 (99)
CAPACITY2	170	18 (7-22)	65.3	98.8	66.8 (7.9)	71.8	2.93 (0.76)	76.9 (13.6)	47.8 (9.8)	378 (82)
INPULSIS1	309	13 (13-13)	77	75	66.9 (8.4)	81.2	2.76 (0.74)	73.9 (15.4)	47.8 (12.3)	-
INPULSIS2	332	13 (13-13)	68.7	55.6	66.4 (7.9)	77.7	2.67 (0.77)	74.3 (16.9)	47.0 (14.5)	-
TOMORROW	344	20 (16-24)	67.2	79.7	65.2 (8.6)	75	2.79 (0.77)	75.5 (15.8)	47.3 (12.5)	415 (110)

Table 4-4 - Baseline participant characteristics for included treatment arms

Baseline FVC % predicted values calculated using standardised global lung initiative (GLI) equations. Values for physiological variables reported in mean (standard deviation) unless otherwise stated. 6MWD, six-minute walk distance, DL_{CO}, gas transfer for carbon monoxide; FVC, forced vital capacity; MRC, medical research council; -, data not available

In treatment arms alone there was a 20% increased risk of overall mortality per 2.5% relative FVC decline over three-months (aHR 1.20 per 2.5% relative FVC decline, 95%CI 1.12;1.28, I^2=18.0%, 6 cohorts, 1602 participants, high certainty), and 46% increased likelihood of disease progression (aOR 1.46 per 2.5% relative FVC decline, 95%CI 1.36;1.57, I^2=34.7%, 1455 participants, high certainty) (Figure 4-13). This was comparable to estimates observed in the placebo arm alone suggesting FVC change over three-months predicts disease outcomes irrespective of treatment.

A.



B.

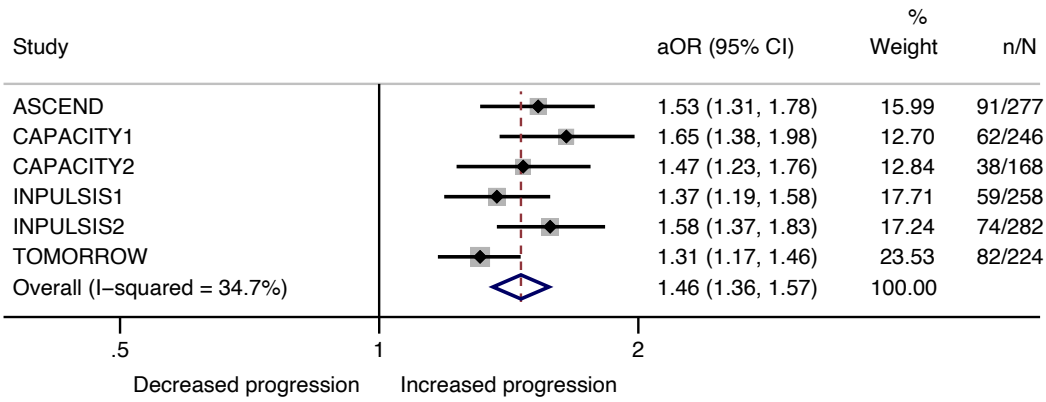


Figure 4-13 - Forest plot of change in FVC (continuous) and outcomes in treatment arms only, per 2.5% relative FVC decline over 3 months

***A**: Adjusted hazard ratios (aHR) for overall mortality with 95% confidence intervals shown per 2.5% decline in FVC over 3 months. Number of patients who died (n) alongside total patients included (N) in the study. **B**: Adjusted odds ratios (aOR) for disease progression with 95% confidence intervals shown per 2.5% decline in FVC over 3 months. Number of progressors (n) alongside total patients included (N) in the study. All estimates were adjusted for baseline values, age, sex, and smoking status.*

To assess whether a treatment effect could be observed at three-months, the pooled change in FVC over three-months was compared between treatment and placebo arms (Figure 4-14). A greater FVC change in 1103 placebo treated individuals compared with 1434 treated individuals (-68.59ml vs. -27.66ml; coefficient 42.9ml; 95%CI 24.0;61.8, p<0.001) was found. Similar differences were observed when FVC was considered using relative percent change (-2.55% vs. -0.99%; p<0.0001).

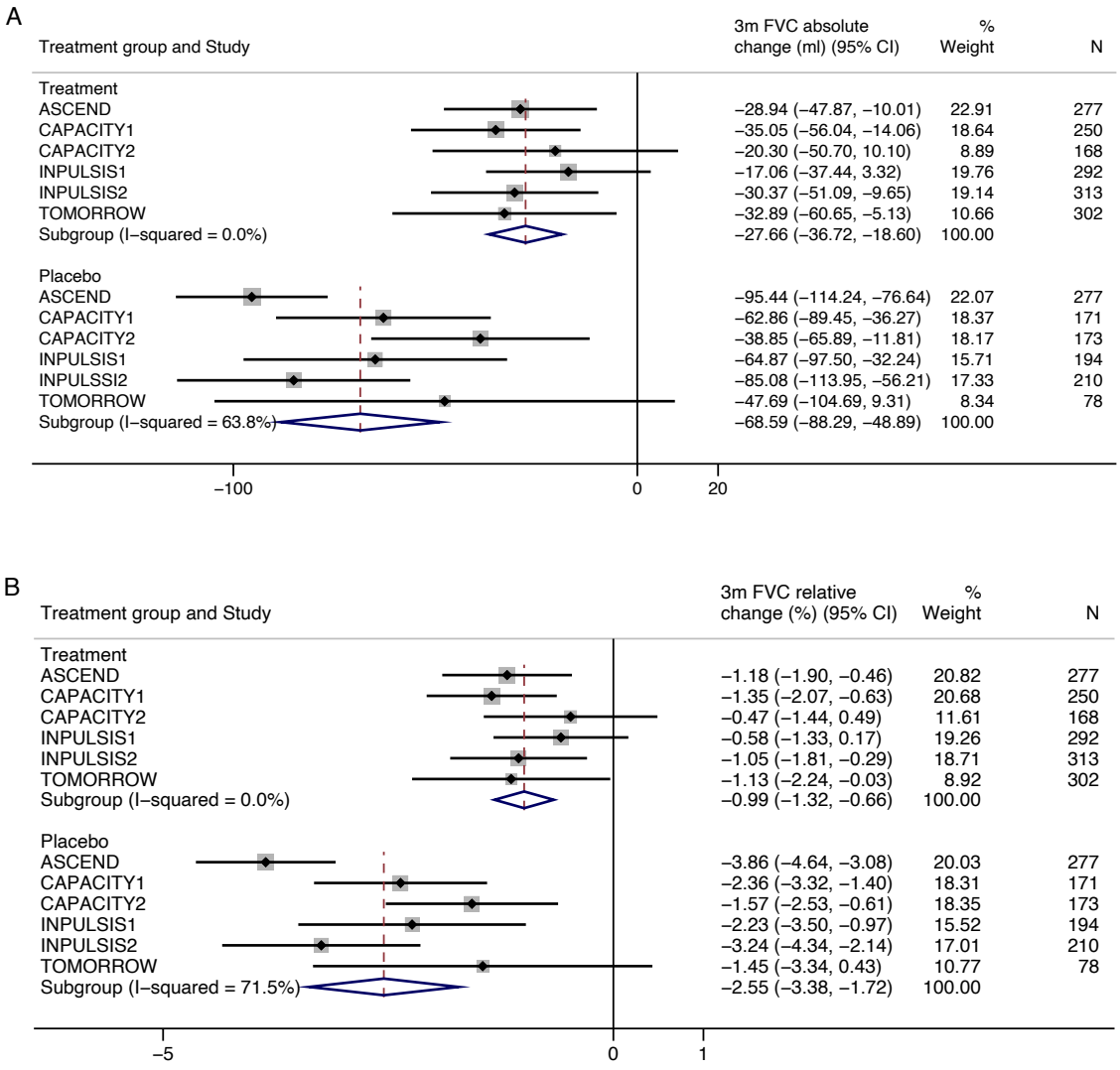


Figure 4-14 - Forest plot of pooled 3m FVC change stratified by placebo and treatment

A: Pooled 3m absolute FVC change (ml) with 95% confidence intervals for placebo and treatment arms. N, total patients included in analysis. B: Pooled 3m relative FVC change (percent) with 95% confidence intervals for placebo and treatment arms. N, total patients included in analysis

Since studies are typically powered on 12-month FVC change, the difference between treatment and placebo arms were compared at 12-months to enable power calculations and sample size estimates for future shortened studies. At 12-months an FVC change of -196.5ml (95%CI -233.1; -159.9) was observed in the placebo arm compared with -113.3ml (95%CI -136.5; -90.2) in individuals who received treatment ($p<0.0001$) (Figure 4-15).

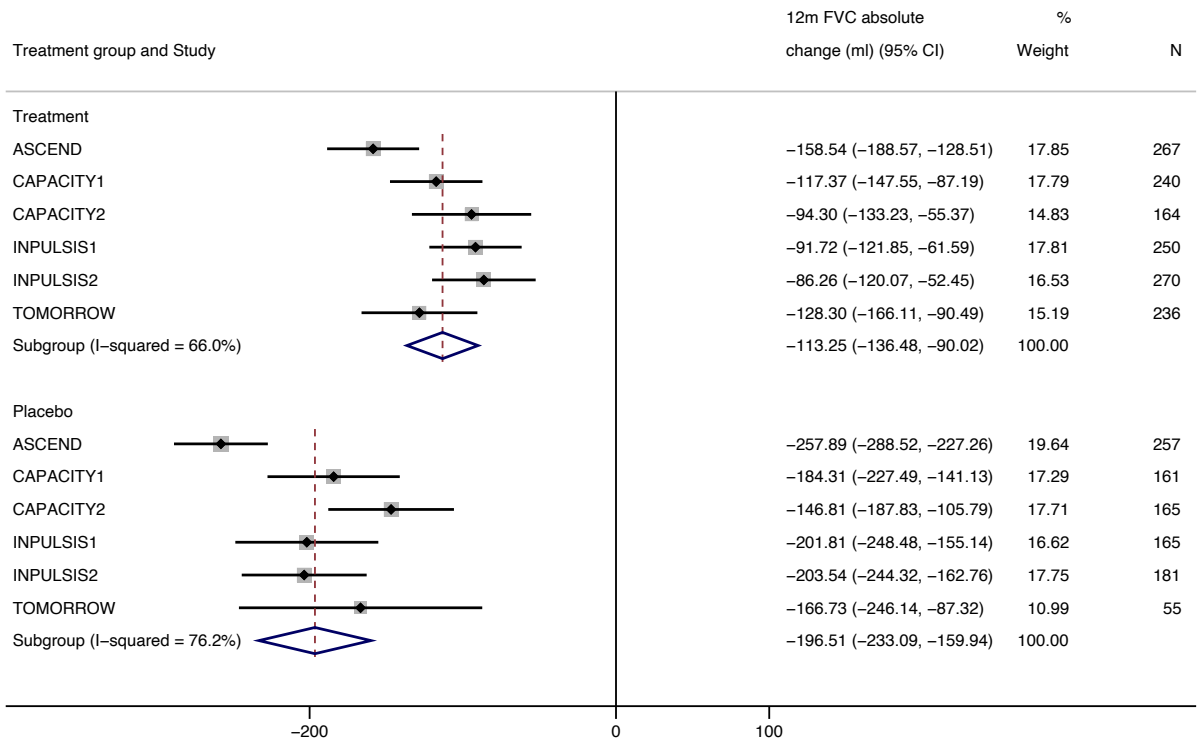


Figure 4-15 - Forest plot for 12-month FVC change by treatment and placebo arms.

Pooled 12m absolute FVC change (ml) with 95% confidence intervals for placebo and treatment arms. N, total patients included in analysis

The difference in FVC change between treatment and placebo arms estimated a weighted standardised difference of 0.22 at three-months and 0.328 at twelve-months, suggesting future trials would require a total sample size of 872 if FVC change at three-months was the endpoint, compared with a sample size of 394 if FVC was measured at twelve-months, assuming 90% power, alpha 0.05, and equal allocation. When studies with modest effect sizes at both three and twelve-months were excluded (TOMORROW and CAPACITY2), an overall standardised difference of 0.273 at three-months and 0.373 at twelve-months was estimated suggesting total sample sizes of 566 and 306 respectively.

4.3.5 Gas transfer for carbon monoxide (DL_{CO})

Baseline DL_{CO} measurement from eleven cohorts demonstrated for every 5% decrement in %predicted DLCO, there was a 24% increased risk of mortality (aHR 1.24, 95%CI 1.14;1.34, I^2=0.0%, 1734 participants) and an 8% increased likelihood of disease progression (aOR 1.08, 95%CI 1.03;1.14, I^2=0.0%, 1512 participants) (Figure 4-16).

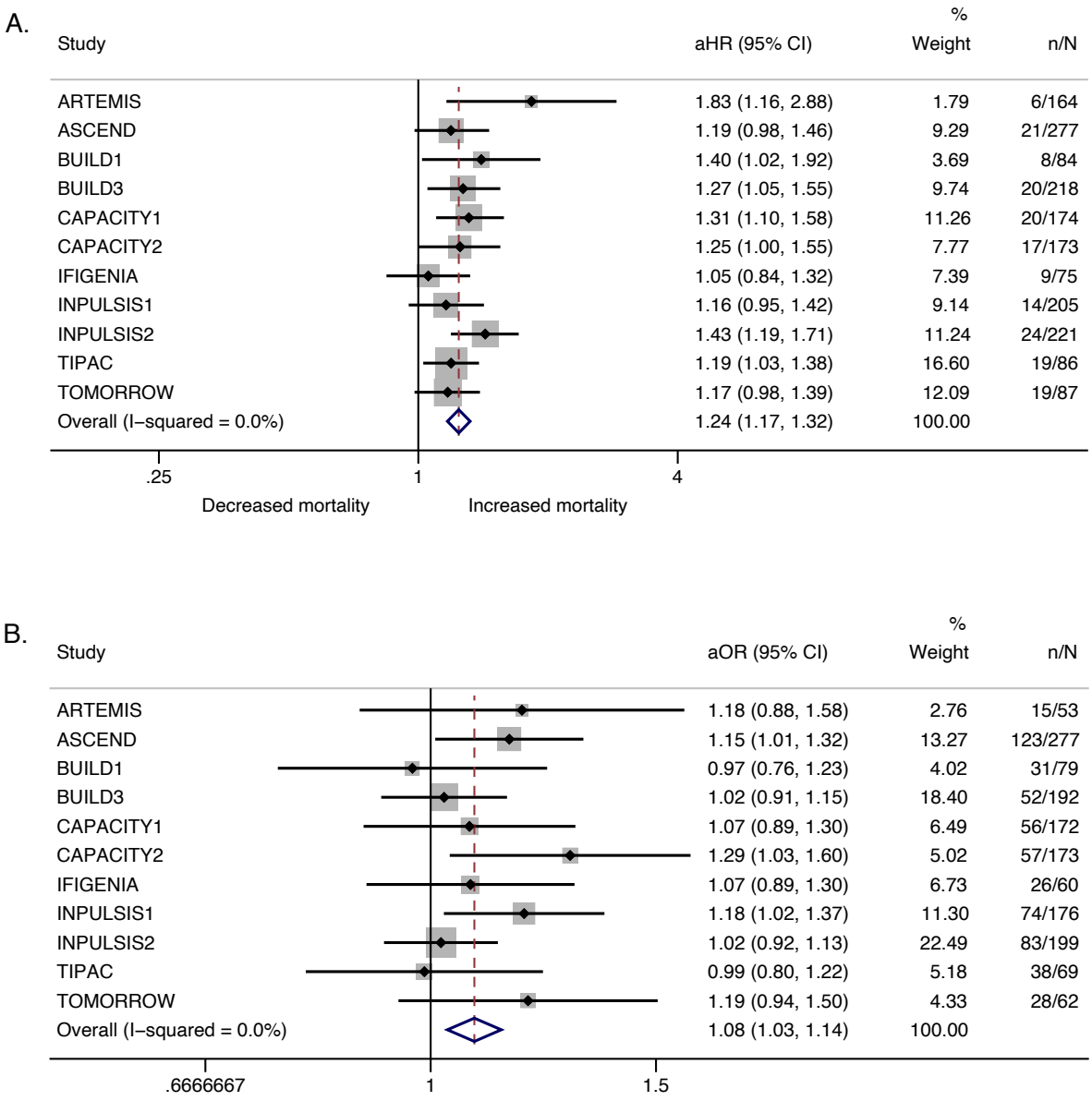


Figure 4-16 - Forest plot for association of outcomes with baseline DL_{CO} per 5% decrement

Number of individuals who died (n) alongside total individuals included (N) in the study. All estimates were adjusted for age, sex, smoking status, and baseline FVC. A. Overall Mortality. B. Disease progression.

Three-month DL_{CO} measurements were available from six cohorts. Meta-analysis estimated for every 2.5% relative decline in DL_{CO}, there was a 7% increased risk of death mortality (aHR 1.07, 95%CI 1.04;1.11, I^2=0.0%, 736 participants) (Figure 4-17). A similar likelihood of disease progression was estimated (aOR 1.08; 95%CI 1.02;1.15, I^2=79.2%, 651 participants), though there was substantial heterogeneity which was not attributable to the factors assessed.

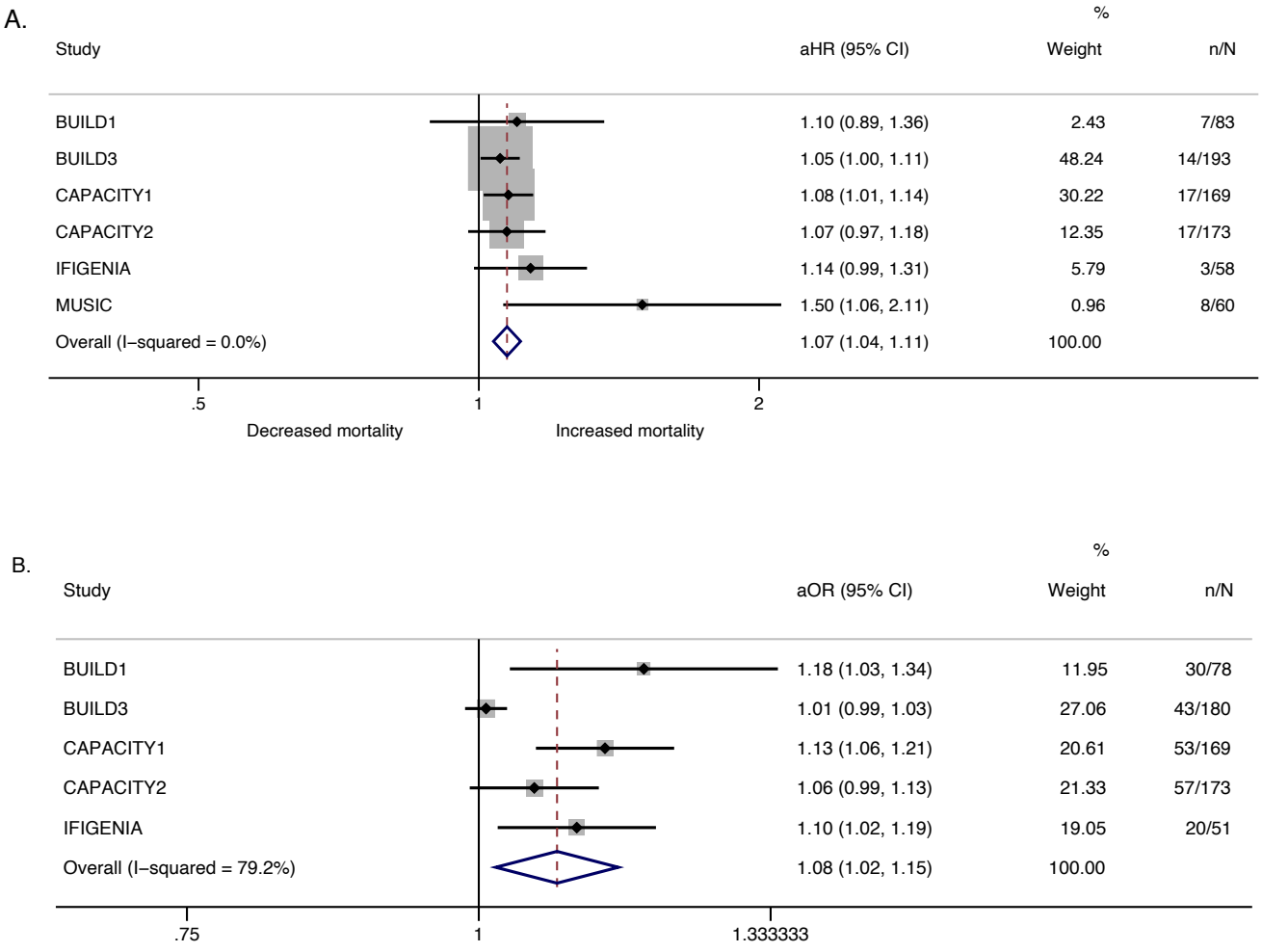


Figure 4-17 - Forest plot of change in DL_{CO} (continuous) and outcomes, per 2.5% relative DL_{CO} decline over 3 months

Number of individuals who died (n) alongside total individuals included (N) in the study. All estimates were adjusted for age, sex, smoking status, baseline DL_{CO} and baseline FVC. A. Overall Mortality. B. Disease progression.

Optimal three-month thresholds were identified to determine mortality and disease progression. A three-month relative decline in DL_{CO} of 10.51% (95%CI 4.14;16.88, I^2=19.9%) for predicting mortality was estimated (Figure 4-18), with a pooled AUROC of 0.64 (95%CI 0.54;0.74, I^2=70.7%) (Figure 4-19). The optimal threshold for predicting disease progression was 7.24% (95%CI 4.63;9.84, I^2=0.0%) with a pooled AUROC of 0.61(95%CI 0.57;0.66, I^2=23.2%)

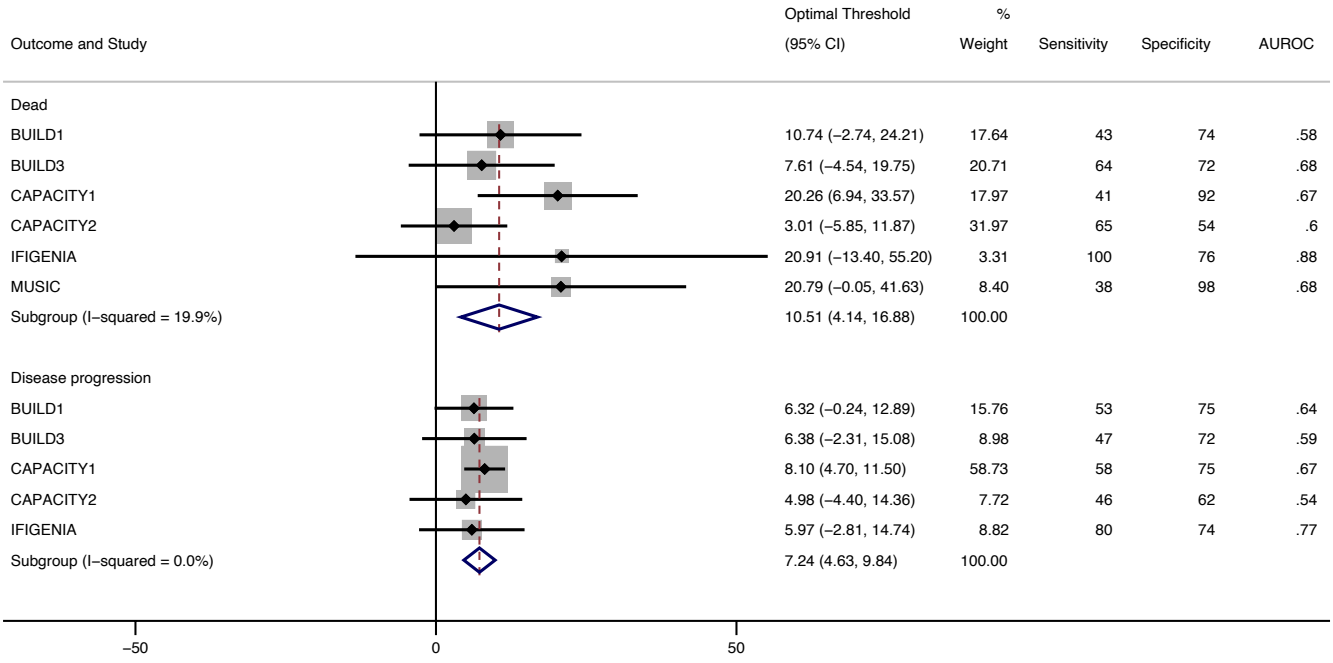


Figure 4-18 - A: Forest plot of pooled optimal thresholds and 95% confidence intervals for 3-month relative DL_{CO} decline in predicting death or disease progression.

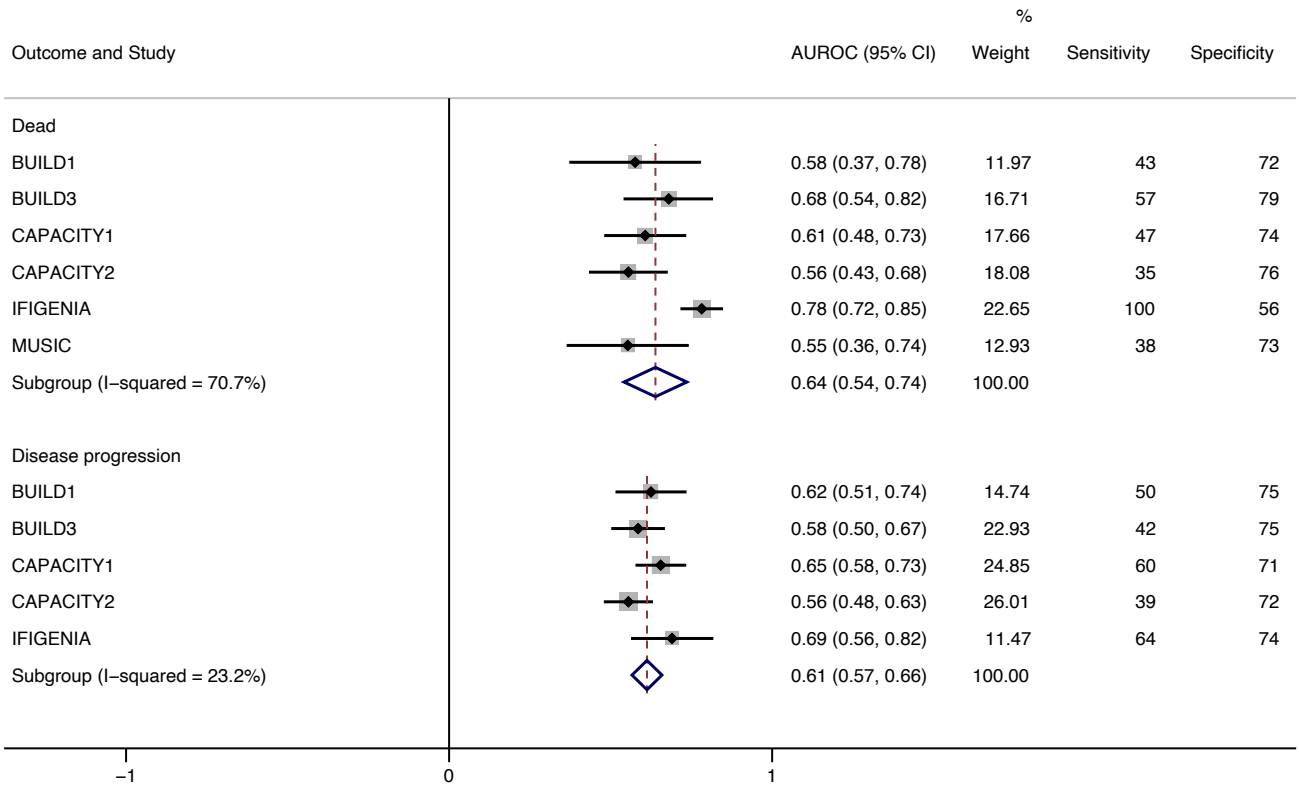


Figure 4-19 - Forest plot of AUROC for overall optimal threshold (10.5% for mortality and 7.2% for disease progression).

The overall optimal threshold was applied to each study to calculate the AUROC, sensitivity and specificity for predicting outcomes.

4.3.6 Six-minute walk distance (6MWD)

Baseline 6MWD measurements and their association with clinical outcomes were available from six cohorts. Meta-analysis estimated a 26% greater risk of mortality (aHR 1.26, 95%CI 1.12;1.42, I^2=0.0%, 828 participants) per 50m decrement in baseline walk distance (Figure 4-20). Estimates for disease progression per 50m decrement were inconclusive (aOR 1.10, 95%CI 0.98;2.24, I^2=40.4%, 718 participants). Study heterogeneity was low, but meta-regression identified studies permitting the inclusion of participants with severe disease (R^2=65.17; p=0.017) and non-incident cases (R^2=65.17; p=0.017) as sources of variability.

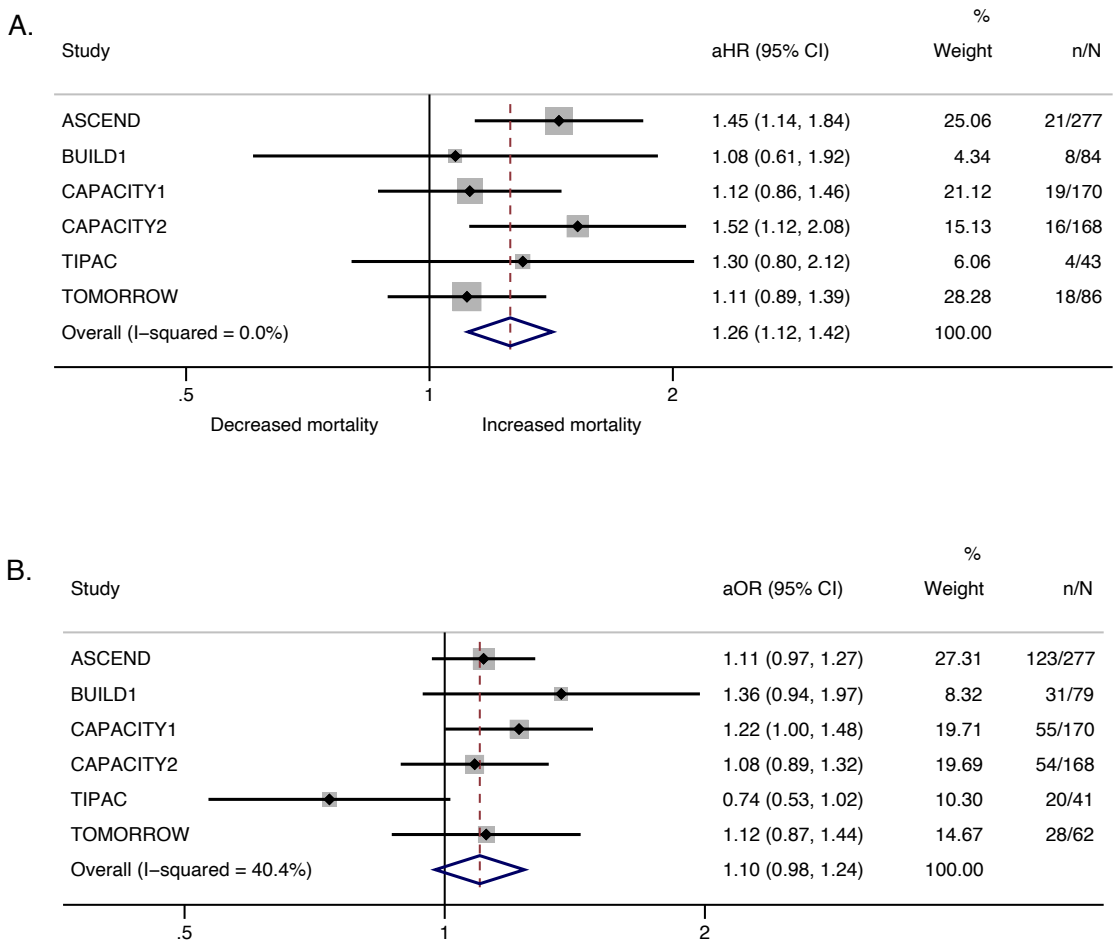


Figure 4-20 - Forest plot for association of outcomes with baseline 6MWD per 50m decrement

Number of individuals who died (n) alongside total individuals included (N) in the study. All estimates were adjusted for age, sex, smoking status, and baseline FVC. A. Overall Mortality. B. Disease progression.

In three-month analyses, 6MWD were available from four cohorts. Longitudinal change, per 20m decline, predicted mortality (aHR 1.09 per 20m decline, 95%CI 1.01;1.17, I^2=0.0%, 696 participants) and disease progression (aOR 1.11 per 20m decline; 95%CI 1.05;1.17, I^2=0.0%, 691 participants) (Figure 4-21). Three-month optimum thresholds for determining clinical outcomes could not be estimated due to insufficient data.

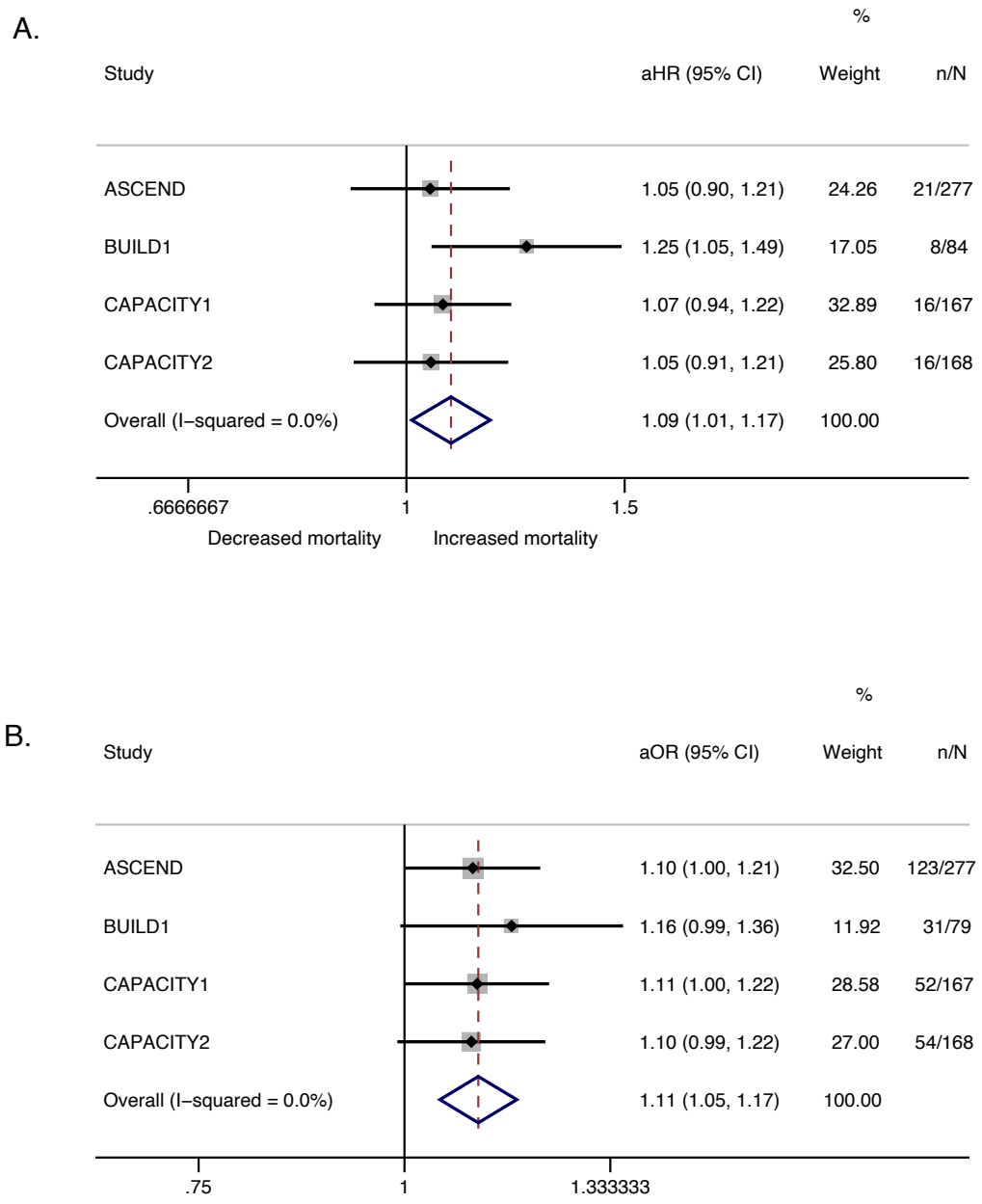


Figure 4-21 - Forest plot of change in 6MWD (continuous) and outcomes, per 20m decline over 3 months

Number of individuals who died (n) alongside total individuals included (N) in the study. All estimates were adjusted for age, sex, smoking status, baseline DL_{CO}, baseline FVC and baseline 6MWD. A. Overall Mortality. B. Disease progression.

4.3.7 Publication bias

Publication bias was assessed per outcome using funnel plots and Egger's test where at least ten cohorts were included in the analysis. Baseline FVC analyses were associated with

publication bias for estimates of overall mortality, and three-month FVC change was associated with publication in estimates of disease progression (Figure 4-22).

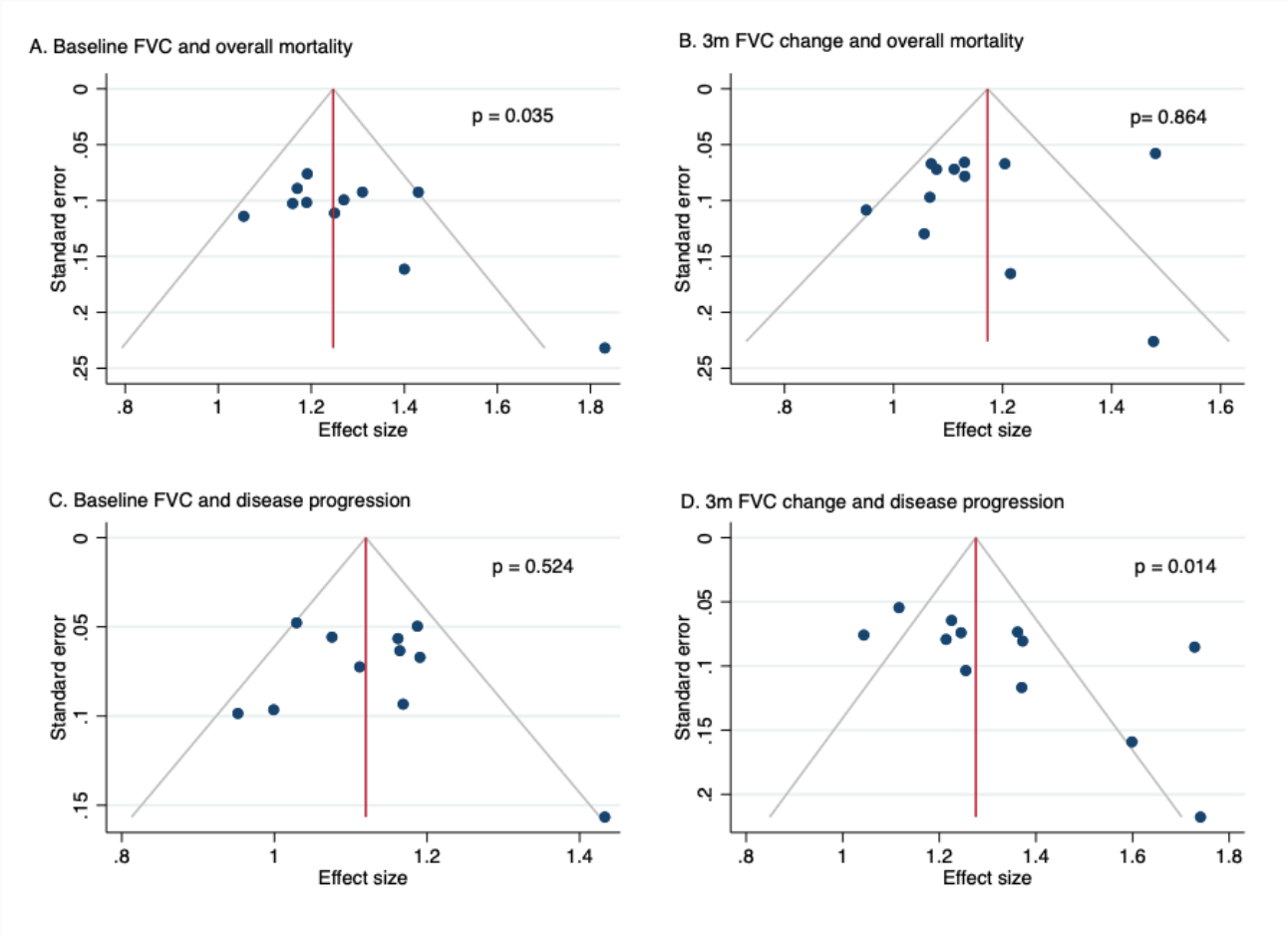


Figure 4-22 - FVC publication bias.

Publication bias assessed using Egger's test where ≥10 studies were included. P values have been included where possible

In estimates of DL_{CO} and 6MWD, there were insufficient studies to enable Egger's test, but visual inspection of funnel plots did not suggest publication bias (Figure 4-23 and Figure 4-24).

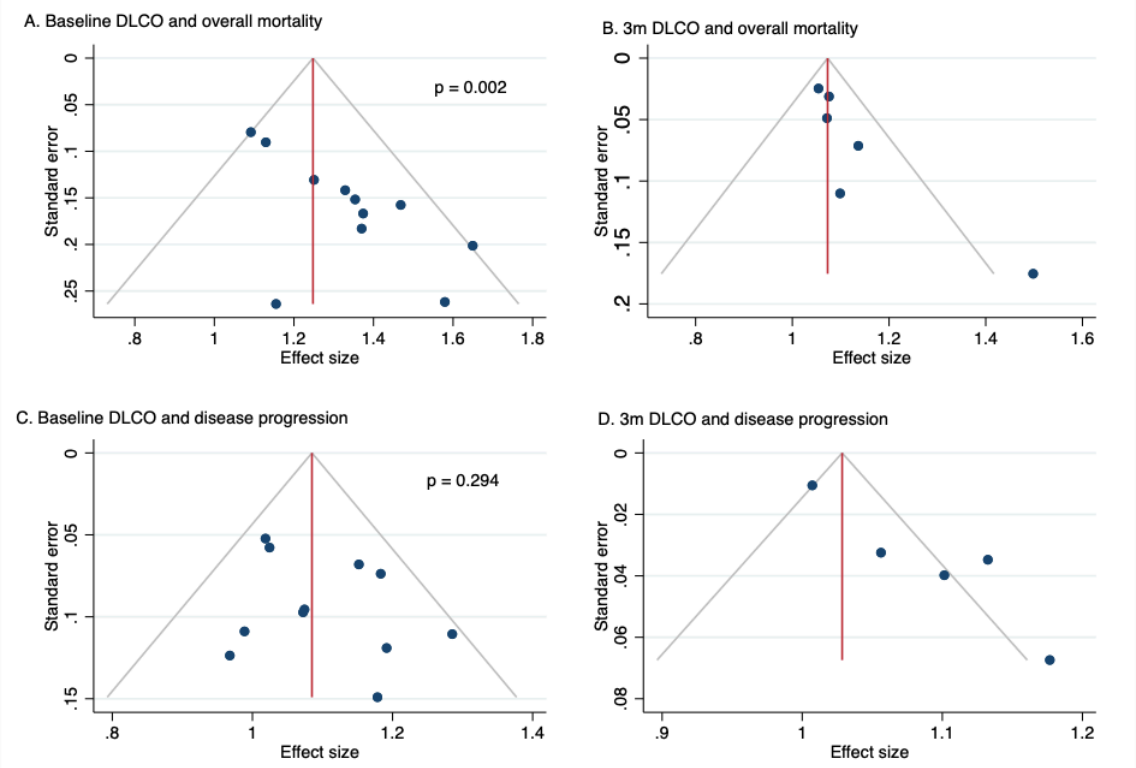


Figure 4-23 - DL_{CO} publication bias.

Publication bias assessed using Egger's test where ≥10 studies were included. P values have been included where possible

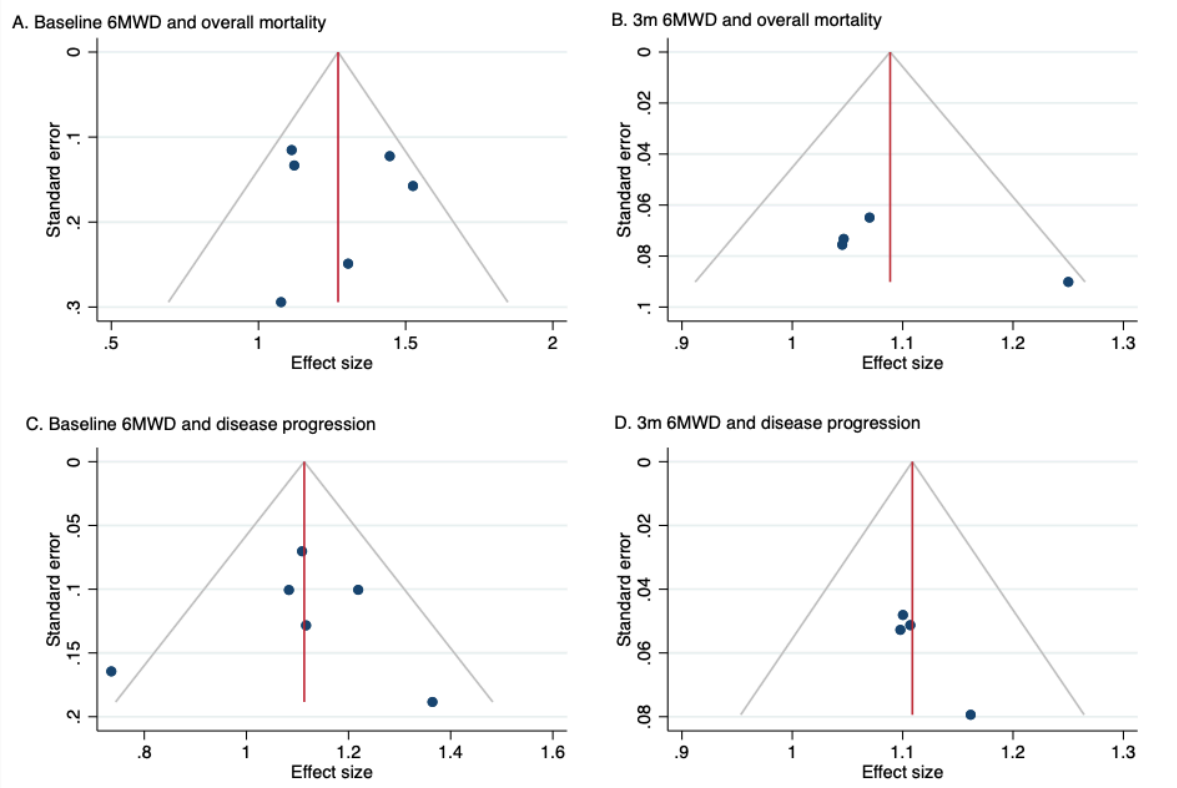


Figure 4-24 - 6MWD publication bias.

Publication bias assessed using Egger's test where ≥10 studies were included. P values have been included where possible

4.3.8 Meta-regression

	Baseline FVC						**3-month change in FVC**				**3-month FVC threshold**				**FVC threshold AUROC**			
Variables	Overall mortality		Disease progression		Change in FVC over 12m		Overall mortality		Disease progression		Overall mortality (>5.7% decline)		Disease progression (3% decline)		Overall mortality		Disease progression	
	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value
FVC vs. non GLI	0.00	0.199	0.00	0.774	17.79	0.191	0.00	0.995	0.00	0.947	8.15	0.163	0.00	0.260	2.84	0.297	6.37	0.175
Concomitant steroid use	0.00	0.413	0.00	0.269	0.00	0.381	2.14	0.343	**31.65**	**0.036**	0.00	0.838	16.67	0.119	0.00	0.584	**34.74**	**0.041**
Inclusion of severe cases	0.00	0.628	0.00	0.811	22.21	0.157	0.00	0.927	5.99	0.304	0.00	0.497	0.00	0.327	0.00	0.644	0.00	0.411
IPF diagnosis within 5 years	4.94	0.475	0.00	0.811	0.00	0.84	0.00	0.859	0.00	0.476	0.00	0.871	0.00	0.476	0.00	0.622	0.00	0.473
	Baseline DL_{CO}						**3-month change in DL_{CO}**				**3-month DL_{CO} threshold**				**DL_{CO} threshold AUROC**			
Variables	Overall mortality		Disease progression				Overall mortality		Disease progression		Overall mortality (>10.5% decline)		Disease progression (>7.2% decline)		Overall mortality		Disease progression	
	R^2 (%)	P value	R^2 (%)	P value			R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value	R^2 (%)	P value
FVC vs. non GLI	0.00	0.294	0.00	0.498			0.00	0.559	**69.57**	**0.025**	100	0.092	0.00	0.770	57.99	0.062	0.00	0.805
Concomitant steroid use	**94.17**	**0.005**	99.99	0.077			98.96	0.575	0.00	0.785	100	0.310	0.00	0.810	**75.13**	**0.017**	0.00	0.720
Inclusion of severe cases	0.00	0.681	0.00	0.686			0.00	0.927	0.00	0.787	100	0.180	0.00	0.77	**100**	**0.000**	0.00	0.805
IPF diagnosis within 5 years	0.00	0.681	0.00	0.811			0.00	0.859	0.00	0.787	N/A	N/A	50.63	0.088	**100**	**0.000**	26.22	0.233
	Baseline 6MWD						**3-month change in 6MWD**											
Variables	Overall mortality		Disease progression				Overall mortality		Disease progression									
	R^2 (%)	P value	R^2 (%)	P value			R^2 (%)	P value	R^2 (%)	P value								
FVC vs. non GLI	N/A	N/A	N/A	N/A			N/A	N/A	N/A	N/A								
Concomitant steroid use	**100**	**0.006**	0.00	0.532			**99.99**	**0.049**	99.85	0.479								
Inclusion of severe cases	0.00	0.479	**65.17**	**0.017**			N/A	N/A	N/A	N/A								
IPF diagnosis within 5 years	0.00	0.916	**65.17**	**0.017**			N/A	N/A	N/A	N/A								

Table 4-5 - Results of meta-regression for variables assessed separated by study outcomes, in placebo arms only

Sample sizes for each outcome shown (n). R2 and p values from meta-regression shown where applicable. Significant p values (p<0.05) are highlighted in red.

4.3.9 GRADE

Several exposure variables and outcomes were assessed, and GRADE was used to rate confidence in estimates. The risk of bias in all estimates was low as exposures were measured objectively for all participants and IPD enabled consistent adjustment for important covariates. All included studies were multi-centre in design and recruited participants according to international consensus criteria, and therefore the results of the meta-analysis are generalisable to the broader IPF population. Publication bias was present for some of the outcomes assessed though in most this could not be formally assessed using Egger's test due to insufficient studies.

The association between baseline FVC and overall mortality was rated with moderate certainty due to publication bias, and high certainty for disease progression estimates. There was evidence of statistical heterogeneity in estimates for change in FVC over three-months for both mortality and disease progression outcomes, leading to a rating of moderate certainty. The association between DL_{CO} and mortality was rated with moderate certainty due to the presence of publication bias, and high certainty for disease progression estimates. Estimates for the change in DL_{CO} over three-months and mortality were rated with high certainty, whilst due to the presence of statistical heterogeneity, estimates for disease progression were rated with moderate certainty. The association between both baseline and three-month change in 6MWD and mortality were rated with high certainty. Effects for baseline 6MWD and disease progression were inconclusive and imprecise, and therefore findings were rated with low certainty. Change in 6MWD and disease progression were rated with moderate certainty due to the presence of study heterogeneity.

4.4 Discussion

An earlier chapter demonstrated a clear association between blood biomarkers and disease outcomes in IPF. MMP-7 measured at baseline, but not the change over three-months was associated with an increased risk of mortality and disease progression. The aim of this chapter was to utilise robust methodology to complement the findings of the blood biomarker review by exploring the role of commonly measured physiological variables, both as prognostic biomarkers and as surrogate endpoints for clinical trials.

4.4.1 Summary of findings

The key findings of this review demonstrate physiological variables measured at baseline and their change over three-months are associated with poorer outcomes in IPF. In placebo arms, baseline FVC, DL_{CO} and 6MWD were independently associated with mortality, whereas FVC and $DL_{CO,}$ but not 6MWD were associated with disease progression. A three-month change in all physiological measurements, particularly FVC were associated with poorer outcomes. Optimal thresholds for three-month change in physiological variables for determining outcomes with the greatest sensitivity and specificity were identified using ROC analysis in placebo arms. Demographic variables including age, gender and smoking status were explored, with age an independent predictor of mortality. GRADE was applied to assess the certainty of findings, and outcomes for the change in lung function are rated with either moderate or high certainty. The association of disease progression and baseline 6MWD was rated with low certainty, whereas estimates of mortality were rated with high certainty.

Findings for three-month change in FVC and disease outcomes were replicated in trial treatment arms, supporting the prognostic significance of three-month FVC change irrespective of anti-fibrotic treatment. Notably, comparing FVC change between placebo and corresponding treatment arms, a benefit from anti-fibrotics could be observed at the early three-month time point.

4.4.2 Implications for clinical practice

The findings of this review support the use of age, baseline lung function and total distance walked as prognostic biomarkers in IPF, whereas sex and smoking status are unlikely to offer additional prognostic value. In longitudinal analysis, a 15% increased risk of mortality and 30% increased likelihood of disease progression was estimated per 2.5% relative decline in FVC over three-months, though on an individual level, test variability must be considered. However, the pre-test probability for disease progression in IPF is high and thus marginal declines are more likely to represent true deterioration than technical variation alone, particularly when associated with increased symptomology or equivocal radiological deterioration. Nonetheless, the findings of this review suggest that short-term change in physiological variables that were previously regarded as evidence of clinically stable disease may be clinically important and worthy of more intense evaluation. Further clinical evaluation should focus on combining with other prognostic markers such as age, radiological scores, and molecular and genetic biomarkers that reflect underlying disease activity. In the UK, anti-fibrotic therapy is licensed for individuals with an FVC between 50-80% predicted.[385] The consideration of anti-fibrotics for mild but progressive IPF as

evidenced by rapid FVC decline over three months, beyond that explained by test variability, may be beneficial and requires further study.

Whilst short-term change in DL_{CO} and 6MWD showed an association with outcomes, the effect size was lower compared with change in FVC. The superiority of serial FVC over DL_{CO} and 6MWD may be explained by its greater reproducibility and less variability[59], though three-month DL_{CO} was missing in 6/12 datasets, which could be non-random and due to severe disease, potentially underestimating effect estimates. The 6MWD offers several advantages over lung function testing, requiring minimal operator skill or special equipment, is inexpensive, and can be flexibly performed in several hospital and community settings[208]. Individually, each of the physiological markers evaluated have distinct advantages suggesting there may be a role for each in the prognostication of IPF.

4.4.3 Implications for future clinical trials

The findings of this review have the potential to streamline future clinical trials in IPF. Several endpoints have been considered for IPF trials including mortality, hospitalisation, acute exacerbations, patient-reported outcomes, and the total distance walked, but none are ideal and are associated with several limitations[405]. Therefore, surrogate markers for mortality including an FVC change over twelve-months are commonly used primary endpoints in interventional trials[54 178 390 391]. However, current trials based on a twelve-month endpoint are lengthy, expensive, and hampered by considerable missing data due to participant death and loss to follow up, often requiring imputation[406]. Moreover, in a condition with poor prognosis,

a twelve-month study limits treatment options and commits a significant period of a subject's remaining life to a clinical trial with attendant hospital visits. An earlier endpoint, although requiring more patients, could mitigate these limitations by supporting objective evaluations in early study termination, minimising lengthy and costly studies of ineffective drugs, and enabling short term placebo-controlled trials to be performed.

In traditional studies enrolment periods can last several years to facilitate recruitment, capture events, and permit open label extensions to determine potential long term adverse effects. In shorter studies requiring larger sample sizes, recruitment periods could be shorter but more intensive. Beyond three months all patients could be offered study drug in an open label extension to determine longer term toxicity in a traditional approach or be randomised to an alternative therapy in an adaptive approach. Furthermore, as recently demonstrated[407], three-month placebo control trials are still feasible in the presence of standard of care raising the prospect of more drugs successfully demonstrating proof of principle in clinical trials.

The findings of this review are particularly pertinent in the anti-fibrotic era where accurate stratification and cohort enrichment strategies are likely to be of greater importance due to reduced rates of disease progression and mortality in those receiving anti-fibrotics. In this study, when FVC decline over three-months was dichotomised according to the cohort median (2.3%), notable differences in FVC decline over twelve-months were observed between groups (280ml vs. 87ml; $p<0.001$). This illustrates that three month declines in lung function are highly predictive of twelve-month change and may permit enrichment into clinical trials based on short term disease behaviour.

4.4.4 Comparison with existing literature

Associations between baseline physiology and disease outcomes in IPF have been demonstrated in smaller studies previously. However, many of these studies have been retrospective analyses of modest sample sizes, have included participants receiving interventional drugs in clinical trials where the effect may be unknown, have been limited by only reporting unadjusted estimates, and have utilised data-dependent thresholds for change in physiology[59]. In a combined clinical trial cohort of well-characterised IPF participants recruited into the placebo arms of the CAPACITY and INSPIRE studies, baseline FVC and 6MWD were independently associated with an increased risk of mortality and composite disease progression outcomes[408]. In a further study of over 1100 participants pooled from the placebo arms of nintedanib and pirfenidone trials (TOMORROW, INPULSIS, ASCEND and CAPACITY), baseline FVC and DL_{CO} were categorised into quartiles, and the risk of death was found to be highest for those subjects in the lower quartiles (FVC < 55% predicted, DL_{CO} < 36% predicted)[409]. The research presented in this chapter helps provide reliable interpretations of effect size in a larger cohort pooled from ten interventional clinical trials performed worldwide, with baseline FVC and 6MWD examined as continuous variables using IPD meta-analysis. The inclusion of several clinical trials broadens the inclusion criteria of participants studied in these analyses, and therefore increases the generalisability of the findings.

Several studies have explored the association of longitudinal change in physiological variables and disease outcomes in IPF. In the largest study to evaluate the relationship between longitudinal change in physiology and disease outcomes in untreated IPF, placebo

arm participants recruited into two interventional trials were pooled. The change in FVC over 24-weeks was associated with an unadjusted increased risk of mortality and composite disease progression outcomes[408]. Another study consisting of both placebo and treatment-arm participants pooled from two clinical trials of interferon-y1b, replicated these findings using categorical variables, with an FVC decline >5%, and a DL_{CO} decline >15% over 24-weeks both independently associated with a two-fold increased risk of death[201]. In a further study of participants recruited into a clinical trial cohort, a 24-week decline greater than 50m in the 6MWD was associated with a fourfold increased risk of death at one year[208]. Taken together these studies consistently demonstrate that the change in physiological variables over 24-weeks can accurately and independently predict poor outcomes in IPF. The present study is the largest to evaluate longitudinal change in physiological variables and its association with disease outcomes in IPF, and the first to establish the prognostic significance of change over a shorter time-period of three-months, whilst concurrently identifying optimal threshold values. Of particular significance, this study is the first study to identify a treatment benefit with current anti-fibrotics as early as three-months. Moreover, unlike previous pooled studies where participants from different studies have been treated as one large cohort, this study is the first to utilise IPD meta-analysis to combine cohorts using a random effects model, and thus account for differences in individual trial populations. IPD meta-analysis enabled all analyses to be performed on a linear scale with consistent adjustment for common confounding factors and standardisation of outcomes and therefore the findings are more likely to be robust.

Studies evaluating demographic factors as prognostic markers in IPF have shown little consistency. In a large clinical trial cohort, neither age nor sex were associated with mortality or disease progression outcomes, though analyses were unadjusted for confounding factors [408]. In a similar clinical trial cohort, an age above 70 years doubled the risk of all-cause mortality in multivariate analysis, compared with participants below 60 years of age, but no associations were found with sex[201]. In the largest placebo cohort to date of approximately 1100 participants, age over 75 years and a previous smoking history rather than current smoking history were associated with an increased risk of death in multivariate analysis.[409] Similar improved outcomes in current smokers were reported in another retrospective study[410], but it is likely these findings represent a "healthy smoker effect" where symptomatic individuals with more severe disease are more likely to cease smoking. In the present study, to overcome the healthy smoker effect, smoking status was categorised into ever and never smoker, and no association was found with either mortality or disease progression. There was a 4% increased risk of death per year increase in age, with age analysed on a continuous scale as this is more likely to be helpful to clinicians, than categorical variables based on arbitrary thresholds. Whilst male sex is known to confer an increased risk of developing IPF, findings from this study suggest sex does not offer additional prognostic insights once IPF is diagnosed.

4.4.5 Limitations

Limitations must be considered in the interpretation of the findings of this study. Importantly, there is a risk of selection bias as the cohort included in this study were recruited into interventional clinical trials with specific inclusion criteria, typically excluding participants

with severe disease or those unlikely to survive the study duration. However, though participants had mild-moderate severity at baseline, several progressed during the period of follow up, and therefore findings are likely to be generalisable to the broader IPF population. Further selection bias can be attributed to the requirement for participants to survive at least three-months after their baseline visit to be included in analyses of longitudinal change, although exclusion on this criterion represented a small proportion of participants (2.3%; 41/1770) and is unlikely to have influenced overall estimates. Accessing data from discrete studies across multiple servers and research environments limited management of missing IPD values, with a two-step IPD meta-analysis design used to facilitate analysis of individual study estimates.

Further limitations include the dependence of secondary endpoints (disease progression and change in FVC at twelve months) on FVC, whilst the exposure variable also included FVC, though at an earlier timepoint. However, the primary endpoint of mortality was not dependent on FVC, and summary estimates remained consistent. Though this is the largest cohort of untreated individuals with IPF, IPD could not be retrieved from 1214 participants. There are two key considerations here. Firstly, data from the majority of important phase three clinical trials In IPF over the precious decade were included, and secondly tabulation of study and participant characteristics suggested there was little difference compared with the included studies, limiting the possibility of availability bias. Moreover, whether IPD was available from a particular trial was unlikely to be influenced by its findings, as the evaluation of physiological prognostic markers was not the objective of any trial.

4.4.6 Future direction

This study identifies several priorities for future research. The AUROC for predicting mortality for both the optimal three-month threshold, and for an FVC of 10% at twelve months were suggestive of relatively poor discriminatory performance. This highlights the importance of identifying more sensitive endpoints, and future studies should combine longitudinal change in physiological biomarkers with radiology and molecular biomarkers in clinical prediction models, to increase the specificity and sensitivity for predicting disease outcomes. Moreover, the prognostic significance of desaturation episodes during six-minute walk tests was not explored, and further research should investigate this further. From a clinical trial perspective, future trials should consider change in FVC over three-months as an endpoint. Short-term change in physiology, both alone and in combination with other biomarkers in clinical prediction models, should be explored in other fibrotic ILDs to ascertain whether three-month change in physiology is a biomarker of progressive fibrosis irrespective of aetiology.

4.5 Summary

This is the largest study to explore the association between physiological variables and disease outcomes in well-characterised individuals with IPF. Key findings demonstrate baseline and three-month change in all physiological variables, particularly FVC offer insights as prognostic biomarkers. Findings for FVC change over three-months are reproduced in treatment arms, and comparisons between treatment and placebo arms demonstrate an observable treatment effect at three-months. The findings from this study have the potential to offer clinical benefits for individual patients and help streamline future clinical trials by utilising FVC change over three-months as a surrogate endpoint in adaptive trial design.

Chapter 5 An observational study to explore biomarkers of progressive fibrotic lung disease

5.1 Introduction

ILD encompasses a heterogeneous group of inflammatory and fibrotic parenchymal lung disorders. As discussed in Chapter 1, a proportion of individuals with non-IPF ILD develop progressive fibrotic phenotypes that show similarities to IPF, raising the possibility of shared pathogenic mechanisms across disease phenotypes regardless of likely aetiology. The majority of blood biomarker studies in ILD have been restricted to participants with IPF, but it is probable these biomarkers reflect distinct fibrotic molecular endotypes that could help define prognostic outcomes and therapeutic strategies regardless of subtype. Though limited studies have identified possible associations between blood biomarkers and clinical outcomes in fibrotic ILDs, further studies are urgently needed to confirm this hypothesis and to further explore the role of blood derived biomarkers in this cohort. Furthermore, the prognostic potential of genetic, radiological, and physiological biomarkers needs exploring in prospective, high-quality longitudinal studies in well-characterised cohorts with fibrotic ILDs.

This chapter describes the ongoing prospective multi-centre “It’s Not Just Idiopathic Pulmonary Fibrosis (INJUSTIS) study”, investigating biomarkers of progressive fibrotic lung disease. My involvement with the INJUSTIS study began with the design and set up, which involved writing the protocol, participant information sheets and seeking ethical approval. Since then, I have been involved with screening and recruiting participants, performing study visits, performing site initiation visits (SIV), coordinating recruiting sites and being the

general first point of contact for any study-related enquiries. In this chapter, alongside the study methodology, I describe the study population recruited to date (6th Aug 2021), including baseline characteristics and longitudinal physiology and quality of life data.

The study protocol has been published in BMJ Open Respiratory Research as part of Khan et al, “The Its Not JUST Idiopathic pulmonary fibrosis Study (INJUSTIS): description of the protocol for a multicentre prospective observational cohort study identifying biomarkers of progressive fibrotic lung disease"[411]. Details of the study protocol have also been made available on ClinicalTrials.gov NCT03670576.

5.1.1 Aims of chapter

1) To describe the methodology for the ongoing INJUSTIS study
2) To describe the clinical features of a cohort with mixed fibrotic ILD
3) To compare clinical features across fibrotic ILDs
4) To evaluate longitudinal disease behaviours in fibrotic ILD to identify the frequency of a progressive fibrotic phenotype

5.2 Methods

5.2.1 Hypothesis

There are shared pathogenic mechanisms in the progression of pulmonary fibrosis irrespective of aetiology.

5.2.2 Study Aims

1) To identify molecular endotypes associated with progressive fibrosis irrespective of aetiology
2) To identify novel blood biomarkers predictive of progressive fibrosis
3) To prospectively validate previously identified blood biomarkers in IPF in well-characterised individuals with fibrotic ILD
4) To investigate gene expression profiles which affect disease progression
5) To explore baseline and short-term change in biomarkers as predictors of disease outcomes
6) To evaluate the usefulness of blinded home handheld spirometry over three-months in predicting disease outcomes
7) To evaluate longitudinal disease behaviours in fibrotic ILD to identify the frequency of a progressive fibrotic phenotype
8) To explore the association between environmental exposures and disease outcomes

5.2.3 Endpoints

The primary endpoint is:

- Disease progression defined as relative forced vital capacity decline ≥ 10% or death within 12 months

Secondary endpoints include:

- All-cause mortality at time of censoring
- Change in DL_{CO} from baseline to 12 months
- Change in 6-minute walk distance from baseline to 12 months
- Change in transcriptomic profiles from baseline to 12 weeks
- Change in home handheld spirometry from baseline to 12 weeks
- Number of respiratory hospitalisations over 2 years
- Change in Quality-of-Life questionnaire scores

5.2.4 Sample size

The power of a study is the probability that a true difference between interventions will be detected and can be used to calculate the minimum sample size required. A power calculation was performed based on data obtained in the PROFILE (Prospective Observation of Fibrosis in the Lung Clinical Endpoints) study of IPF[124]. Of all the blood biomarkers evaluated over three-months, MMP-7 was the most conservative with the lowest threshold for change, and thus powering on MMP-7 ensures analyses for other blood biomarkers have adequate power. Power calculation demonstrated 100 participants with stable disease and 100 participants with progressive disease would be sufficient to detect dynamic change in biomarkers over 3 months with 80% power and 5% type 1 error rate. Thus 200 participants with non-IPF fibrotic ILD split equally between the four diagnostic groups are being recruited. Alongside individuals with fibrotic ILD, 50 with IPF will be recruited to benchmark progressive fibrotic lung disease but will not be included in the final analysis. All participants

with non-IPF fibrotic ILD will be analysed collectively, although exploratory analyses will be performed to guide further study.

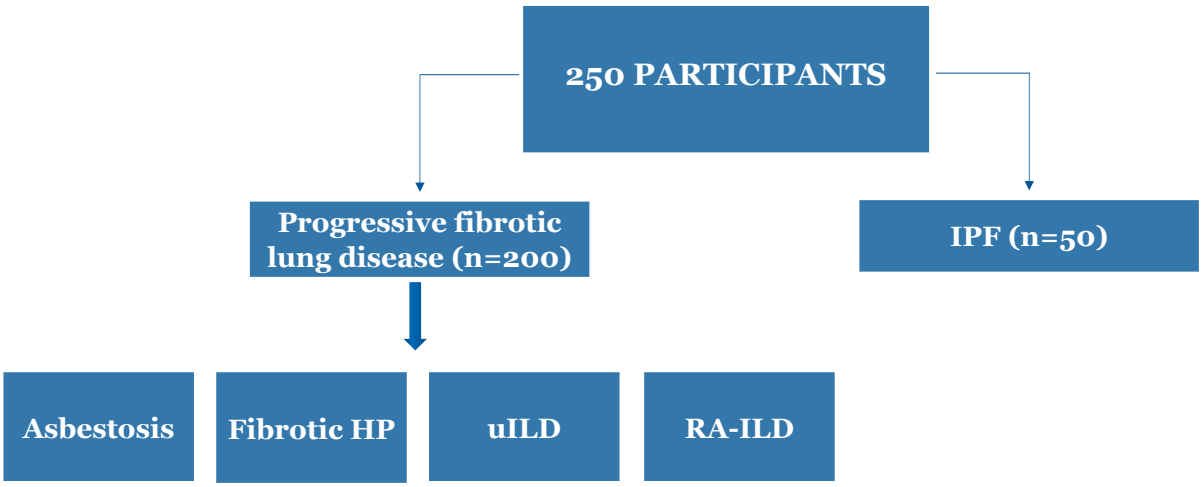


Figure 5-1 - Flow diagram demonstrating planned study recruitment

5.2.5 Study population

5.2.5.1 Inclusion criteria

- Adults aged ≥ 18 years
- Diagnosed after 1st March 2017 (diagnostic HRCT or surgical lung biopsy)
- An MDT diagnosis of fibrotic ILD defined as the presence of reticulation and traction bronchiectasis, associated with one of the following subgroups:
    - Idiopathic Pulmonary Fibrosis (diagnosed according to international consensus criteria)
    - Asbestosis (appropriate asbestos exposure history)
    - Fibrotic hypersensitivity pneumonitis (with or without the identification of an inciting antigen)

- Rheumatoid arthritis (formal rheumatologist diagnosis)
- Unclassifiable ILD (unclassified fibrotic disease despite extensive clinical and radiological examination)

5.2.5.2 Exclusion criteria

- Participation in an interventional clinical trial
- Asymptomatic interstitial lung abnormalities (ILA) and normal lung function
- Change in clinical phenotype from initial radiological diagnosis to screening

5.2.6 Study regimen

5.2.6.1 Visit details

Participants are being followed up over 2 years with visits at baseline, 3, 12 and 24 months. The baseline visit is combined with the screening visit, which includes assessing the suitability of participants according to the inclusion criteria. Detailed participant information including age, gender, smoking history, and ethnicity are being recorded, alongside details of co-morbidities, family history and medication history. The case report form also includes a detailed occupational exposure and job history and categorises jobs according to skill e.g., professional. All participants are having routine bloods consisting of full blood count and kidney/liver function. Where available, historic results for angiotensin converting enzyme (ACE), avium precipitins and autoantibodies including extractable nuclear antigen (ENA), rheumatoid factor (RhF), anti-citrullinated protein antibody (anti-CCP), antinuclear antibody (ANA), anti-neutrophilic cytoplasmic antibody (ANCA) are being collected. An overview of participant flow through the study is provided (Figure 5-2):

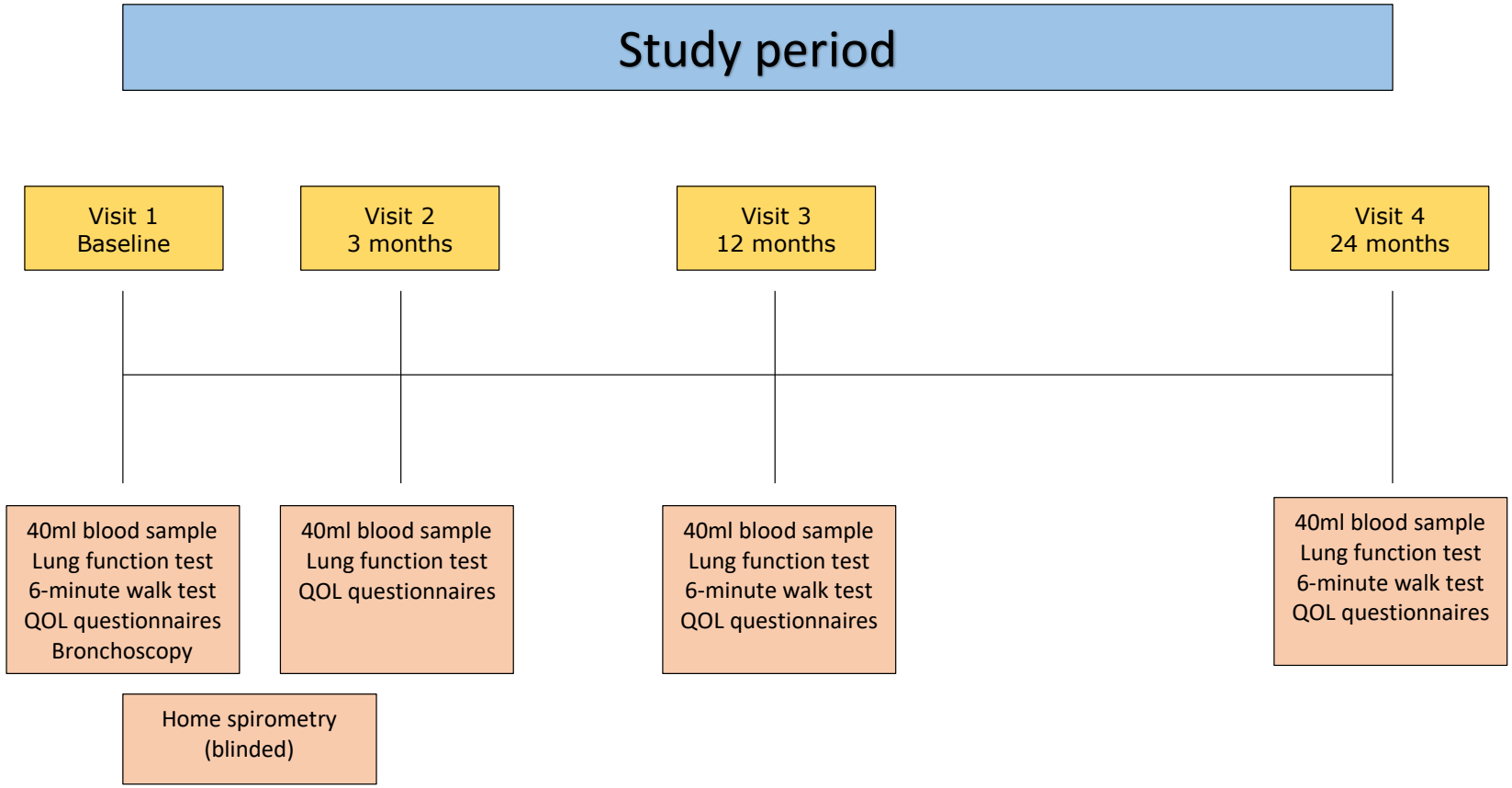


Figure 5-2 - Participant flow through study.

Participants will attend for four visits in total. At each visit participants are offered a 40ml blood sample for biomarkers, lung function testing including spirometry and gas transfer, 6-minute walk test at baseline, 12 and 24 months, and quality of life questionnaires. Participants recruited in Nottingham only are offered a bronchoscopy for bronchoalveolar lavage. Home spirometry is offered to all participants for the first three months of the study

5.2.6.2 Questionnaires

Participants are being asked to complete five individual questionnaires at each visit. Each of the questionnaires are included in appendix 10.8.

5.2.6.2.1 IPF Prognostic Assessment and Referral to Care

The IPF Prognostic Assessment and Referral to Care (I-PARC) questionnaire is a concise distress questionnaire that was developed using the Sheffield Profile for Assessment and Referral to Care (SPARC) holistic tool[412 413]. The questionnaire consists of 11 items that measure the level of distress or bother over the past one month including questions relating to symptoms and questions relating to independence and activities of daily living. Each is scored out of three (0=not at all; 1=a little bit; 2=quite a bit; 3=very much) and combined to produce an overall distress score. A greater score indicates high levels of distress. In the PROFILE cohort[412], I-PARC distress scores were negatively correlated with both FVC % predicted and DL_{CO} % predicted and were associated with an increased risk of mortality and disease progression in IPF.

5.2.6.2.2 King's Brief ILD Questionnaire

The King's Brief ILD Questionnaire (K-BILD) is an interstitial-disease specific 15-item health related quality of life questionnaire designed to measure the impact of lung disease in three domains: psychological, breathlessness and activities, and chest symptoms[414]. Responses are recorded on a seven-point Likert scale, which are used to calculate weighted scores

using logit transformation for each domain, alongside an overall total score. Scores range from 0-100, with higher scores indicating better health related quality of life.

5.2.6.2.3 European Quality of Life 5-Dimensions 5-Levels

The European Quality of Life 5-Dimensions 5-Levels questionnaire (EQ-5D-5L) is a generic multidimensional questionnaire that enables measurement of health-related quality of life and calculation of quality-adjusted-life-years[415]. The tool contains five dimensions that are each scored out of five: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Scores from each of the dimensions are combined to produce a five-digit code, which can also be represented using a single summary number termed the index value. The index value is derived by attaching weights to levels in each of the dimensions, with a higher value indicating a greater health related quality of life. The EQ-5D-5L also includes a visual analogue scale scored between 0 and 100, where 100 represents the best health possible, and 0 the worst.

5.2.6.2.4 Leicester Cough Questionnaire

The Leicester Cough Questionnaire (LCQ) is a self-completed quality of life measure designed to assess the impact of cough severity over the previous two weeks[414]. It consists of 19 items, each scored with a seven-point Likert response scale, that assess the impact of cough on three main domains: physical (eight items), psychological (seven items) and social (four items). The mean score for each domain is calculated and adding together for an overall total score. Higher scores indicate better cough-related quality of life.

5.2.6.2.5 Medical Research Council Dyspnoea Scale

The Medical Research Council (MRC) dyspnoea scale consists of five statements used to grade the impact of perceived breathlessness on activities of daily living[416].

MRC Grade	*Degree of breathlessness related to activities*
1	Breathlessness with strenuous exercise
2	Short of breath when hurrying on the level or walking up a slight hill
3	Walks slower than contemporaries on the level or stops for breath at own pace
4	Stops for breath after walking 100m
5	Too breathless to leave the house or breathless when dressing

Figure 5-3 - Medical Research Council (MRC) dyspnoea scale

5.2.7 Blood

At each of the four visits, blood samples of up a total of 40ml are being collected (Table 5-1). Full blood count (FBC) tubes are sent to local NHS labs for processing, whilst the remaining blood bottles are stored on dry ice during transport and processed by the research team according to a standardised protocol (Figure 5-4 to Figure 5-6). Batch collection of samples from sites is arranged periodically for long-term storage at the University of Nottingham.

Sample	**Quantity**
Full Blood Count	1 X 2ml
Serum	3 X 5ml
Plasma (Lithium heparin)	3 X 4ml
DNA PAXgene (baseline only)	2 X 2.5ml
RNA PAXgene	2 X 2.5ml

Table 5-1 - Blood samples taken at each visit.

DNA PAXgene only collected at baseline visit, whilst the remaining bloods are collected at all four visits.

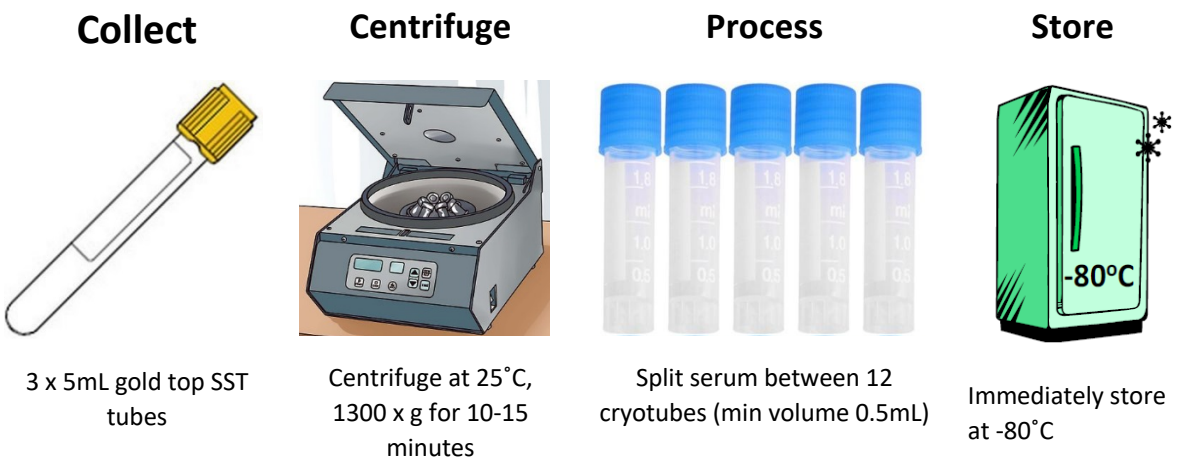


Figure 5-4 - Procedure for processing of serum samples

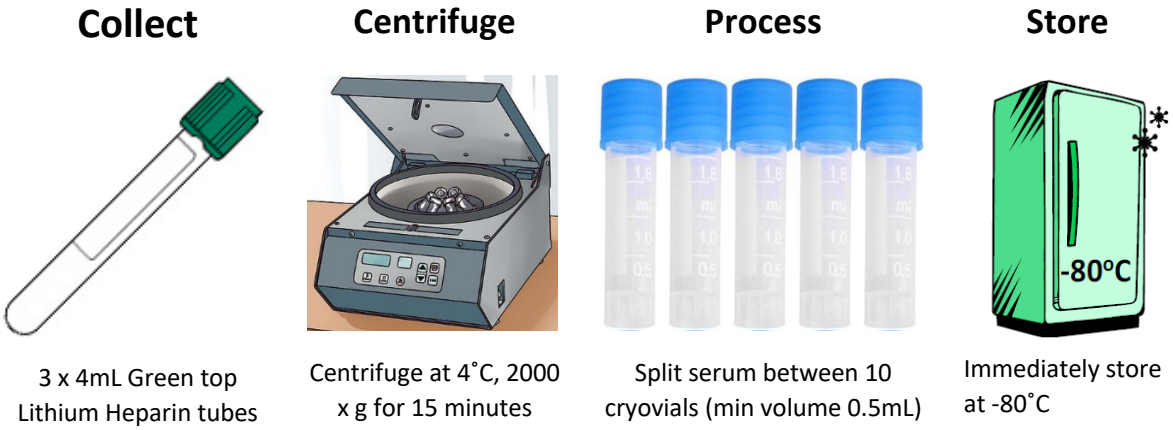


Figure 5-5 - Procedure for processing plasma samples

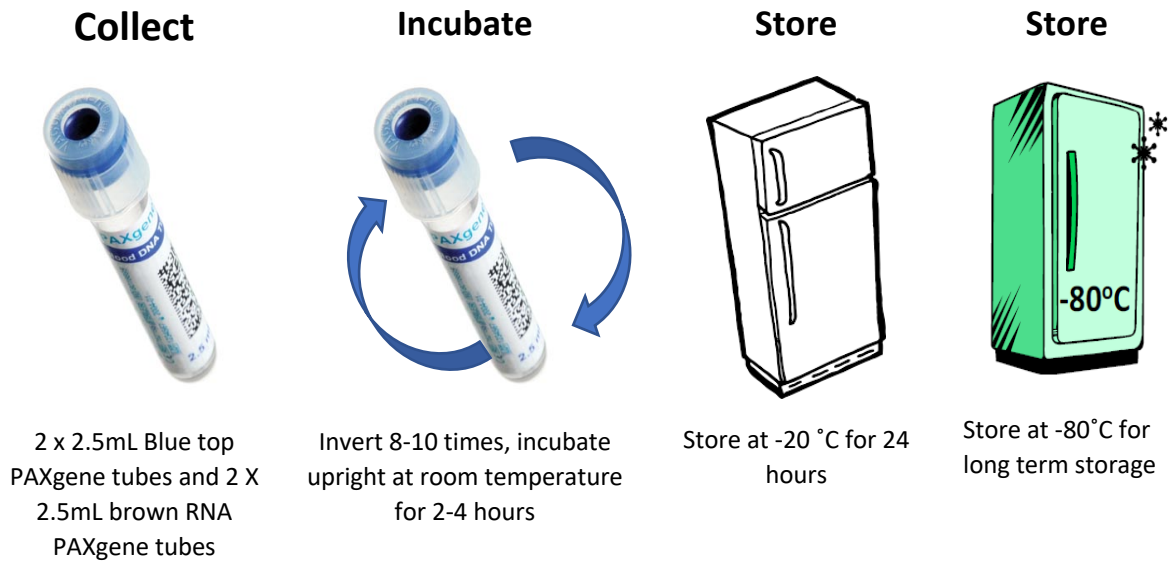


Figure 5-6 - Procedure for processing DNA and RNA samples

5.2.8 Physiology investigations

Lung function including spirometry and DL_{CO} are being performed at each visit according to ATS/ERS standards and consensus recommendations[417 418]. The best of three technically acceptable manoeuvres is recorded and FVC reference values are calculated using standardised GLI equations as described in section 4.2.5. The 6MWT is performed according to a standardised protocol consisting of two cones placed 10m apart on a flat surface. The test measures the total distance walked in six-minutes, whilst recording oxygen saturations.

5.2.9 Home spirometry

Participants are being offered a home hand-held spirometer which connects via Bluetooth to a smartphone enabled app and provided with training on using the spirometer alongside written instructions. They are asked to perform a daily, single, blinded, forced expiratory manoeuvre for three months. The study protocol was amended during the COVID-19 pandemic to necessitate participation in home spirometry for new study recruits. Further details of home spirometry have been described in section 6.3.

5.2.10 Bronchoscopy

Bronchoscopy and bronchoalveolar lavage (BAL) are being offered to participants recruited in Nottingham only, according to BTS guidelines and a standardised research protocol (Appendix 10.9). Bronchoscopies are performed by me with appropriate supervision by an NHS respiratory consultant and assistance from at least two endoscopy nurses. Following standard procedures for patient preparation, sedation and intubation, the bronchoscope is inserted into the right middle lobe and up to 60ml of normal saline slowly injected, before

being gently aspirated into a lavage trap. The process is repeated up to four times in total instilling a maximum of 240ml normal saline. BAL fluid is separated into three aliquots, with approximately 50ml in aliquot A, 15ml in aliquot B and the remainder in aliquot C. A combined protease and phosphatase inhibitor cocktail is added (0.9ml phosphatase and 0.045ml of protease inhibitor per 15ml of BAL) to aliquot B before all aliquots are centrifuged at 300g 4°C for 10 minutes. Following centrifugation, the resultant cell pellet from Aliquot A is resuspended and cell count is performed, whilst the remaining two aliquots are frozen at -80°C for future cell protein and RNA analysis.

5.2.11 COVID-19 amendments

The disturbance caused by the COVID pandemic has led to a significant impact on the study, with recruitment and follow up visits particularly affected. As a consequence of lockdown restrictions and personal safety concerns from an extremely vulnerable cohort, numerous follow up visits have been missed or are incomplete. To assess the extent of COVID-related missing data, all recruiting sites have been asked to provide reasons for missing visits on a protocol deviation form. Moreover, recruitment of new participants was halted between March 2020 and April 2021 in line with guidance from research and innovation departments, as staff were redeployed to high priority COVID research, or were shielding long term. Since the formal restart, several sites have been unable to resume recruitment due to the impact of COVID on their research departments.

A further challenge has been accessing respiratory physiology departments for lung function testing. Respiratory physiology departments have faced huge backlogs and have run

reduced services to facilitate sterilisation of rooms and equipment in between patients. In an interim analysis performed in July 2020, home spirometry correlated well with hospital spirometry and non-inferiority of measurements was demonstrated (described further in section 6.3). To help mitigate the effects of reduced lung function services and considering the interim analysis findings, the steering committee approved the alteration of the study primary endpoint to include an FVC decline > 10% over 12 months measured using either hospital or home spirometry. Participation in home spirometry was accordingly altered from an optional component to mandatory for inclusion, and participants were asked to perform three-months of blinded readings as per the initial study protocol, but also to perform home spirometry readings one week either side of their follow up visits, irrespective of whether they attended for hospital spirometry.

5.2.12 Ethics/R&D approval

The study received ethical approval from the Nottingham Research Ethics Committee on 2nd July 2018, with reference number 18/EM/0139. A steering committee was established consisting of the chief investigator (Professor Gisli Jenkins), study statistician, independent chair, research officer, clinicians, patient representatives and myself. The steering committee meet every 4 months to oversee the study.

5.2.13 Patient and public involvement

The Action for Pulmonary Fibrosis (APF) charity have been consulted throughout the study design and sit on the study steering committee as patient representatives. All patient facing documents and study publications have been reviewed by patient representatives.

5.2.14 Recruitment sites

A total of 25 sites across the UK have been involved or will be involved with the recruitment of participants, though not all sites have recruited their first participant at the time of censoring. Each site required local research and development approvals.

5.2.15 Analysis

Demographic factors including age, sex and smoking status were assessed for IPF and non-IPF and compared using t tests for means, Wilcoxon rank-sum tests for medians and Fisher's test for proportions. Baseline physiological measurements including FVC percent predicted, DL_{CO} percent predicted, and 6MWD were estimated and compared between groups. Baseline physiological variables were additionally dichotomised into two severity groups (FVC above or less than 80% predicted, DL_{CO} above or less than 55% predicted, and 6MWD above or less than 300m) according to commonly used criteria and the ILD GAP model [419]. The mean relative change in FVC and DL_{CO} over three and 12-months was estimated and compared between IPF and non-IPF. Lung function changes over three-months were dichotomised relative to the median to overcome limited sample sizes, and proportions of participants in each group were compared between IPF and non-IPF. The Quality of life (QoL) questionnaire scores stratified at baseline, and their change over 12-months categorised into stable/improved or worse, were reported for IPF and non-IPF. The change in KBILD questionnaire scores was assessed using a minimal clinically important difference (MCID) threshold of 5-unit change based on published data[420]. Disease progression defined as an FVC relative decline ≥ 10% or death at 12 months, and mortality censored at one year, was estimated, and compared between IPF and non-IPF. Associations for mortality between

subgroups were assessed using log-rank tests. Further exploratory analyses were performed for individual ILD subgroups for all demographic factors, physiological variables and QoL scores. A p value < 0.05 was considered statistically significant for all analyses.

5.3 Results

5.3.1 Recruitment

191 participants with fibrotic ILD were recruited between November 2018 and August 2021 across 21 sites in the UK. Due to the pandemic, recruitment was halted for the period in between March 2020 and April 2021 but has since slowly resumed at several sites.

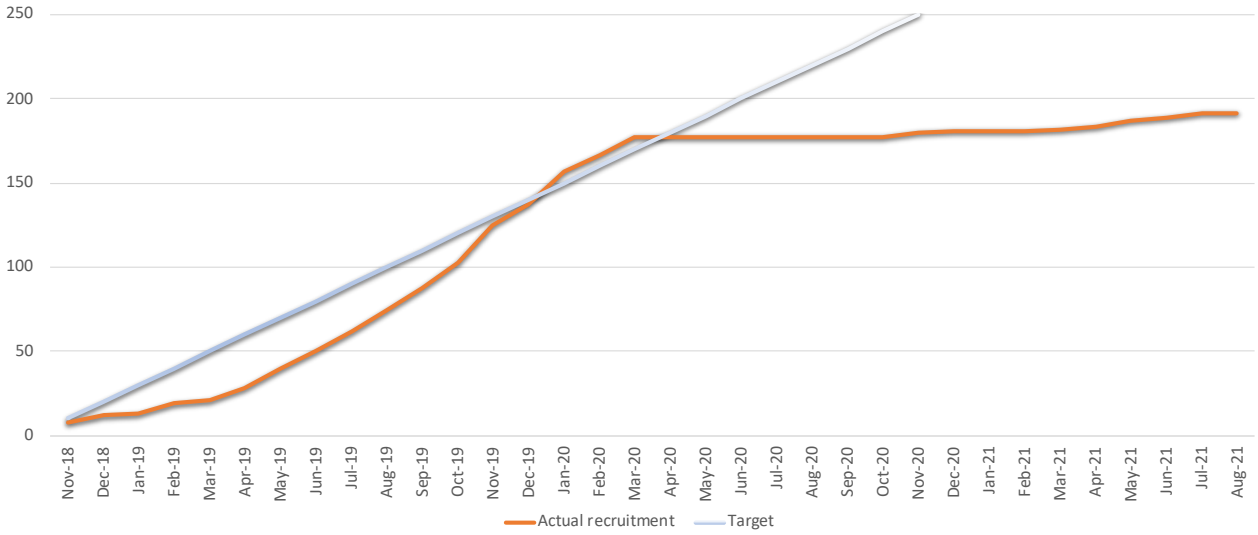


Figure 5-7 – Summary of recruitment at time of censoring

121 of the 191 participants are currently under study follow-up. Of the remaining 70 participants, 29 have completed the study, nine have withdrawn, and 32 have died, with COVID-19 listed as the cause of death in seven participants. The number of participants recruited in each of the subgroups are as follows: IPF, n=63; asbestosis, n=37; fibrotic HP, n=37; RA-ILD, n=27; unclassifiable ILD, n=30.

Site	Target	All	IPF	Non-IPF
Royal United Hospitals of Bath	10	1	0	1
Heartlands Hospital, University Hospitals of Birmingham	10	0	0	0
Blackpool Teachings Hospitals NHS Trust	20	9	4	5
North Bristol NHS Trust	20	7	3	4
Burton Hospital, University Hospitals of Derby and Burton	20	4	2	2
University Hospital of Coventry and Warwickshire	30	4	1	3
Royal Derby Hospital, University Hospitals of Derby and Burton	70	18	4	14
Royal Devon and Exeter NHS Trust	25	7	4	3
Hammersmith Hospital, Imperial College NHS Trust	TBC	0	0	0
Medway Maritime Hospital, NHS Foundation Trust	20	2	0	2
Kingsmill hospital, Sherwood Forest Hospitals	32	12	0	12
Kingston Hospital NHS Foundation Trust	15	2	1	1
North Manchester General Hospital, Manchester University NHS Foundation Trust	25	6	6	0
Royal Victoria Infirmary, Newcastle-upon-Tyne Hospitals NHS Foundation Trust	10	8	5	3
University Hospitals of North Tees	10	5	2	3
Northumbria Healthcare NHS Foundation Trust	20	5	2	3
Nottingham City Hospital **(host site)**	40	50	8	42
Sheffield Teaching Hospitals NHS Foundation Trust	15	3	0	3
South Tyneside district hospital, NHS Foundation Trust	23	12	5	7
St. Georges University Hospitals NHS Foundation Trust	10	11	2	9
Taunton and Somerset NHS Foundation Trust	25	0	0	0
Wigan Infirmary, Wigan Teaching Hospitals NHS Foundation Trust	20	10	4	6
New Cross Hospital, The Royal Wolverhampton NHS Trust	10	10	6	4
Worcestershire Royal Hospital, NHS Trust	24	5	4	1
Wythenshawe Hospital, University Hospitals of Manchester	20	0	0	0
Total		**191**	**63**	**128**

Table 5-2 – Summary of recruiting sites including number of participants recruited by each centre.

5.3.2 Missing data

Several missed visits can be attributed to the COVID-19, with reasons including local and national restrictions, cancellation of non-urgent research visits, staff redeployment to high priority studies, staff shortages from self-isolation, and participant choice to shield and not attend hospital. Over half of participants have missing 12-month spirometry data, and about two-thirds have missing 12-month gas transfer. Table 5.3 illustrates the extent of collected data:

	Baseline	**3-month visit**	**12-month visit**	**24-month visit**
N	191	170	146	50
Spirometry performed, n (%)	189 (98.9)	114 (67.1)	60 (41.1)	27 (54.0)
DLCO performed, n (%)	180 (94.2)	104 (61.2)	45 (30.8)	13 (26.0)
6MWD performed, n (%)	182 (95.3)	-	39 (26.7)	22 (44.0)
QoL data collected, n (%)	191 (100.0)	147 (86.5)	105 (71.9)	34 (68.0)
Bloods, n (%)	185 (96.9)	131 (77.1)	68 (46.6)	36 (72.0)

Table 5-3 – Summary of collected data

N denotes the total number of participants who should have available data at each of the visits at the point of censoring. Total (N) does not exclude participants who have withdrawn from the study or died.

5.3.3 Home spirometry

104 participants (54.5%) consented to blinded home spirometry and further analyses are presented in section 6.3. Measurements obtained from home spirometry were used to calculate FVC values at follow up visits, where hospital spirometry was missing, in a hierarchical approach. 27 participants had additional 3-month home spirometry measurements, and 18 participants had 12-month measurements (Table 5-4).

	Hospital spirometry	Total (Hospital + Home)
Baseline FVC	189	190
3m FVC	114	141
12m FVC	60	78
24m FVC	27	29

Table 5-4 - Summary of spirometry data obtained.

Hospital spirometry was preferred, but where unavailable, home spirometry values were used to calculate Δ3m and Δ12m FVC.

5.3.4 IPF vs. non-IPF

5.3.4.1 Baseline demographics

63 participants with IPF and 128 with non-IPF were recruited and comparisons between the two groups are included in this section. The baseline demographics of the 191 participants are shown in Table 5-5, and the age distribution in Figure 5-8. No differences were observed in demographics between non-IPF and IPF ILD. The mean age of participants was in the seventh decade, with most male and of white ethnicity, and over half had a smoking history. A family history of ILD in a first or second degree relative was reported in fewer participants with non-IPF compared with IPF (8.6% vs. 15.9%), though differences were not significant. The median time from diagnosis to study recruitment was shorter in non-IPF ILD (5.1 months vs. 8.8 months; p=0.03). No differences in baseline characteristics were observed between participants with IPF and progressive non-IPF.

	All	IPF	Non-IPF	P value (IPF vs non IPF)	Progressive non-IPF	P value (IPF vs prog non-IPF
n	191	63	128		26	
Age, mean (SD)	72.5 (8.1)	72.6 (7.9)	72.4 (8.3)	0.83	71.8 (8.0)	0.64
Male, (%)	137 (71.7)	48 (76.2)	89 (69.5)	0.22	19 (73.1)	0.79
Ethnicity, No. (%),						
White	182 (95.3)	63 (100)	119 (93)		24 (92.3)	
Black	3 (1.6)	0	3 (2.3)	0.14	1 (3.9)	0.08
Asian	6 (3.1)	0	6 (4.7)		1 (3.9)	
Smoking status, No (%)						
Current/Ex	126 (66.0)	38 (60.3)	84 (68.7)	0.26	21 (80.8)	0.09
Never	65 (34.0)	25 (39.7)	40 (31.3)		5 (19.2)	
Family history of ILD, No. (%)	21 (11)	10 (15.9)	11 (8.6)	0.15	2 (7.7)	0.50
Time since diagnosis, months (median IQR)	5.6 (2.1-12.2)	8.8 (3.2-15.2)	5.1 (1.9-10.2)	0.03	7.4 (1.8-11.1)	0.70

Table 5-5 – Baseline demographics of recruited participants stratified by IPF and non-IPF.

All values presented as absolute numbers and percentages in brackets. Age presented using mean and standard deviation, and time to diagnosis in median and IQR. n denotes the total number of participants included in each group. T-test used to compare means, Wilcoxon rank sum to compare medians, and Fisher's tests to compare proportions. Significant p values using a threshold of 0.05 highlighted in red. Further analyses restricted to participants with progressive non-IPF ILD performed and comparisons with IPF presented using p values.

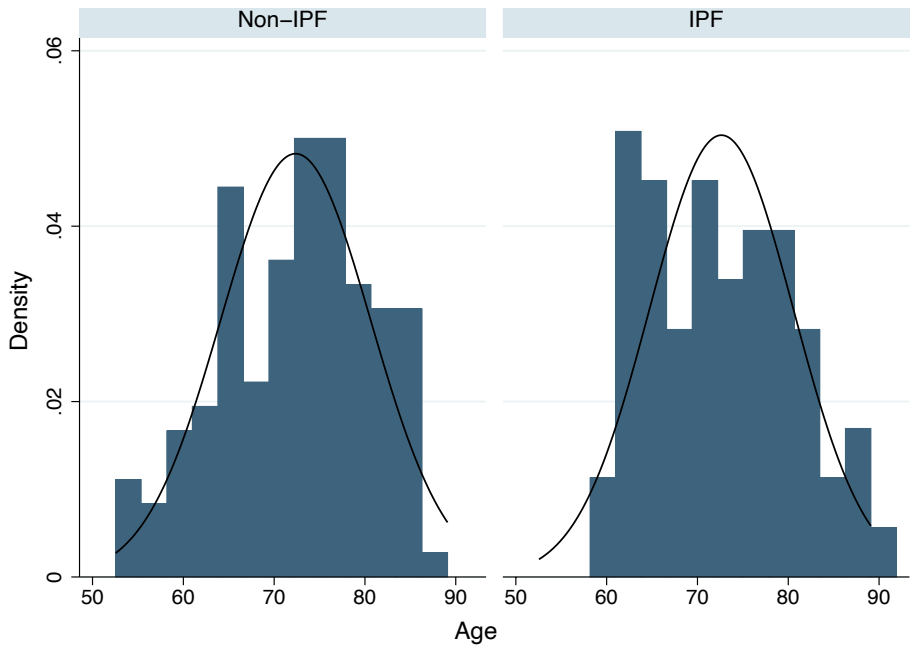


Figure 5-8 - Age distribution of included participants stratified by non-IPF and IPF

5.3.4.2 Baseline physiology

Baseline physiological variables including FVC, DL_{CO} and 6MWD are reported in Table 5-6. In both IPF and non-IPF, baseline FVC percent predicted (81.0% vs 82.2%; p=0.68), DL_{CO} (55.0% vs. 57.3%; p=0.39) and total six-minute distance walked (321m vs. 315m; p=0.72) were well matched. A greater proportion of participants with IPF had moderate-severe impairment of their gas transfer (DL_{CO} < 55% predicted) compared with non-IPF, though differences were not statistically significant. Participants with progressive non-IPF had more severe lung function impairment compared with IPF, but differences were not statistically significant.

	Baseline Physiological variable	All	IPF	Non-IPF	P value (IPF vs non IPF)	Prog non-IPF	P value (IPF vs prog non-IPF)
FVC	n	189	62	127		25	
	FVC, L (SD)	2.90 (0.88)	2.97 (0.89)	2.87 (0.87)	0.45	2.83 (1.06)	0.54
	FVC % pred (SD)	81.8 (18.3)	81.0 (17.7)	82.2 (18.6)	0.68	76.6 (20.3)	0.98
	Mild, >80% predicted, No. (%)	97 (51.3)	33 (53.2)	64 (50.4)	0.76	9 (36.0)	0.16
	Moderate/Severe (≤80%), No. (%)	92 (48.6)	29 (47.6)	63 (49.6)		16 (64.0)	
DL_{CO}	n	180	58	122		23	
	DLCO, %predicted (SD)	56.6 (16.9)	55.0 (15.9)	57.3 (15.3)	0.39	49.3 (16.1)	0.15
	Mild, >55% predicted (%)	92 (51.1)	26 (44.8)	66 (54.1)	0.27	7 (30.4)	0.24
	Moderate/Severe ≤55% predicted	88 (48.9)	32 (55.2)	56 (45.9)		16 (69.6)	
6MWD	n	182	58	124		26	
	Mean distance (SD)	317 (109)	321 (124)	315 (101)	0.72	336 (106)	0.58
	>300m, No. (%)	103 (56.6)	31 (53.5)	72 (58.1)	0.63	17 (65.4)	0.35
	≤300m, No. (%)	79 (43.4)	27 (46.5)	52 (41.9)		9 (34.6)	

Table 5-6 - Baseline physiological variables (FVC, DL_{CO}, 6MWD) of recruited participants stratified by IPF and non-IPF.

Baseline physiology was stratified according to frequently used criteria in ILD management and/or ILD GAP criteria. Values presented as absolute numbers and percentages in brackets, or as mean and standard deviations. Comparisons between IPF and ILD subtype were performed using t-tests for means and fishers test for proportions, p values presented. n denotes the total number of participants included in each group. Further analysis restricted to non-IPF participants with progressive disease were performed, and p values presented.

5.3.4.3 Baseline FBC

Haemoglobin (Hb) levels were lower in non-IPF compared with IPF (136g/L vs. 144g/L; p=0.003), but no differences between groups were observed for either the platelet, white cell count or its differentials.

FBC	All	IPF	Non-IPF	P value (IPF vs non IPF)	Prog non-IPF
Hb	138 16)	144 (13)	136 (16)	0.003	137 (14)
WCC	8.4 (2.4)	8.5 (2.2)	8.4 (2.5)	0.81	9.1 (2.5)
Neutrophils	5.6 (2.1)	5.5 (2.0)	5.6 (2.3)	0.74	6.2 (2.5)
Lymphocytes	1.8 (0.8)	1.9 (0.8)	1.8 (0.7)	0.21	1.9 (0.7)
Monocytes	0.7 (0.3)	0.7 (0.3)	0.7 (0.3)	0.45	0.7 (0.3)
Eosinophils	0.27 (0.)	0.29 (0.21)	0.27 (0.29)	0.61	0.27 (0.27)
Platelets	248 (65)	246 (62)	249 (67)	0.75	240 (49)

Table 5-7 – Baseline full blood count (FBC) stratified by IPF and non-IPF.

P values to compare means between IPF and non-IPF obtained using t-tests. Significant p values using a threshold of 0.05 highlighted in red.

5.3.4.4 Baseline QoL questionnaires

All participants had baseline questionnaire scores for five separate items. For each questionnaire, there was no difference in scores between IPF, non-IPF, and progressive non-IPF.

	Baseline	All	IPF	Non-IPF	P value (IPF vs non IPF)	Prog non-IPF	P value (IPF vs prog non IPF)
	N, total	191	63	128		26	
MRC	Median (IQR)	2 (2-2)	2 (2-2)	2 (2-2)	0.39	2.5 (2-3)	0.53
	Low ≤ median, No. (%)	114 (60.3)	38 (62.3)	76 (59.4)	0.75	13 (50.0)	0.80
	High > median, No (%)	75 (39.7)	23 (37.7)	52 (40.6)		13 (50.0)	
IPARC	Median (IQR)	9 (4-15)	8.5 (4-13)	9 (4-17)	0.71	10 (5-15)	0.35
	Low ≤ median, No. (%)	102 (54)	34 (54.8)	68 (53.5)	0.88	11 (42.3)	0.59
	High > median, No (%)	87 (46)	28 (45.2)	59 (46.5)		15 (57.7)	
EQ5D5L	Median (IQR)	0.75 (0.62-0.88)	0.76 (0.66-0.88)	0.74 (0.60-0.88)	0.27	0.68 (0.60-0.82)	0.11
	Low ≤ median, No. (%)	98 (51.3)	30 (47.6)	68 (53.1)	0.54	16 (61.5)	0.25
	High > median, No (%)	93 (48.7)	33 (52.4)	60 (46.9)		10 (38.5)	
KBILD	Mean (SD)	59.05 (14.91)	58.26 (10.24)	59.43 (16.74)	0.61	55.21 (10.4)	0.21
	Low ≤ mean (SD)	102 (54)	35 (56.5)	67 (52.8)	0.65	18 (69.2)	0.34
	High > mean (SD)	87 (46)	27 (43.5)	60 (47.2)		8 (30.8)	
LCQ	Median (IQR)	17.43 (14.1-19.4)	17.34 (14.1-19.3)	17.46 (13.8-19.5)	0.96	17.53 (15.6-19.52)	0.53
	Low ≤ median, No. (%)	94 (49.7)	32 (52.5)	62 (48.4)	0.64	11 (42.3)	0.48
	High > median, No (%)	95 (50.3)	29 (47.5)	66 (51.6)		15 (57.7)	

Table 5-8 - Baseline questionnaire scores of recruited participants stratified by IPF and non-IPF.

Median and means calculated using all participants. T-tests used to compare means, Wilcoxon rank-sum to compare medians and Fisher tests to compare proportions between IPF and non-IPF, with p values presented. Further analysis restricted to non-IPF participants with progressive disease were performed, and p values presented.

5.3.4.5 Longitudinal change in physiology

141 and 99 participants had available three-month FVC and DL_{CO} respectively. Participants with non-IPF ILD had a mean relative increase in FVC of 0.21% (SD 26.69) and DL_{CO} decrease of 0.41% (22.44) over three-months, whereas in IPF, there was a mean FVC relative decline of 2.62% (SD 7.71), and a DL_{CO} decline of 4.52% (SD 16.67). Differences in lung function

change between groups were not statistically significant. The median FVC decline across all participants over three-months was 1.3%, with similar proportions of non-IPF and IPF demonstrating a greater than median decline over three-months. Similar observations were noted for the median DL_{CO} decline over three-months.

	Change in Physiological variables	All	IPF	Non-IPF	P value (IPF vs non IPF)
FVC	3-months, n	141	42	99	
	Relative percent change, mean (SD)	-0.63 (22.76)	-2.62 (7.71)	0.21 (26.69)	0.50
	Relative percent decline > 5%, n (%)	44 (31.2%)	15 (35.7%)	29 (29.3%)	0.55
	Relative percent decline > median (1.3%), n (%)	71 (50.4)	22 (52.4)	49 (49.5)	0.85
DL_{CO}	3-months, n	99	32	67	
	Relative percent change, mean (SD)	-1.73 (20.76)	-4.52 (16.67)	-0.41 (22.44)	0.36
	Relative percent decline > median (1.7%), n (%)	48 (48.9)	15 (46.9)	33 (49.3)	0.83

Table 5-9 - Change in physiology (FVC and DL_{CO}) over three-months stratified by IPF and non-IPF.

n denotes the number of participants included, with relative decline presented using mean and SD. Number of participants with changes above denoted thresholds presented using absolute numbers and percentages in brackets. T-tests used to compare means, and Fisher's test to compare proportions between IPF and non-IPF. Further analyses were performed using the overall cohort median calculated separately for FVC and DL_{CO}

Fewer participants had available 12-month FVC and DL_{CO} measurements, and though there was a numerically greater mean decline in FVC, DL_{CO} and 6MWD in IPF compared with IPF, the difference was not statistically significant. A similar proportion of participants in both groups had a relative FVC decline greater than 10% over 12-months (38.1% vs. 22.8%; p=0.25). The mean 12-month decline in 6MWD was 57.33m (SD 77.77) in IPF compared with 36.7m (SD 67.96) in non-IPF (p=0.51).

Physiological variables over 12-months		All	IPF	Non-IPF	P value (IPF vs non IPF)
FVC	12-months, n	78	21	57	
	Relative percent change, mean (SD)	-3.33 (13.70)	-5.99 (19.2)	-2.35 (11.1)	0.30
	Absolute ml change, mean (SD)	-123.0 (399.7)	-187.2 (521.9)	-99.4 (346.9)	0.39
	Relative decline > 10%, n (%)	21 (26.9%)	8 (38.1%)	13 (22.8%)	0.25
DL_{CO}	12-months, n	44	10	34	
	Relative percent change, mean (SD)	-2.78 (22.69)	-5.13 (31.07)	-2.09 (20.13)	0.71
6MWD	12-months, n	39	6	33	
	Absolute change, metres, mean (SD)	-39.87 (68.86)	-57.33 (77.77)	-36.70 (67.96)	0.51

Table 5-10 - Change in physiology (FVC, DLCO and 6MWD) over 12-months stratified by IPF and non-IPF.

n denotes the number of participants included, with relative decline presented using mean and SD for FVC and DL_{CO}. 6MWD decline presented using metres. T-tests used to compare means, and Fisher's test to compare proportions between IPF and non-IPF with p values presented.

5.3.4.6 Longitudinal change in QoL questionnaires scores

113 participants provided longitudinal questionnaire data over a follow up period of 12 months. A greater proportion of non-IPF had worsening of their MRC scores compared with IPF, with an overall greater mean increase (mean change of 0.27, SD 0.56 vs. mean change of 0, SD 0.47; p=0.02). For the remaining questionnaires, there were no differences in change over 12-months between IPF and non-IPF.

Questionnaire scores over 12-months		All	IPF	Non-IPF	P value (IPF vs non IPF)
	N, total	113	28	85	
MRC	Mean change (SD)	0.20 (0.55)	0 (0.47)	0.27 (0.56)	0.02
	Stable/Improve, No. (%)	82 (72.6)	25 (89.3)	57 (67.1)	0.03
	Worse, No. (%)	31 (27.4)	3 (10.7)	28 (32.9)	
IPARC	Mean change (SD)	2.15 (6.11)	3.29 (5.12)	1.78 (6.39)	0.26
	Stable/Improve, No. (%)	46 (40.7)	10 (35.7)	36 (42.4)	0.66
	Worse, No. (%)	67 (59.3)	18 (64.3)	49 (57.6)	
EQ5D5L	Mean change (SD)	-0.08 (0.19)	-0.11 (0.24)	-0.07 (0.18)	0.31
	Stable/Improve, No. (%)	46 (41.1)	12 (41.4)	34 (41)	1.00
	Worse, No. (%)	66 (58.9)	17 (58.6)	49 (59)	
KBILD	Mean change (SD)	-3.93 (11.47)	-5.48 (10.45)	-3.42 (11.81)	0.41
	> 5-unit change (%)	47 (42.0)	14 (50.0)	33 (39.3)	0.38
	< 5-unit change (%)	65 (58.0)	14 (50.0)	51 (60.7)	
LCQ	Median change (IQR)	0 (-1.88-1.35)	-0.25 (-2.42-1.62)	0.02 (-1.54-1.04)	0.88
	Stable/Improve, No. (%)	57 (51.8)	13 (46.4)	44 (53.7)	0.52
	Worse, No. (%)	53 (48.2)	15 (53.6)	38 (46.3)	

Table 5-11 - Change in QoL questionnaires over 12-months stratified by IPF and non-IPF.

The change in questionnaire scores over 12-months were stratified into stable/improve or worse. The KBILD questionnaire was stratified by the reported minimal clinically important difference. Comparisons between IPF and non-IPF were performed and p values are presented.

5.3.4.7 Disease outcomes

Greater proportions of individuals with IPF had disease progression (58.6% vs 37.1%), and mortality at one year (14.3% vs 10.2%) though differences did not reach significance.

Outcomes	Diagnosis	N	Outcome, n	Outcome, %	P value (IPF vs non. IPF)
Disease progression	IPF	29	17	58.6	0.07
	Non-IPF	70	26	37.1	
Mortality	IPF	63	9	14.3	0.23
	Non-IPF	128	13	10.2	

Table 5-12 - Number of individuals with disease progression or mortality stratified by IPF and non-IPF.

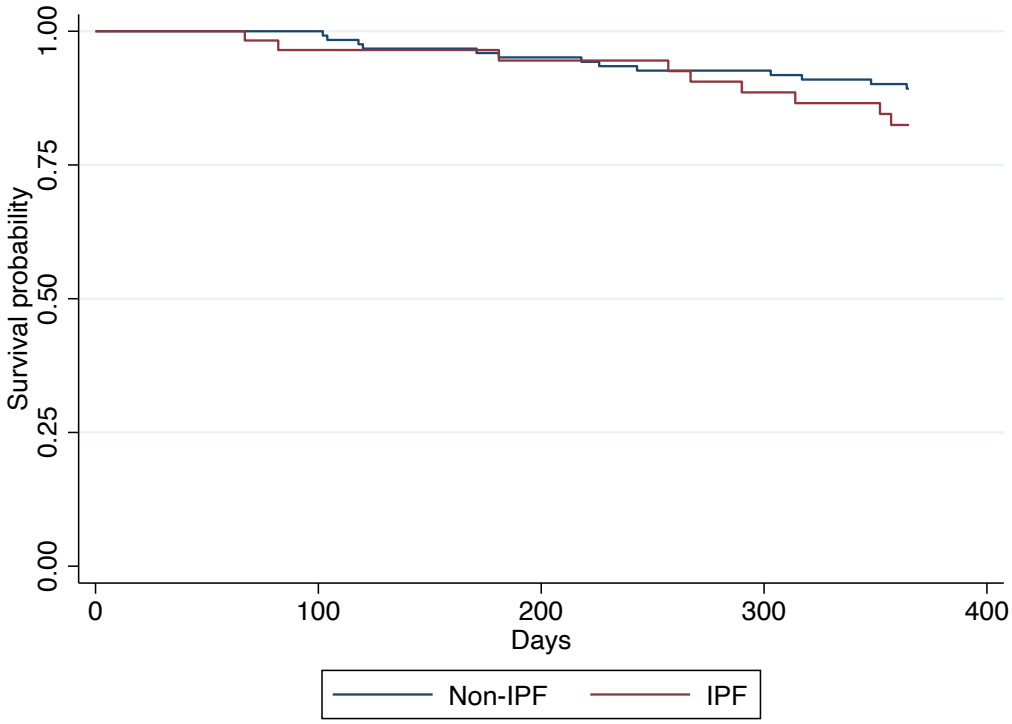


Figure 5-9 - Survival curve of IPF compared with non-IPF

5.3.5 Comparison of ILD subtypes

In this section comparisons between ILD subgroups and IPF for each of the biomarkers (demographic, physiological and QoL) are presented to identify similarities and differences.

5.3.5.1 Baseline demographics

The mean age of participants was similar across all subgroups. Participants with asbestosis were more likely to be male, whereas there was a female preponderance in RA-ILD.

	IPF	Asbestosis	Asbestosis vs IPF (p value)	HP	HP vs IPF (p value)	RA-ILD	RA-ILD vs IPF (p value)	uILD	uILD vs IPF (p value)
n	63	37		34		27		30	
Age, mean (SD)	72.6 (7.9)	75.3 (6.5)	0.09	70.2 (9.2)	0.18	71.5 (7.9)	0.53	71.9 (8.7)	0.70
Male, No. (%)	48 (76.2)	36 (97.3)	0.01	21 (61.8)	0.16	12 (44.4)	0.01	20 (66.7)	0.45
Ethnicity No. (%),									
White	63 (100)	37 (100)		29 (85.3)		25 (92.6)		28 (93.3)	
Black	0	0	1.00	0	0.004	1 (3.7)	0.09	2 (6.7)	0.10
Asian	0	0		5 (14.7)		1 (3.7)		0	
Smoking status									
Current/ex	38 (60.3)	26 (70.3)	0.39	21 (61.8)	1.00	20 (74.1)	0.24	21 (70)	0.49
Never	25 (39.7)	11 (29.7)		13 (38.2)		7 (25.9)		9 (30)	
Family history of ILD, No. (%)	10 (15.9)	1 (2.7)	0.05	5 (14.7)	1.00	1 (3.7)	0.16	4 (13.3)	1.00
Time since diagnosis, months	8.8 (3.2-15.2)	4.8 (2.5-6.7)	0.05	6.8 (1.9-14.2)	0.38	4.9 (2.1-11.1)	0.17	5.2 (1.6-7.4)	0.04

Table 5-13 -Baseline demographics of recruited participants stratified by ILD subtypes

All values presented as absolute numbers and percentages in brackets. Age presented using mean and standard deviation, and time to diagnosis in median and IQR. n denotes the total number of participants included in each group. T-test used to compare means, Wilcoxon rank sum to compare medians, and Fisher's tests to compare proportions. Significant p values using a threshold of 0.05 highlighted in red.

5.3.5.2 Baseline physiology

In comparisons of baseline lung function with IPF, participants with HP had more severe impairment of FVC and DL_{CO}, whereas milder lung function impairment was observed in RA-ILD, though neither of these findings were significant. No differences in baseline six-minute walk distance were found between the subgroups.

Physiological variable		IPF	Asbestosis	Asb vs IPF (p value)	HP	HP vs IPF (p value)	RA-ILD	RA-ILD vs IPF (p value)	uILD	uILD vs IPF (p value)
Baseline FVC	n	62	37		34		26		30	
	FVC % pred (SD)	81.0 (17.7)	83.9 (16.0)	0.41	74.0 (19.0)	0.08	87.5 (16.2)	0.11	84.5 (21.0)	0.40
	Mild, >80% predicted, No. (%)	33 (53.2)	21 (56.8)	0.84	13 (38.2)	0.20	15 (57.7)	0.82	15 (50.0)	0.83
	Moderate/Severe ≤80%), No. (%)	29 (47.6)	16 (43.2)		21 (61.8)		11 (42.3)		15 (50.0)	
Baseline DL_{CO}	n	58	35		32		25		30	
	DLCO, %predicted (SD)	55.0 (15.9)	61.3 (19.1)	0.09	50.3 (17.1)	0.19	60.0 (15.9)	0.19	58.1 (14.9)	0.39
	Mild, >55% predicted (%)	26 (44.8)	20 (57.1)	0.29	12 (37.5)	0.66	17 (68.0)	0.06	17 (56.7)	0.37
	Moderate/Severe ≤55%predicted	32 (55.2)	15 (42.9)		20 (62.5)		7 (32.0)		13 (43.3)	
Baseline 6MWD	n	58	35		34		27		28	
	Mean distance (SD)	321 (124)	289 (99)	0.20	321 (96)	0.98	317 (120)	0.71	328 (115)	0.43
	>300m, No. (%)	31 (53.5)	18 (51.4)	1.00	21 (61.8)	0.52	14 (51.9)	1.00	19 (67.9)	0.25
	<300m, No. (%)	27 (46.5)	17 (48.6)		13 (38.2)		13 (48.1)		9 (32.1)	

Table 5-14 – Baseline physiological variables (FVC, DL_{CO}, 6MWD) stratified by ILD subtypes

Baseline physiology was stratified according to frequently used criteria in ILD management and/or ILD GAP criteria. Values presented as absolute numbers and percentages in brackets, or as mean and standard deviations. Comparisons between IPF and ILD subtype were performed using t-tests for means and fishers test for proportions, p values presented. n denotes the total number of participants included in each group.

5.3.5.3 Baseline FBC

Lower haemoglobin counts were observed in HP and RA-ILD relative to IPF, and a suppressed eosinophil count was seen in RA-ILD.

FBC	IPF	Asbestosis	Asb vs IPF (p value)	HP	HP vs IPF (p value)	RA-ILD	RA-ILD vs IPF (p value)	uILD	uILD vs IPF (p value)
Hb	144 (13)	140 (19)	0.32	136 (14)	0.01	128 (15)	<0.001	140 (13)	0.21
WCC	8.5 (2.2)	7.7 (2.0)	0.11	9.4 (2.7)	0.09	8.8 (2.3)	0.54	7.6 (2.5)	0.11
Neutrophils	5.5 (2.0)	4.9 (1.7)	0.13	5.9 (2.4)	0.03	5.7 (2.1)	0.18	5.3 (2.0)	0.20
Lymphocytes	1.9 (0.8)	1.8 (0.6)	0.27	1.8 (0.8)	0.56	1.8 (0.8)	0.33	1.8 (0.7)	0.41
Monocytes	0.7 (0.3)	0.6 (0.3)	0.53	0.7 (0.3)	0.91	0.6 (0.3)	0.56	0.6 (0.3)	0.29
Eosinophils	0.27 (0.27)	0.37 (0.41)	0.20	0.25 (0.25)	0.42	0.18 (0.16)	0.02	0.24 (0.20)	0.30
Platelets	246 (62)	242 (63)	0.76	251 (55)	0.67	272 (86)	0.11	234 (62)	0.40

Table 5-15 - Baseline full blood count (FBC) stratified by ILD subtypes

P values to compare means between IPF and non-IPF obtained using t-tests. Significant p values using a threshold of 0.05 highlighted in red.

5.3.5.4 Baseline QoL questionnaires

No differences in baseline QoL questionnaire scores were observed between the ILD subtypes, though there was indication (non-statistical) of reduced symptoms in uILD compared with IPF, as measured by IPARC and KBILD scores.

		IPF	Asbestosis	Asb vs IPF (p value)	HP	HP vs IPF (p value)	RA-ILD	RA-ILD vs IPF (p value)	uILD	uILD vs IPF (p value)
	N, total	61	37		34		27		30	
MRC	Median (IQR)	2 (2-2)	2 (2-2)	0.92	2 (2-2)	0.88	2 (1-2)	0.30	2 (2-2)	0.11
	Low (< median), No. (%)	38 (62.3)	19 (51.4)	0.29	19 (55.9)	0.54	18 (66.7)	0.70	20 (66.7)	0.69
	High (> median), No (%)	23 (37.7)	18 (48.7)		15 (44.1)		9 (33.3)		10 (33.3)	
IPARC	Median (IQR)	8.5 (4-13)	10 (5.5-17.5)	0.40	9.5 (5-17)	0.32	9 (3-20)	0.72	6 (3-11)	0.22
	Low (≤ median) No. (%)	34 (54.8)	17 (47.2)	0.53	17 (50)	0.67	14 (51.8)	0.82	20 (66.7)	0.37
	High (> median) No. (%)	28 (45.2)	19 (52.8)		17 (50)		13 (48.2)		10 (33.3)	
EQ5D5L	Index value, median (IQR)	0.76 (0.66-0.88)	0.73 (0.64-0.80)	0.24	0.75 (0.53-0.88)	0.58	0.69 (0.40-0.84)	0.07	0.81 (0.59-1)	0.66
	Low (≤ median) No. (%)	98 (51.3)	22 (59.5)	0.30	17 (50.0)	0.84	17 (63.0)	0.25	12 (40.0)	0.51
	High (> median) No. (%)	93 (48.7)	15 (40.5)		17 (50.0)		10 (37.0)		18 (60.0)	
KBILD	KBILD total, mean (SD)	58.26 (10.24)	56.44 (15.12)	0.48	56.02 (12.96)	0.35	62.78 (19.61)	0.16	64.13 (18.79)	0.06
	KBILD < mean (SD)	35 (56.5)	23 (62.2)	0.68	19 (55.9)	1.00	11 (40.7)	0.25	14 (48.3)	0.51
	KBILD > mean (SD)	27 (43.5)	14 (37.8)		15 (44.1)		16 (59.3)		15 (51.7)	
LCQ	LCQ median (IQR)	17.34 (14.1-19.3)	17.45 (14.4-18.8)	0.77	16.88 (12.8-19.1)	0.46	18.54 (14.7-19.9)	0.29	17.13 (14.2-20.1)	0.75
	LCQ < median, No. (%)	32 (52.5)	18 (48.6)	0.84	18 (52.9)	1.00	10 (37)	0.25	16 (53.3)	1.00
	LCQ > median, No. (%)	29 (47.5)	19 (51.4)		16 (47.1)		17 (63)		14 (46.7)	

Table 5-16 - Baseline questionnaire scores of recruited participants stratified by ILD subtypes

Comparisons between ILD subtypes and IPF performed using Fisher's test for proportions, Wilcoxon test for medians and t-test for means.

5.3.5.5 Longitudinal change in physiology

The mean change in FVC over three-months was similar in each of the subgroups, except RA-ILD where an increase of 12.21% was observed, albeit with substantial variation (SD 56.66). However, over 12-months, there was a mean FVC relative decline of 6.19% (SD 11.92) in RA-ILD which was comparable to IPF (5.99%, SD 19.2). A smaller mean decline in FVC was observed in asbestosis over 12-months compared with IPF (0.54% vs 5.99%), though the difference was not statistically significant (p=0.35). In comparison of gas transfer measurements between ILD subtypes, no differences were noted at either 3-months, or with more restricted sample sizes at 12-months. The mean decline in six-minute walk distance was greatest in HP (88.38m, SD 66.52, p=0.44) with comparable changes in the other subtypes, except uILD, where a significant increase in 6MWD was observed.

Physiological change over 3 months		IPF	Asbestosis	Asb vs IPF (p value)	HP	HP vs IPF (p value)	RA-ILD	RA-ILD vs IPF (p value)	uILD	uILD vs IPF (p value)
FVC	3-months, n	42	27		32		20		20	
	Relative percent change, mean (SD)	-2.62 (7.71)	-1.74 (5.57)	0.61	-4.07 (10.93)	0.50	12.21 (56.66)	0.10	-2.28 (5.80)	0.86
	Relative percent decline > 5%	15 (35.7%)	8 (29.6%)	0.30	12 (37.5)	0.28	4 (20%)	0.92	5 (25%)	0.13
	Relative percent decline > 1.3%	22 (52.4)	12 (44.4)	0.62	17 (53.1)	1.00	9 (45.0)	0.79	11 (55.0)	1.00
DL_{CO}	3-months, n	32	16		23		13		15	
	Relative percent change, mean (SD)	-4.52 (16.67)	-4.99 (17.73)	0.93	5.07 (7.09)	0.17	-0.76 (7.97)	0.44	-3.58 (9.08)	0.84
	Relative percent decline > 1.7%	15 (46.9)	8 (50.0)	1.00	12 (52.2)	0.79	5 (38.5)	0.75	8 (53.3)	0.76

Table 5-17 - Change in physiology (FVC and DL_{CO}) over three-months stratified by IPF and non-IPF.

n denotes the number of participants included, with relative decline presented using mean and SD. Number of participants with changes above denoted thresholds presented using absolute numbers and percentages in brackets. T-tests used to compare means, and Fisher's test to compare proportions between IPF and non-IPF. Further analyses were performed using the overall cohort median calculated separately for FVC and DL_{CO}

Physiological change over 12 months		IPF	Asbestosis	Asb vs IPF (p value)	HP	HP vs IPF (p value)	RA-ILD	RA-ILD vs IPF (p value)	uILD	uILD vs IPF (p value)
FVC	12-months, n	21	14		18		11		14	
	Relative percent change, mean (SD)	-5.99 (19.2)	-0.54 (11.73)	0.35	-2.39 (8.54)	0.47	-6.19 (11.92)	0.97	-1.08 (13.03)	0.41
	Absolute ml change, mean (SD)	-187.2 (521.9)	-67.9 (353.8)	0.46	-62.9 (259.4)	0.36	-259.7 (448.6)	0.70	-51.9 (351.1)	0.40
	Relative decline > 10%, n (%)	8 (38.1%)	3 (21.4%)	0.30	4 (22.2%)	0.28	4 (36.4%)	0.92	2 (14.3%)	0.13
DL_{CO}	12-months, n	10	6		12		6		10	
	Relative percent change, mean (SD)	-5.13 (31.07)	-12.45 (22.54)	0.62	-3.51 (4.80)	0.88	8.11 (22.22)	0.38	-0.29 (6.66)	0.69
6MWD	12-months, n	6	8		8		8		9	
	Absolute change, metres, mean (SD)	-57.33 (77.77)	-59.75 (58.62)	0.95	-88.38 (66.52)	0.44	-26.0 (45.79)	0.36	20.22 (52.91)	0.04

Table 5-18 - Change in physiology (FVC, DL_{CO}, 6MWD) over 12-months stratified by IPF and non-IPF.

n denotes the number of participants included, with relative decline presented using mean and SD. Number of participants with changes above denoted thresholds presented using absolute numbers and percentages in brackets. T-tests used to compare means, and Fisher's test to compare proportions.

5.3.5.6 Longitudinal change in QoL questionnaires

In longitudinal analysis over 12-months, a significant increase in MRC scores relative to IPF were observed for asbestosis and RA-ILD. No difference in scores over 12-months for the remaining questionnaires were demonstrated across the ILD subtypes.

Questionnaire scores over 12-months		IPF	Asbestosis	Asb vs IPF (p value)	HP	HP vs IPF (p value)	RA-ILD	RA-ILD vs IPF (p value)	uILD	uILD vs IPF (p value)
	N, total	28	26		20		17		21	
MRC	Mean change (SD)	0 (0.47)	0.38 (0.57)	0.01	0.2 (0.52)	0.17	0.29 (0.69)	0.10	0.18 (0.50)	0.19
	Stable/Improve, No. (%)	25 (89.3)	15 (57.8)	0.01	15 (75)	0.25	10 (58.8)	0.03	17 (77.3)	0.28
	Worse, No. (%)	3 (10.7)	11 (42.3)		5 (25)		7 (41.2)		(22.7)	
IPARC	Mean change (SD)	3.29 (5.12)	1.78 (6.58)	0.35	2.75 (5.92)	0.74	0.29 (8.27)	0.14	2.05 (4.93)	0.40
	Stable/Improve, No. (%)	10 (35.7)	13 (48.2)	0.42	7 (35)	1.00	8 (47.1)	0.54	8 (38.1)	1.00
	Worse, No. (%)	18 (64.3)	14 (51.9)		13 (65)		9 (52.9)		13 (61.9)	
EQ5D5L	Mean change (SD)	-0.11 (0.24)	-0.11 (0.18)	0.93	-0.09 (0.22)	0.75	-0.01 (0.14)	0.12	-0.03 (0.16)	0.21
	Stable/Improve, No. (%)	46 (41.1)	9 (34.6)	0.78	7 (35)	0.77	8 (47.1)	0.77	10 (50)	0.57
	Worse, No. (%)	66 (58.9)	17 (65.4)		13 (65)		9 (52.9)		10 (50)	
KBILD	Mean change (SD)	-5.48 (10.45)	-4.66 (10.71)	0.77	-2.68 (12.05)	0.39	-3.09 (17.05)	0.57	-2.77 (1.83)	0.33
	> 5-unit change (%)	14 (50.0)	14 (51.9)	1.00	13 (65.0)	0.38	12 (75.0)	0.13	12 (57.1)	0.77
	< 5-unit change (%)	14 (50.0)	13 (48.2)		7 (35.0)		4 (25.0)		9 (42.9)	
LCQ	Median change (IQR)	-0.25 (-2.42-1.62)	0.03 (-1.54-1.2)	0.81	-0.45 (-3.71-1.69)	0.90	0.43 (-0.25-0.79)	0.66	-0.13 (-1.13-0.33)	0.92
	Stable/Improve, No. (%)	13 (46.4)	14 (56)	0.59	9 (47.4)	1.00	11 (64.7)	0.36	10 (47.6)	1.00
	Worse, No. (%)	15 (53.6)	11 (44)		10 (52.6)		6 (35.3)		11 (52.4)	

Table 5-19 - Change in QoL questionnaires over 12-months stratified by ILD subtype

Comparisons between ILD subtypes and IPF performed using Fisher's test for proportions, Wilcoxon test for medians and t-test for means.

5.3.5.7 Disease outcomes

There were fewer deaths and disease progression events in participants with asbestosis compared with IPF. No statistically significant differences in mortality or disease progression were observed for the other ILD subtypes.

Outcomes	IPF	Asbestosis	Asb vs IPF (p value)	HP	HP vs IPF (p value)	RA-ILD	RA-ILD vs IPF (p value)	uILD	uILD vs IPF (p value)
Disease progression	17/29 (58.6%)	4/15 (26.7%)	0.06	11/25 (44%)	0.41	6/13 (46.2%)	0.52	5/17 (29.4%)	0.07
Overall mortality	9/63 (14.3%)	1/37 (3.0%)	0.04	7/34 (20.6%)	0.20	2/27 (7.4%)	0.26	3/30 (10.0%)	0.44

Table 5-20- Number of individuals with disease progression or mortality stratified by IPF and non-IPF

Log-rank tests used to estimate p values for mortality, and Fisher's test for disease progression.

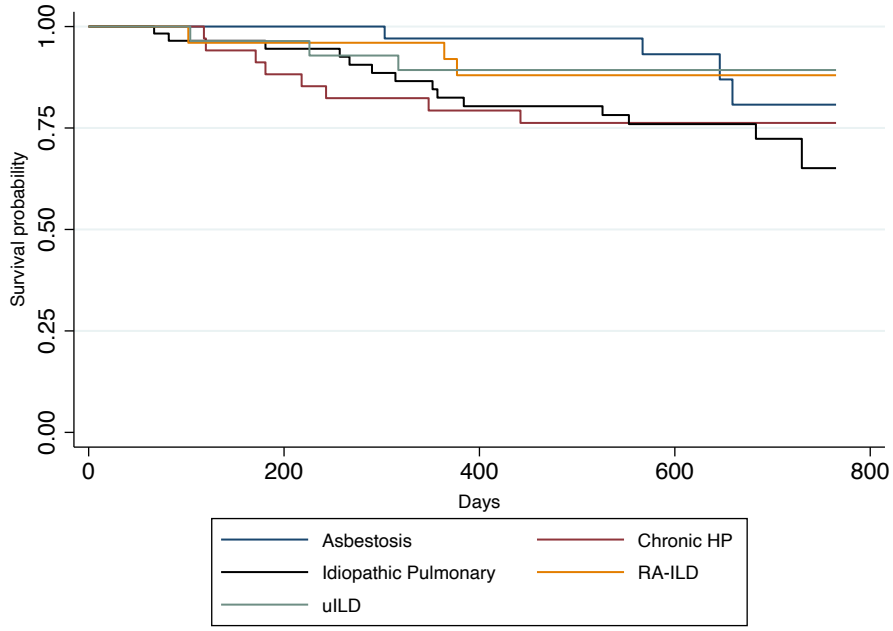


Figure 5-10 - Survival curve of ILD subtypes

5.4 Discussion

5.4.1 Summary of findings

The INJUSTIS study is the largest observational prospective study of non-IPF fibrotic ILD to date. Interim analysis demonstrated several similarities in disease behaviour between IPF and non-IPF. Most participants with non-IPF ILD tended to be in their seventh decade, male, white and with a previous smoking history. Baseline disease severity assessed using lung physiology and the burden of symptoms scored using QoL scores were well-matched across IPF and non-IPF individuals, with mild disease observed in most. When longitudinal disease behaviour was assessed over twelve months, the change in QoL scores were comparable across IPF and non-IPF, but greater declines in lung function were noted in IPF, though differences were not significant. In evaluation of disease outcomes, a substantial proportion of individuals (37.1%) with non-IPF fibrotic ILD had evidence of disease progression at one year, compared with 58.6% observed in IPF, the prototypic progressive fibrotic lung disease. When baseline characteristics and disease severity were analysed in a limited number of non-IPF individuals with established progressive disease, there was greater lung function impairment compared with the remainder of the cohort, though differences were not statistically significant. These findings emphasise the importance of identifying biomarkers that can predict disease outcomes before there is established and irreversible fibrosis.

Exploratory analysis comparing IPF with individual ILD subtypes suggested fewer deaths and disease progression events in asbestosis and uILD, though sample sizes were limited, and further study is required. Specifically in asbestosis there was increased symptom burden relative to IPF, measured using QoL scores at baseline and over 12-months, and reduced six-

minute walk distances, suggesting disease behaviour in asbestosis may be associated with a distinct clinical phenotype. Individuals with HP demonstrated more severe baseline lung function impairment and progressed more rapidly over 3-months relative to IPF, but increased progression was not maintained at 12-months.

5.4.2 Comparison with existing literature

Previous longitudinal studies in non-IPF fibrotic ILD are scarce and INJUSTIS represents the first prospective non-interventional longitudinal study of a mixed cohort of non-IPF, with IPF participants recruited simultaneously for benchmarking purposes. Compared with other interventional longitudinal studies in non-IPF, our cohort of participants were older, and a greater proportion were males[57 164 421]. The mean absolute FVC decline over 12-months in non-IPF ILD was 99mls, which was comparable to the RELIEF study where the FVC decline over 48-weeks in the placebo arm comprising a heterogenous group of fibrotic ILD was -114.4ml[421]. In the placebo arm of the INBUILD study, participants demonstrated a greater mean FVC decline over 52-weeks of -187.8ml, which was similar to the FVC decline observed in the placebo arms of landmark IPF trials including CAPACITY and INPULSIS. However, participants in the INBUILD study were required to meet progression criteria over the preceding 24 months, whereas in the INJUSTIS study, no such enrichment for progressors was applied. Furthermore, our cohort consisted of individuals with milder disease as evidenced by a mean baseline FVC of 82.2% predicted compared with an FVC of 69.3% the INBUILD study. In longitudinal follow up of questionnaire scores, the mean absolute decline in the total score on the KBILD questionnaire over 12-months was 3.42 points, which was greater than the 0.79 points observed in the INBUILD cohort. A possible explanation may be

the milder disease severity of our cohort as evidenced by a baseline KBILD score of 59.4 vs. 52.3, which meant participants started with lower symptom burden, and thus had more to lose on the KBILD questionnaire scoring. Approximately 10% of participants with non-IPF ILD in our study died at one-year censoring, which is slightly higher than the 8% mortality reported in the RELIEF study and 5.1% in the INBUILD study. A handful of deaths in our cohort were attributable to COVID-19 which may help explain the differences in mortality observed. Regardless, the absolute number of individuals who died was small, and thus differences in mortality between studies are unlikely to be of particular significance.

5.4.3 Limitations

The interim results of the INJUSTIS study should be interpreted in the context of several limitations. The COVID-19 pandemic has had considerable impact on the study with several missed follow up visits. As this was a descriptive analysis of the cohort, no imputation was applied for missing values, though this should be considered during the final analysis, especially as 24-month visit data is expected to be available in the future as the impact of the pandemic eases. Disease progression is a key study endpoint, and for the purposes of the interim analysis to maximise 12-month FVC data to correctly categorise progressors, home spirometry measurements were used in lieu of missing hospital spirometry. Whilst consistency of measurement would be preferable, home spirometry has been demonstrated to be an accurate and reliable alternative to hospital spirometry in fibrotic ILD, with further details presented in Chapter 6. To enable robust analyses and the correct application of imputation algorithms in the final dataset, reasons for missed visits are being collected.

Mechanisms underlying acute exacerbations and progressive disease remain unclear, but viral infections may represent potential triggers. As participants with ILD were generally shielding throughout the pandemic, exposure to agents which may have otherwise influenced disease progression were absent. Conversely, several individuals succumbed to COVID-19 who otherwise may have not represented disease progressors. To overcome these limitations in the final analysis, subgroup analyses according to pre-pandemic, during-pandemic and post-pandemic will be performed, and the findings compared.

A further limitation is possible confounding from anti-fibrotics and immunomodulatory therapies in IPF and non-IPF respectively, which may have altered individual progression status. Less than a quarter of IPF participants were receiving anti-fibrotics (14/63), but a greater proportion with non-IPF were receiving corticosteroids or immunomodulatory therapies (51/128). However, there remains insufficient evidence to suggest immunomodulatory therapies alter the rate of progression in fibrotic ILD, so this is unlikely to have significantly impacted the results. Nonetheless, future analysis should include adjustment for treatments that may be confounders.

Change in biomarkers (physiology and QoL scores) from baseline to 3 and 12-months were reported, but participants who died before reaching these time-points were excluded without imputation, potentially underestimating the mean change. Survivor bias influences longitudinal data, as those participants who survive the longest contribute the most data. However, the number of participants who died at one-year was modest (11.5%) with similar

proportions of deaths in IPF and non-IPF, and therefore it is unlikely the absence of longitudinal data due to death would significantly alter comparisons across IPF and non-IPF.

The possibility of lead-time bias must be considered. Certain ILD subtypes are more likely to be detected earlier in their disease course due to the rigorous screening associated with their underlying initial disease, such as RA-ILD in rheumatoid-arthritis. Conversely individuals with IPF and other idiopathic interstitial pneumonias typically have worsening symptoms over several years before they are diagnosed. An earlier diagnosis can result in lead-time bias which distorts the estimation of survival time from a particular disease. Moreover, in this cohort, once diagnosed, individuals with IPF had a greater median time from MDT to enrolment in the study compared with individuals with non-IPF. A greater time to diagnosis has the potential to enrich the cohort for prevalent cases over incident cases, who generally have milder forms of disease and thus can also represent a source of survival bias. The delay in recruitment attributable to the COVID-19 pandemic is further likely to enrich for prevalent cases and this limitation must be considered upon analysis of the complete dataset. Further sensitivity analyses for survival estimates using the date of diagnosis as time zero rather than date of enrolment may help mitigate this limitation.

A further limitation associated with diagnosis is the lack of central review and independent verification of CT images. Clinical phenotyping of ILD can be challenging, particularly in non-IPF ILD, where there remains an absence of consensus diagnostic guidelines. Although, CT imaging holds a key role in defining fibrotic ILD alongside the presence/absence of other supporting clinical features, agreement between radiologists is often poor[39], and diagnostic

decisions are often deferred to the MDT, which remains the “gold standard”. To ensure our study cohort were representative of the broader non-IPF ILD population, and to maximise the generalisability of our findings, an MDT discussion was mandatory for study inclusion.

An important consideration for the analysis of the complete dataset is the power calculation. Initial power calculations assumed 50% of individuals would demonstrate disease progression regardless of disease subtype. However, interim data presented in this chapter suggests approximately one-third (37.1%) of the non-IPF cohort developed disease progression, and therefore the study may be underpowered. Since power calculations were based on change in MMP-7 over three-months which is the most conservative biomarker, it is likely the study will be powered for the majority of biomarkers of interest.

5.5 Summary

INJUSTIS is an ongoing prospective multi-centre observational cohort that aims to evaluate longitudinal disease behaviour in fibrotic non-IPF ILD, evaluate the role of commonly measured variables as prognostic biomarkers, and identify blood biomarkers associated with progressive fibrotic lung disease irrespective of aetiology. This chapter details the study methodology, provides a recruitment update, and presents baseline and longitudinal interim data. Key findings suggest a significant proportion of individuals with non-IPF fibrotic ILD have progressive phenotypes that are comparable in disease behaviour in IPF. These findings emphasise the importance of identifying biomarkers irrespective of ILD subtype that can predict disease outcomes before there is established and irreversible fibrosis

Chapter 6 An investigation into biomarkers of poor outcomes in interstitial lung disease

6.1 Introduction

In the previous chapter, I reported details of the INJUSTIS study methodology, a recruitment update and a descriptive analysis of the cohort recruited to date. This chapter is divided into two sections, and both evaluate the relationship between biomarkers and disease outcomes in fibrotic ILD utilising INJUSTIS interim data. In chapter 4, I explored the association between demographics and physiological measurements with disease outcomes in individuals with IPF using IPD meta-analysis. The key findings suggested baseline and three-month change in physiological variables, particularly FVC, were accurate indicators of poor outcomes. It is likely such biomarkers offer prognostic insights beyond IPF to other fibrotic ILDs, though the vast majority of previous studies exploring the association between demographics and outcomes in ILD have been retrospective single-centre cohorts. INJUSTIS offers a prospective longitudinal cohort allowing serial assessment of respiratory health to identify factors associated with poor outcomes. In the first section of this chapter, I examine demographic factors, questionnaire scores and physiology, both at baseline and their change over 3-months, to ascertain their association with outcomes in fibrotic ILD.

The second section evaluates the role of home spirometry in fibrotic ILD. Home spirometry offers opportunity for more frequent lung function measurement and earlier detection of disease behaviour, thus offering potential as a prognostic biomarker and early-phase clinical trial endpoint in fibrotic ILD. An increasing number of studies in IPF have evaluated the use of home spirometry and found changes in FVC as early as three-months can predict disease

progression and survival, but little data exists regarding the acceptance of daily spirometry in non-IPF [183 205 422 423]. The intention here is to evaluate the prognostic potential of home spirometry in the INJUSTIS cohort of mixed fibrotic ILD. Furthermore, considering the COVID-19 pandemic, I investigate the feasibility and reliability of home spirometry as an alternative to hospital spirometry. The key findings have been published as part of "Clinical Utility of Home versus Hospital Spirometry in Fibrotic ILD: Evaluation following INJUSTIS Interim Analysis" in the Annals of the ATS Journal.

6.1.1 Aims of chapter

1) To investigate the association between demographic factors (age, sex, and smoking history) and disease outcomes in fibrotic ILDs
2) To investigate the role of physiology as prognostic markers by examining the association between baseline and short-term change and clinical outcomes
3) To investigate the role of QoL scores as prognostic markers in fibrotic ILD by examining the association between baseline and short-term change in measurements and clinical outcomes
4) To explore the association of above commonly measured variables with outcomes separately in IPF and non-IPF to identify commonalities for poor disease outcomes
5) To assess the feasibility of home spirometry as an alternative to hospital spirometry
6) To evaluate the prognostic potential of home spirometry in fibrotic ILD
7) To determine the feasibility of home spirometry as an endpoint in non-IPF interventional trials

6.2 Relationship of biomarkers with outcomes

6.2.1 Methods

The relationship between measured variables with disease progression defined as an FVC relative decline ≥ 10% or death at 12 months, and mortality censored at one year, was estimated, and compared between IPF and non-IPF. Baseline physiological measurements were dichotomised into two severity groups (FVC above or less than 80% predicted, DL_{CO} above or less than 55% predicted, and 6MWD above or less than 300m) according to commonly used criteria and the ILD GAP model [419]. QoL scores were dichotomised according to the median to overcome limited sample sizes. Baseline physiological measurements and QoL scores and their association with disease progression were estimated using Fisher's test, and association with mortality estimated using the log-rank test alongside Kaplan-Meier survival curves, with a p value < 0.05 considered statistically significant. Change over three-months relative to the median was stratified into low and high for physiological variables, and into stable vs. worse for QoL scores to overcome limited sample sizes, and associations with outcomes were estimated. A MCID of a 5-unit change in total score was applied specifically for the KBILD questionnaire.

6.2.2 Results

6.2.2.1 Baseline demographics

Age, gender, or smoking status were not associated with mortality (Table 6-1) or disease progression (Table 6-2) in fibrotic ILD. In IPF specifically, females tended to have poorer outcomes compared with males, though this association was not statistically significant. A greater proportion of participants with no previous smoking, and participants above the age of 65 had poorer outcomes in IPF compared with non-IPF, though absolute differences were small. For the remaining demographic factors, the association with outcomes was similar in both IPF and non-IPF.

Demographics	**Overall mortality, No. (%)**	**P value (mortality)**	**IPF mortality, No. (%)**	**Non-IPF mortality, No. (%)**	**P value (IPF vs. non-IPF)**
Age					
≤65	7/42 (16.7)	0.11	1/13 (7.7)	6/29 (20.7)	0.50
>65	15/149 (10.1)		8/50 (16.0)	7/99 (7.1)	0.049
Gender					
Male	16/137 (11.7)	0.99	6/48 (12.5)	10/89 (11.2)	0.58
Female	6/54 (11.1)		3/15 (20.0)	3/39 (7.7)	0.15
Smoking					
Current/ex	13/126 (10.3)	0.33	3/38 (7.9)	10/88 (11.4)	0.68
Never	9/65 (13.9)		6/25 (24.0)	3/40 (7.5)	0.03

Table 6-1 - Association of demographic factors with mortality.

Demographic factors were stratified, and the number of deaths tabulated with logrank tests applied to test associations with mortality and p values presented. Further analyses comparing IPF and non-IPF were performed with p values shown for comparisons.

Demographics	Overall disease progressors, No. (%)	P value (disease progression)	IPF disease progression, No. (%)	Non-IPF disease progression, No. (%)	P value (IPF vs. non-IPF)
Age					
≤65	11/25 (44.0)	1.00	3/5 (60.0)	8/20 (40.0)	0.62
>65	32/74 (43.2)		14/24 (58.3)	18/50 (36.0)	0.08
Gender					
Male	30/72 (41.7)	0.65	11/21 (52.4)	19/51 (37.3)	0.30
Female	13/27 (48.2)		6/8 (75.0)	7/19 (36.8)	0.10
Smoking					
Current/ex	29/62 (46.8)	0.41	8/15 (53.3)	21/47 (44.7)	0.77
Never	23/37 (62.2)		9/14 (64.3)	5/23 (21.7)	0.02

Table 6-2 – Association of demographic factors and disease progression.

Demographic factors were stratified, and the number of disease progression events tabulated with Fishers tests to test associations with disease progression and p values presented. Further analyses comparing IPF and non-IPF were performed with p values shown for comparison.

6.2.2.2 Baseline physiology

An FVC below 80% predicted at baseline compared with an FVC > 80% was associated with increased mortality (19.6% vs. 2.1%; p<0.001) and disease progression (57.1% vs. 27.1%; p=0.004), with no differences between IPF and non-IPF. Baseline FVC evaluated as a continuous variable adjusted for age, sex, and smoking status, was associated with a 34% increased risk of mortality (aHR 1.34; 95%CI 1.16-1.54) and a 16% increased likelihood of disease progression (aOR 1.16; 95%CI 1.03-1.31), for each 5% decrement in percent predicted FVC.

A baseline DL_{CO} below 55% predicted was associated with increased mortality (15.9% vs 2.2%) and disease progression (52.0% vs. 25.6%; p=0.01), with no differences observed between IPF and non-IPF. DL_{CO} as a continuous variable adjusted for age, sex, smoking

status, and baseline FVC was associated with a 37% increased risk of mortality (aHR 1.37; 95%CI 1.09-1.72) and 19% increased likelihood of disease progression (aOR 1.19; 95%CI 1.03-1.38) per 5% relative decrement. 6MWD, either dichotomised using a threshold of 300m or evaluated as a continuous variable was not associated with disease outcomes in either IPF or non-IPF.

Baseline physiology	Overall mortality, No. (%)	P value (mortality)	IPF mortality, No. (%)	Non-IPF mortality, No. (%)	P value (IPF vs. non-IPF)
FVC					
Mild >80% predicted	2/97 (2.1)	<0.001	1/33 (3.0)	1/65 (1.6)	0.52
Moderate/Severe ≤80% predicted	18/92 (19.6)		7/29 (24.1)	11/63 (17.5)	0.30
DLCO					
Mild, >55% predicted (%)	2/92 (2.2)	<0.001	1/26 (3.9)	1/66 (1.5)	0.40
Moderate/Severe ≤55% predicted	14/88 (15.9)		5/32 (15.6)	9/56 (16.1)	0.77
6MWD					
>300m, No. (%)	10/103 (9.7)	0.33	3/31 (9.7)	7/72 (9.7)	0.83
≤300m, No. (%)	11/79 (13.9)		5/27 (18.5)	6/52 (11.5)	0.28

Table 6-3 - Association of baseline physiology and overall mortality.

Baseline physiology was stratified according to frequently used criteria in ILD management and/or ILD GAP criteria, and the number of deaths were tabulated. Associations for physiological variables and mortality were estimated using the logrank test and presented using p values. Further analyses comparing IPF and non-IPF were performed with p values shown for comparisons.

Baseline physiology	Overall disease progressors, No. (%)	P value (disease progression)	IPF disease progression, No. (%)	Non-IPF disease progression, No. (%)	P value (IPF vs. non-IPF)
FVC					
Mild >80% predicted	13/48 (27.1)	0.004	4/13 (30.8)	9/35 (25.7)	0.73
Moderate/Severe ≤80% predicted	28/49 (57.1)		12/15 (80.0)	16/34 (47.1)	0.06
DLCO					
Mild, >55% predicted (%)	11/43 (25.6)	0.01	4/10 (40.0)	7/33 (21.2)	0.25
Moderate/Severe ≤55% predicted	26/50 (52.0)		10/16 (62.5)	16/34 (47.1)	0.37
6MWD					
>300m, No. (%)	25/62 (40.3)	0.52	8/16 (50.0)	17/46 (40.0)	0.39
≤300m, No. (%)	17/35 (48.6)		8/12 (66.7)	9/23 (39.1)	0.16

Table 6-4 - Association of baseline physiology and disease progression.

Baseline physiology was stratified according to frequently used criteria in ILD management and/or ILD GAP criteria, and the number of disease progression events (defined as an FVC relative decline ≥ 10% or death within 12 months) were tabulated. Associations for physiological variables and disease progression were estimated using the Fisher's test and presented using p values. Further analyses comparing IPF and non-IPF were performed with p values shown for comparisons.

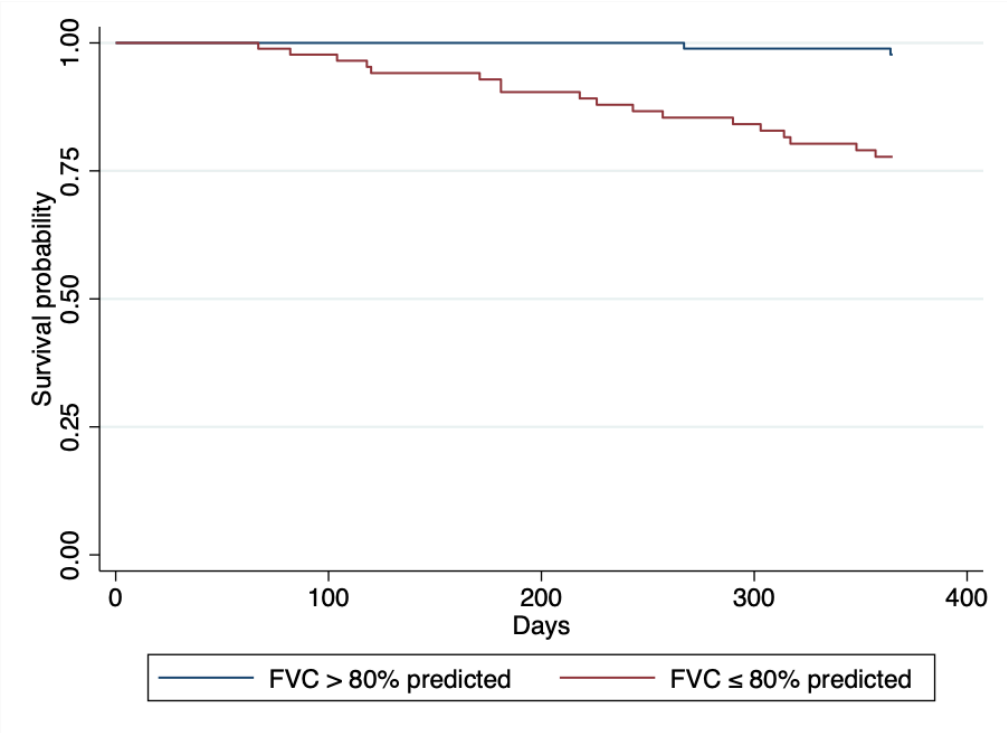


Figure 6-1 - Survival curve for the association between baseline FVC and mortality for all individuals with fibrotic ILD

Survival curves were stratified by a baseline FVC of 80% predicted based on commonly used criteria in ILD management.

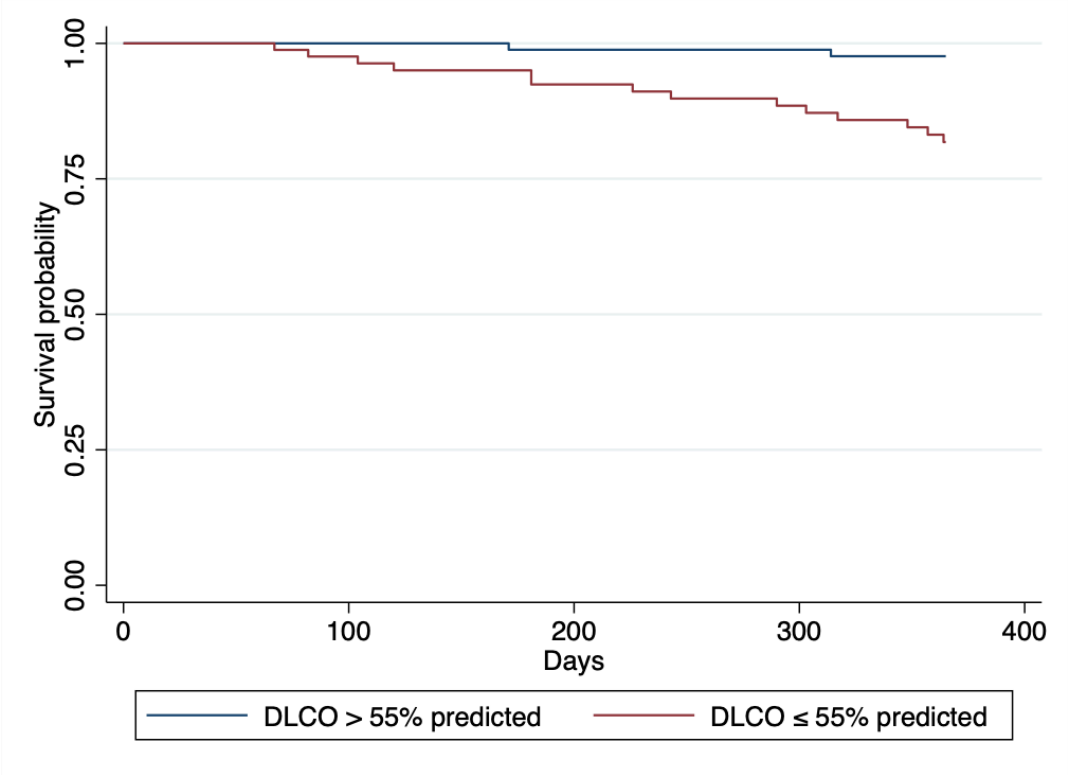


Figure 6-2 - Survival curve for the association between baseline DL_{CO} and mortality for all individuals with fibrotic ILD

Survival curves were stratified by a baseline DL_{CO} of 55% predicted based on ILD GAP criteria.

6.2.2.3 Baseline QoL questionnaires

The association of disease outcomes with baseline questionnaires scores stratified by the mean or median were investigated (Table 6-5 and 6-6). A high MRC score and a low K-BILD score suggested an increased likelihood of disease progression, though findings did not reach statistical significance. Differences in proportions of participants with disease progression stratified by baseline IPARC, LCQ and EQ5D5L were noted between IPF and non-IPF. None of the questionnaires were associated with increased mortality.

Baseline QoL	Overall mortality, No. (%)	P value (mortality)	IPF mortality, No. (%)	Non-IPF mortality, No. (%)	P value (IPF vs. non-IPF)
MRC					
Low (≤median)	11/114 (9.7)	0.28	5/38 (13.2)	6/76 (7.9)	0.26
High (>median)	11/75 (14.7)		4/23 (17.4)	7/52 (13.5)	0.50
IPARC					
Low (≤median)	12/102 (11.8)	0.96	7/34 (20.6)	5/68 (7.4)	0.02
High (>median)	10/87 (11.5)		2/28 (7.1)	8/59 (13.6)	0.49
KBILD					
Low (≤mean)	13/102 (12.8)	0.54	3/35 (8.6)	10/67 (14.9)	0.51
High (>mean)	9/87 (10.3)		6/27 (22.2)	3/60 (5.0)	0.006
LCQ					
Low (≤median)	10/94 (10.6)	0.70	6/32 (18.8)	4/62 (6.5)	0.04
High (>median)	12/95 (12.6)		3/29 (10.3)	9/66 (13.6)	0.89
EQ5D5L					
Low (≤median)	10/98 (10.2)	0.65	2/30 (6.7)	8/68 (11.8)	0.66
High (>median)	12/93 (12.9)		7/33 (21.2)	5/60 (8.3)	0.06

Table 6-5 - Association of baseline QoL questionnaires and overall mortality.

Baseline questionnaires were stratified by the mean or median and the number of deaths were tabulated. Associations for each questionnaire and mortality were estimated using the log-rank test and presented using p values. Further analyses comparing IPF and non-IPF were performed with p values shown for comparisons.

Baseline QoL	Overall disease progressors, No. (%)	P value (disease progression)	IPF disease progression, No. (%)	Non-IPF disease progression, No. (%)	P value (IPF vs. non-IPF)
MRC					
Low (≤median)	24/66 (36.4)	0.055	11/20 (55.0)	13/46 (28.3)	0.053
High (>median)	19/33 (57.6)		6/9 (66.7)	13/24 (54.2)	0.70
IPARC					
Low (≤median)	24/59 (40.7)	0.54	13/21 (61.9)	11/38 (38.9)	0.03
High (>median)	19/40 (47.5)		4/8 (50.0)	15/32 (46.9)	1.00
KBILD					
Low (≤mean)	28/53 (52.8)	0.07	10/14 (71.4)	18/39 (46.2)	0.13
High (>mean)	15/46 (32.6)		7/15 (46.7)	8/31 (25.8)	0.19
LCQ					
Low (≤median)	24/49 (49.0)	0.31	13/16 (81.3)	11/33 (33.3)	0.002
High (>median)	19/50 (38.0)		4/13 (30.8)	15/37 (40.5)	0.74
EQ5D5L					
Low (≤median)	20/46 (43.5)	1.00	4/9 (44.4)	16/37 (43.2)	1.00
High (>median)	23/53 (43.4)		13/20 (65.0)	10/33 (30.3)	0.02

Table 6-6 - Association of baseline QoL questionnaires and disease progression.

Baseline questionnaires were stratified by the mean or median and the number of disease progression events (defined as an FVC relative decline ≥ 10% or death within 12 months) were tabulated. Associations for each questionnaire and disease progression were estimated using the Fisher's test and presented using p values. Further analyses comparing IPF and non-IPF were performed with p values shown for comparisons.

6.2.2.4 Longitudinal change in physiology

The median relative decline in FVC over three-months was 1.3%, with a change greater than the median associated with both increased mortality (18.3% vs. 2.9%; p=0.001) and disease progression (53.3% vs. 29.0%; p=0.03), regardless of diagnosis. An FVC decline over 3-months greater than an arbitrarily defined threshold of 5% was observed in approximately one-third of participants and was associated with poor outcome in both IPF and non-IPF. When 3-month FVC change was considered as a continuous variable and adjusted for age, gender, smoking status, and baseline FVC, there was a 29% increased risk of mortality per

2.5% relative FVC decline (aHR 1.29; 95% CI 1.11-1.49), but no unequivocal association with disease progression (aOR 1.11, 95%CI 0.97-1.27). In analyses restricted to non-IPF, three-month FVC change was associated with mortality (aHR 1.23; 95%CI 1.04-1.45), but not disease progression (aOR 1.05; 95%CI 0.94-1.17).

The median DL_{CO} change over three-months was estimated at 1.7%, with changes above the median not associated with increased mortality or disease progression. In analyses of DL_{CO} as a continuous variable adjusted for age, sex, smoking status, baseline FVC and baseline DL_{CO}, change in DL_{CO} over 3-months per 2.5% relative decline was associated with increased mortality (aHR 1.08; 95%CI 1.00-1.18), but not conclusively with disease progression (aOR 1.08; 95%CI 0.99-1.17), with similar estimates observed in analyses restricted to non-IPF.

Physiology	Relative change over 3-months	Overall mortality, No. (%)	P value (mortality)	IPF mortality, No. (%)	Non-IPF mortality, No. (%)	P value (IPF vs. non-IPF)
FVC	Low (≤1.3%)	2/70 (2.9)	0.001	0/20 (0.0)	2/50 (4.0)	1.00
	High (>1.3%)	13/71 (18.3)		4/22 (18.2)	9/49 (18.4)	1.00
	<5%	4/97 (4.1)	<0.001	0/27 (0.0)	4/70 (5.7)	0.22
	≥5%	11/44 (25.0)		4/15 (26.7)	7/29 (24.1)	0.97
DL_{CO}	Low (≤1.7%)	4/51 (7.8)	0.44	1/17 (5.9)	3/34 (8.8)	0.73
	High (>1.7%)	6/48 (12.5)		1/15 (6.7)	5/33 (15.2)	0.39

Table 6-7 - Association of three-month change in physiology and overall mortality.

The relative change over three-months in FVC and DL_{CO} stratified by the cohort median was tabulated according to the number of deaths. Associations for three-month change in physiology and mortality were assessed using the log-rank test and p values presented. Further analyses comparing IPF and non-IPF were performed with p values shown for comparisons.

Physiology	Relative change over 3-months	Overall disease progressors, No. (%)	P value (disease progression)	IPF disease progression, No. (%)	Non-IPF disease progression, No. (%)	P value (IPF vs. non-IPF)
FVC	Low (≤1.3%)	11/38 (29.0)	0.03	2/7 (28.6)	9/31 (29.0)	1.00
	High (>1.3%)	24/45 (53.3)		10/14 (71.4)	14/31 (45.2)	0.12
	<5%	17/56 (30.4)	0.002	3/12 (25.0)	14/44 (31.8)	0.74
	≥5%	18/27 (66.7)		9/9 (100.0)	9/18 (50.0)	0.01
DL_{CO}	Low (≤1.7%)	12/31 (38.7)	0.46	4/9 (44.4)	8/22 (36.4)	0.70
	High (>1.7%)	16/33 (48.5)		6/10 (60.0)	10/23 (43.5)	0.47

Table 6-8 - Association of three-month change in physiology and disease progression.

The relative change over three-months in FVC and DL_{CO} stratified by the cohort median was tabulated according to the number of disease progression events (defined as an FVC relative decline ≥ 10% or death within 12 months). Associations for three-month change in physiology and disease progression were assessed using the Fisher's test and p values presented. Further analyses comparing IPF and non-IPF were performed with p values shown for comparisons.

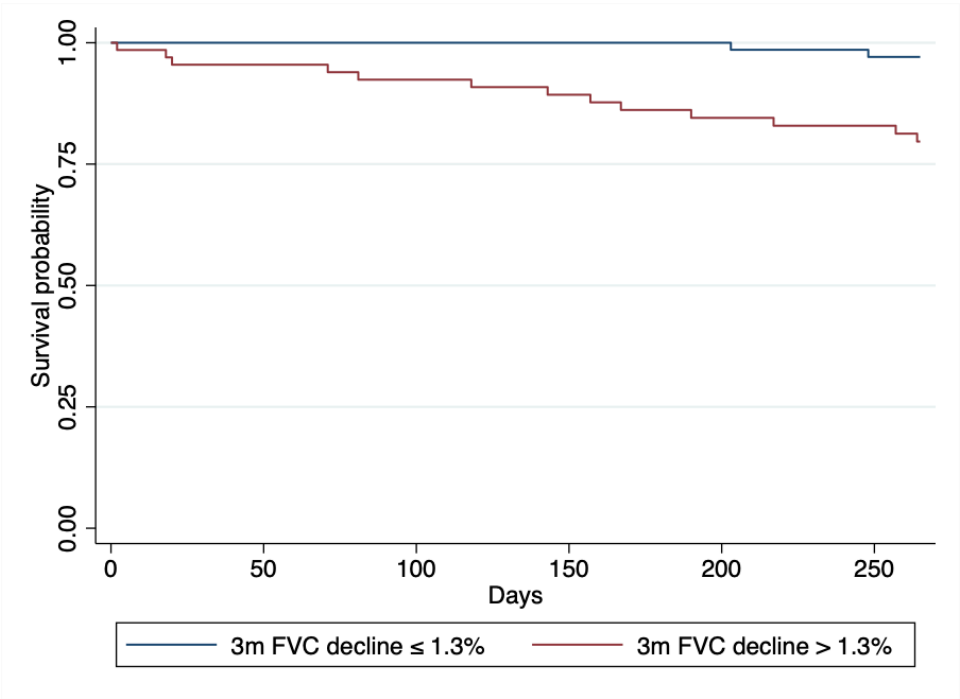


Figure 6-3 - Survival curve for the association between three-month FVC change and mortality for all individuals with fibrotic ILD

The survival curve was stratified by the overall cohort median decline over three-months (1.3%)

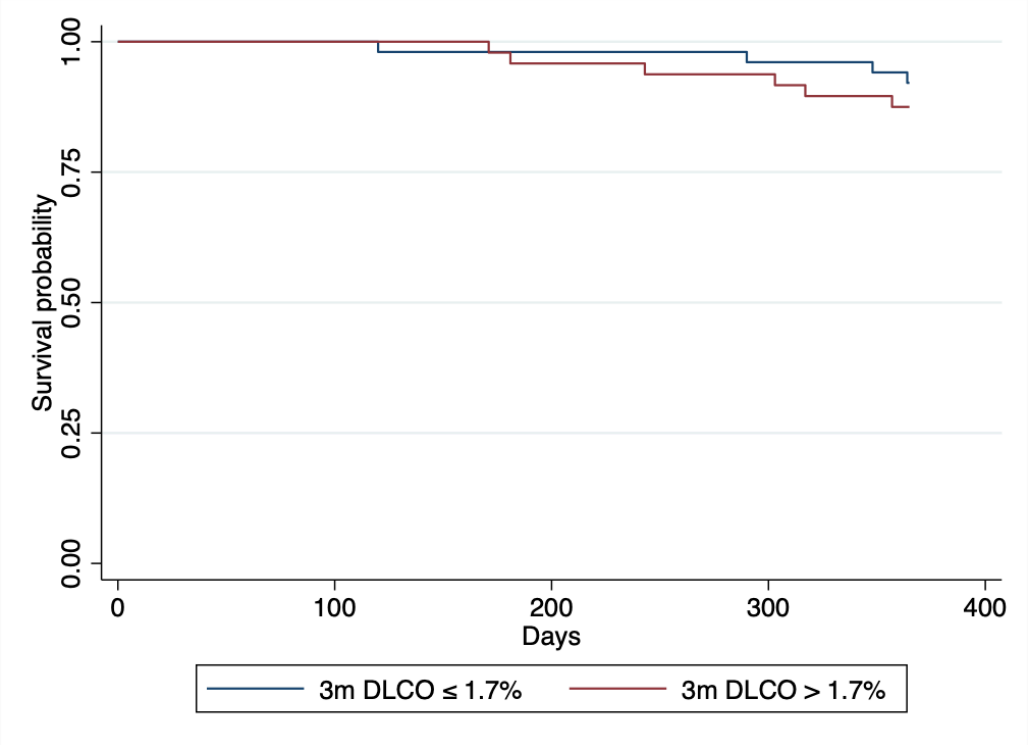


Figure 6-4 - Survival curve for the association between three-month DL_{CO} change and mortality for all individuals with fibrotic ILD.

The survival curve was stratified by the overall cohort median decline over three-months (1.7%)

6.2.2.5 Longitudinal change in QoL questionnaires

Worsening IPARC scores over three-months were associated with increased mortality (Table 6-9), but not disease progression (Table 6-10), with no differences observed between IPF and non-IPF. Longitudinal change in the other questionnaires were not associated with disease outcomes.

Change over 3 months in QoL	Overall mortality, No. (%)	P value (mortality)	IPF mortality, No. (%)	Non-IPF mortality, No. (%)	P value (IPF vs. non-IPF)
MRC					
Stable/Improve	13/122 (10.7)	0.12	5/40 (12.5)	8/82 (9.8)	0.66
Worse	5/23 (21.7)		2/4 (50.0)	3/19 (15.8)	0.20
IPARC					
Stable/Improve	4/75 (5.3)	0.01	2/20 (10.0)	2/55 (3.6)	0.30
Worse	13/68 (19.1)		4/24 (16.7)	9/44 (20.5)	0.75
KBILD					
>5-unit change	6/44 (13.6)	0.66	3/16 (18.8)	3/28 (10.7)	0.38
< 5-unit change	11/100 (11.0)		3/28 (10.7)	8/72 (11.1)	0.92
LCQ					
Stable/Improve	8/72 (11.1)	0.60	4/23 (17.4)	4/49 (8.2)	0.26
Worse	10/70 (14.3)		3/20 (15.0)	7/50 (14.0)	0.98
EQ5D5L					
Stable/Improve	9/84 (10.7)	0.48	4/25 (16.0)	5/59 (8.5)	0.38
Worse	9/63 (14.3)		3/21 (14.3)	6/42 (14.3)	0.95

Table 6-9 - Association of three-month change in physiology and mortality.

The change in questionnaire scores over three-months were stratified into stable/improve or worse, and numbers of deaths were tabulated. The KBILD questionnaire was stratified by the reported minimal clinically important difference. The association between the change in each questionnaire and mortality was calculated using the logrank test and presented using p values. Further analyses were performed to compare IPF and non-IPF, and p values are shown for comparison.

Change over 3 months in QoL	Overall disease progressors, No. (%)	P value (disease progression)	IPF disease progression, No. (%)	Non-IPF disease progression, No. (%)	P value (IPF vs. non-IPF)
MRC					
Stable/Improve	31/71 (43.7)	1.00	13/24 (54.2)	18/47 (38.3)	0.22
Worse	6/15 (40.0)		2/3 (66.7)	4/12 (33.3)	0.53
IPARC					
Stable/Improve	17/44 (38.6)	0.51	6/12 (50.0)	11/32 (34.4)	0.49
Worse	19/40 (47.5)		8/14 (57.1)	11/26 (42.3)	0.51
KBILD					
>5-unit change	14/29 (48.3)	0.49	8/11 (72.7)	6/18 (33.3)	0.06
< 5-unit change	22/56 (39.3)		6/15 (40.0)	16/41 (39.0)	1.00
LCQ					
Stable/Improve	18/40 (45.0)	0.83	9/14 (64.3)	9/26 (34.6)	0.10
Worse	18/44 (40.9)		5/12 (41.7)	13/32 (40.6)	1.00
EQ5D5L					
Stable/Improve	18/47 (38.3)	0.39	7/15 (46.7)	11/32 (34.4)	0.52
Worse	19/39 (48.7)		8/12 (66.7)	11/27 (40.7)	0.18

Table 6-10 - Association of three-month change in QoL questionnaires and disease progression.

The change in questionnaire scores over three-months were stratified into stable/improve or worse, and numbers of disease progressors and non-disease progressors were tabulated. Disease progression was defined as an FVC relative decline of at least 10% or death at 12 months. The KBILD questionnaire was stratified by the reported minimal clinically important difference. The association between changes in each questionnaire and disease progression was calculated and presented using p values. Further analyses were performed to compare IPF and non-IPF, and p values are shown for comparison.

6.3 Home spirometry in Fibrotic ILDs

6.3.1 Methods

6.3.1.1 Study subjects

Participants with MDT confirmed fibrotic ILD recruited into the INJUSTIS study who possessed a smartphone were offered home spirometry. Hospital spirometry measurements according to international guidelines[418] were simultaneously collected at baseline, 3, 12 and 24 months. Participants were followed until death, study completion (24 months), or until they were censored on 6th August 2021.

6.3.1.2 Details of spirometer

Eligible and consenting participants were provided with a portable handheld spirometer (MIR Spirobank Smart) linked via Bluetooth to a smartphone application. The MIR Spirobank Smart spirometer meets the requirements of the ATS/ERS task force and is approved as a medical device in Europe (CE0476) and the United States[424]. The spirometer measures peak expiratory flow (PEF), forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and volume expired in initial 6 seconds (FEV6). The volume accuracy is ± 3% or 50mL (as stated on product data sheet) and flow accuracy is ± 5% or 200mL/s. During the forced expiratory manoeuvre, data were transferred in real time to the participant's smartphone, with no data displayed on the spirometer itself. All spirometry data measurements were immediately transferred from the participant's mobile device to secure cloud storage.

6.3.1.3 Participant instructions

The patientMpower application was downloaded onto the participant's smartphone and face-to-face training alongside step-by-step written instructions were provided. Participants were asked to perform a single forced expiratory manoeuvre at approximately the same time each day for 105 days. 105 was selected as there was a two-week window around the three-month visit, and thus ensured all eligible participants performed home spirometry until their study visit. All FVC measurements were recorded in litres to two decimal places. Spirometry measurements were blinded for the first 105 days, with visual feedback using a rotating windmill provided to ensure an acceptable blow. Readings were unblinded if participants continued to use their home spirometer beyond day 105.

6.3.1.4 Statistics

Means, medians, and proportions were used to describe the study population, and comparisons between IPF and non-IPF were made using t-tests, Wilcoxon rank-sum tests, and Fisher's test respectively. Home spirometry readings falling within the upper and lower centile of aggregated group data based on FVC %predicted values were excluded to limit effects of substandard blows. Baseline measurements were calculated as the mean of daily readings obtained during the first seven days. Three-month measurements were calculated as the mean of readings obtained between days 90 and 96. Correlation coefficients between home and hospital spirometry for corresponding timepoints were assessed using Pearson correlation and intra-class correlation coefficients in a two-way random effects model. Bland-Altman plots were generated to assess the number of measurements that were

outside the 95% limits of agreement. Adherence was determined by the number of days where a participant provided at least one reading divided by 105 days. To assess reliability, a weekly coefficient of variation (CoV) was estimated where three or more daily values were provided and the median for all participants plotted. The overall CoV for the duration of the 15 weeks was calculated.

Linear regression was performed using all available values between baseline and days 28, 90 and 365, without any imputation of missing values. Annualised decline in FVC was calculated as the percentage change relative to baseline values. The rate of change in FVC at specified time points (28 days and 3-months defined as 90 days) was categorised using thresholds of 5% and 10% relative to baseline values, and cox proportional hazards and logistic regression were applied to test the association with disease outcomes. The association between FVC change per percent decline was tested using a multivariate cox regression model adjusted for age, sex, smoking status, and baseline FVC. Disease progression was defined as an FVC relative decline ≥ 10% or death at 12 months. A p value < 0.05 was considered statistically significant for all analyses. All analyses were performed using Stata v.16 (StataCorp, College Station, TX, USA).

6.3.2 Results

6.3.2.1 Baseline demographics

101 participants with fibrotic ILD were included in this interim analysis (Table 6-11), of which 32 had IPF (32%) and 69 had non-IPF ILD (68%). The majority of participants in both groups were male, the mean age was 69.8±8 years respectively, and approximately two-thirds had a previous smoking history. The mean FVC was 2.95±0.85L, with milder severity of disease observed in IPF vs. non-IPF (3.26L vs 2.81L; p=0.01), though there were no differences in DL_{CO} and 6MWD. The median adherence calculated as number of readings/105, was 79% (IQR 53-93%) and was non-statistically higher in non-IPF compared with IPF.

Demographics	All	IPF	Non-IPF	P value (IPF vs. non IPF)
Baseline, N	101	32	69	
Male, n (%)	72 (71)	26 (81)	46 (67)	0.16
Mean age (sd)	69.8 (8.0)	70.7 (7.5)	69.4 (8.2)	0.51
Smoking history, n (%)				
Current/Ex	65 (64.4)	18 (56.3)	47 (68.1)	0.38
Never	36 (35.6)	14 (43.8)	22 (31.9)	
Hospital FVC, litres (sd)	2.95 (0.85)	3.26 (0.85)	2.81 (0.82)	0.01
FVC, % predicted (sd)	80.1 (17.5)	83.8 (14.5)	78.3 (18.6)	0.13
DL_{CO}, % predicted (sd)	55.1 (16.3)	54.9 (14.3)	55.2 (17.2)	0.98
6MWD, m (sd)	328 (109)	328 (134)	329 (98)	0.96
Median Adherence, % (IQR)	79% (53-93)	74% (47-91)	81% (61-94)	0.39

Table 6-11 - Baseline demographic information for included participants.

Baseline demographics shown for all participants, and stratified by IPF and non-IPF, with p values used for comparison. Values shown in mean (SD) unless otherwise stated.

6.3.2.2 Coefficient of variation (CoV)

The median coefficient of variation (CoV) for all participants was 5.94% (IQR 3.77-10.20%). A slightly higher CoV was observed in the phenotypically more diverse and larger non-IPF ILD cohort (CoV 6.59%, 95%CI 4.31-11.31%) compared with IPF (CoV 4.38, 95%CI 2.95-7.41) (Figure 6-5).

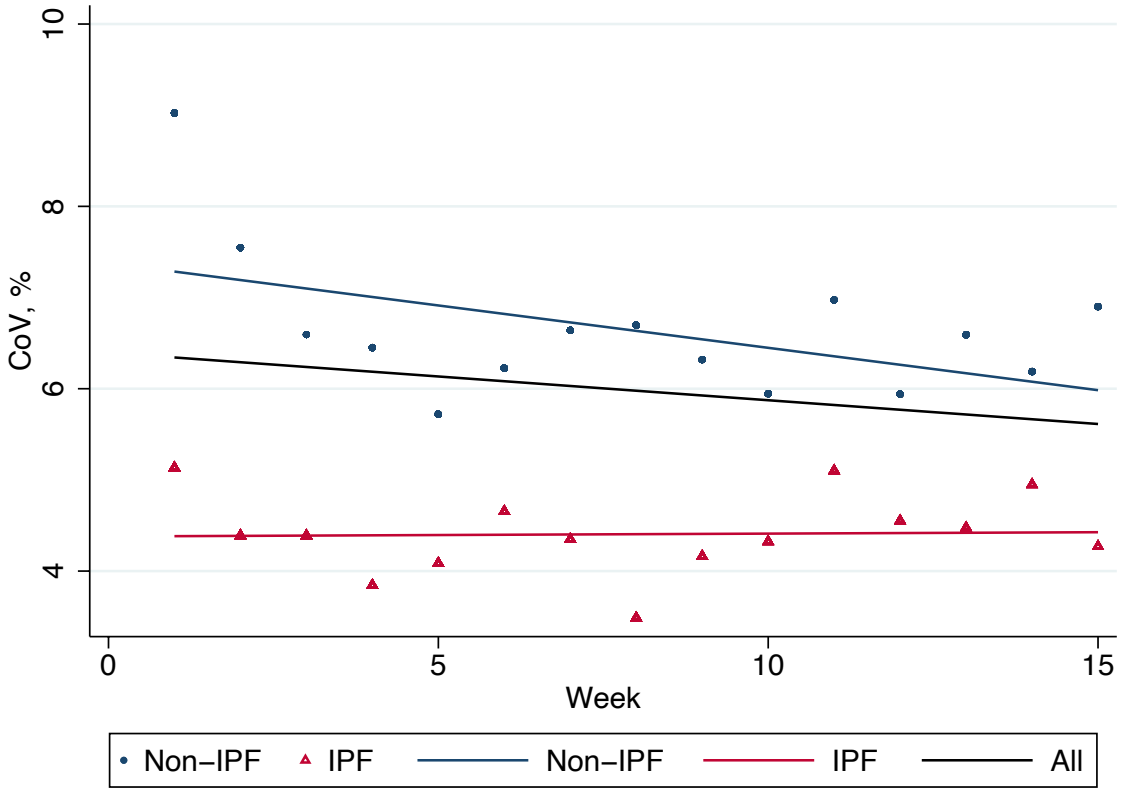


Figure 6-5 - Weekly coefficient of variation (CoV) (%) in home spirometry across study weeks.

Red and blue scatter points represent median CoV in IPF and non-IPF group, respectively. Line of best fit shown for IPF and non-IPF separately, and overall line of best fit.

6.3.2.3 Comparison with hospital spirometry

Home spirometry measurements within the upper and lower percentile of aggregated group data (below 1.1L or above 5.9L) were excluded to limit effects of inadequate expiratory efforts, leaving a total of 6919 measurements. The mean of daily home readings obtained between during the first seven days were compared with hospital spirometry. The mean hospital baseline FVC was 2.94±0.86L, compared with a mean home spirometry baseline of 2.68±0.92L (Table 6-12). High correlation between hospital and home spirometry was observed (r=0.87), with an intra-class coefficient of 0.91 (95% CI 0.80-0.95) (Figure 6-6). Bland-Altman plots confirmed 92.1% of home spirometry values were within agreement limits of hospital values (Figure 6-9). Three-month FVC readings were compared by calculating the mean of home spirometry values obtained between day 90 and 96 and compared with hospital spirometry performed during the three-month INJUSTIS visit. Similar to baseline observations, home spirometry underestimated FVC values (mean 2.76±1.04L) relative to hospital spirometry (mean 2.92±0.96L), though there was high correlation (r=0.84), and an intra-class coefficient of 0.90 (95% CI 0.83-0.95). Bland-Altman plots demonstrated 91.3% of values were within agreements limits (Figure 6-7). Similar results were obtained when analyses were restricted to participants with non-IPF only (Table 6-13).

	N	Comparison			Agreement		Pearson correlation			Intra-class coefficient (95%CI)
		Mean Hosp. (SD)	Mean Home (SD)	Mean diff (SD)	n outside limits	% Within limits	r	R2	P	
Baseline	101	2.94 (0.86)	2.68 (0.92)	-0.26 (0.46)	8	92.1	0.87	0.76	<0.001	0.91 (0.80;0.95)
3 months	46	2.92 (0.96)	2.76 (1.03)	-0.16 (0.57)	4	91.3	0.84	0.70	<0.001	0.90 (0.83;0.95)

Table 6-12 - Comparison of FVC shown in litres after FVC <1st and >99th percentile excluded, for all participants.

Agreement after values plotted on Bland-Altman plot, with n the total number of participants with values outside limits. Correlation presented between hospital (hosp.) and home spirometry.

	N	Comparison			Agreement		Pearson correlation			Intra-class coefficient (95%CI)
		Mean Hosp. (SD)	Mean Home (SD)	Mean diff (SD)	n outside limits	% Within limits	r	R2	P	
Baseline	69	2.79 (0.83)	2.50 (0.89)	-0.29 (0.46)	6	91.3	0.86	0.74	<0.001	0.90 (0.73;0.95)
3 months	46	2.79 (0.97)	2.56 (0.90)	-0.23 (0.54)	1	97.0	0.84	0.70	<0.001	0.90 (0.77;0.95)

Table 6-13 - Comparison of FVC shown in litres after FVC <1st and >99th percentile excluded, for non-IPF only.

Agreement after values plotted on Bland-Altman plot, with n the total number of participants with values outside limits. Correlation presented between hospital (hosp.) and home spirometry.

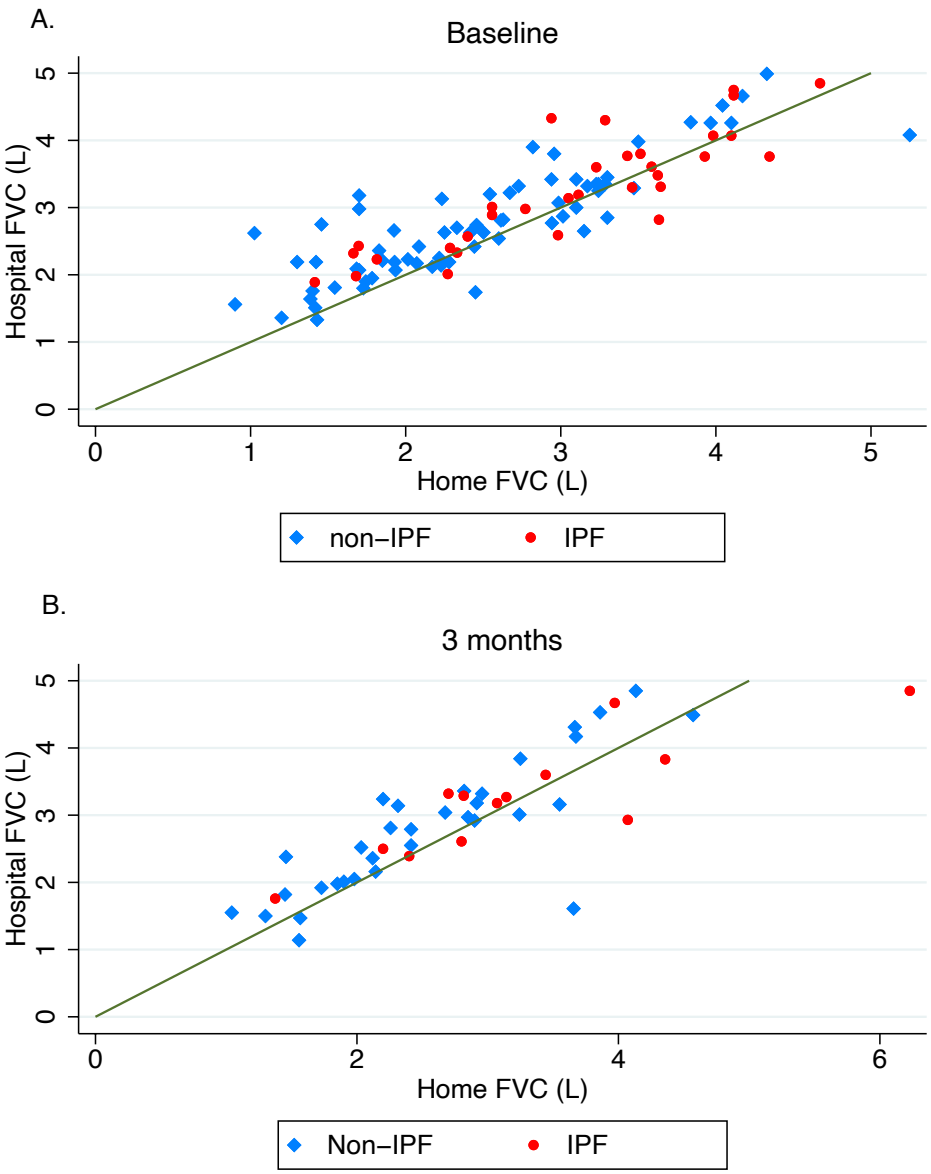


Figure 6-6 - A. Correlation of home and hospital spirometry.

Baseline measurements were calculated as the mean of daily readings obtained during the first seven days. Three-month measurements were calculated as the mean of readings obtained between days 90 and 96. FVC (litres) measurements at baseline (A) and 3 months (B) coloured differently for IPF and non-IPF. Black reference line represents y=x.

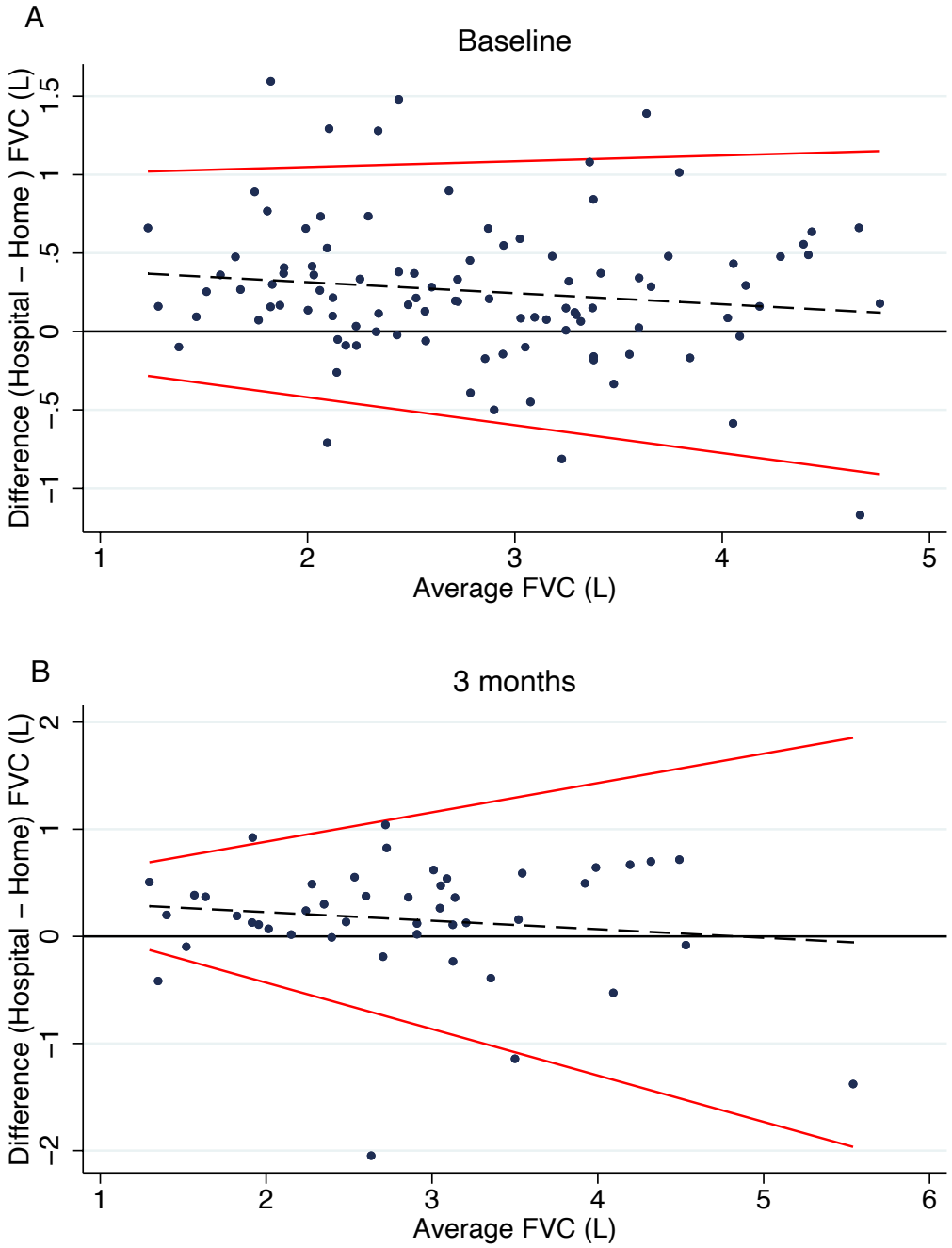


Figure 6-7 - Bland Altman plot for baseline and 3 months.

Mean difference of hospital relative to home spirometry was 0.26L (SD 0.46) at baseline and 0.16L (SD 0.57) at 3 months. The red lines represent the 95% limits of agreement. Baseline measurements were calculated as the mean of daily readings obtained during the first seven days. Three-month measurements were calculated as the mean of readings obtained between days 90 and 96.

6.3.2.4 Patterns of disease behaviour

Linear regression estimated the mean rate of annual decline in FVC as 133.2ml (SD 1141.1) in non-IPF, 79.5ml (SD 2575.6) in IPF, and 116.2ml (SD 1711.4) in all participants (Figure 6-8). The mean relative decline at one year in %predicted FVC from baseline was 7.91% (SD 41.13) in non-IPF, 8.91% (SD 75.62) in IPF, and 8.22% (SD 55.02) in all participants.

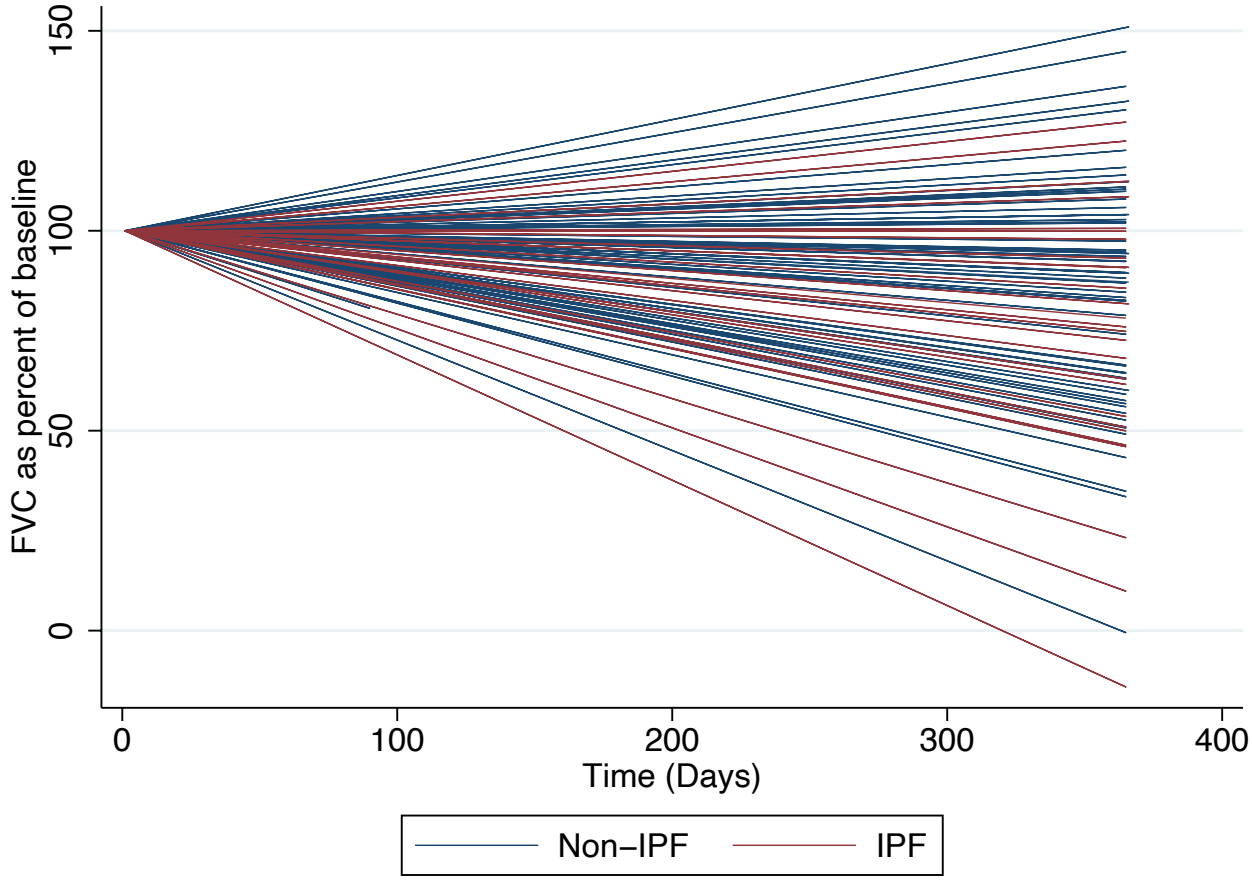


Figure 6-8 – Rate of decline in FVC estimated using linear regression.

All available readings used between baseline and day 365 without imputation. Blue lines represent participants with non-IPF, and red lines represent IPF participants.

6.3.2.5 Predicting outcomes

The change in FVC at specified time points (28 days and three-months) and the association with mortality and disease progression was initially determined using FVC thresholds. The rate of relative FVC change from baseline to 28 days was predictive of outcome when assessed using a threshold of 5% (Table 6-14 and Table 6-15). An FVC decline greater than 5% observed in 39 participants (40%), was associated with an increased risk of mortality and disease progression (Figure 6-9). In multivariate analysis adjusted for age, sex, smoking status, and baseline FVC, an FVC relative decline greater than 5% over 28 days was associated with increased mortality (aHR 2.58; 95%CI 1.01-6.62) and disease progression (aOR 5.55; 95%CI1.66-18.57).

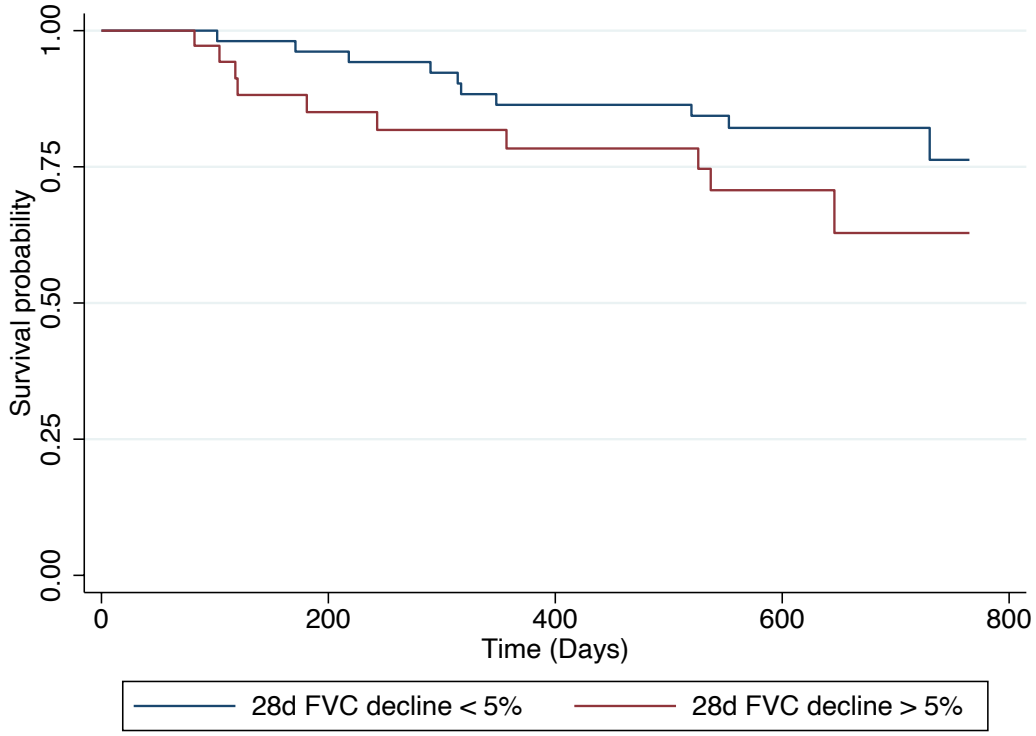


Figure 6-9 - Relationship between 28 days FVC using a dichotomised threshold of 5%.

Rate of change was calculated using linear regression analysis of all points between baseline and 28 days. Rate of change is reported relative to baseline values.

At three-months a relative decline greater than 5% and 10% was observed in 58 participants (61.1%), and 42 participants (44.2%) respectively. Neither 5% nor 10% relative FVC decline at three-months were associated with mortality, but both thresholds were associated with an increased likelihood of disease progression in multivariate analysis (aOR 4.70, 95%CI1.37;16.07 and aOR 10.75, 95%CI 2.82;40.92 respectively).

Physiology	Relative change over 3-months	Overall mortality, No. (%)	P value (mortality)	IPF mortality, No. (%)	Non-IPF mortality, No. (%)	P value (IPF vs. non-IPF)
28 days	<5%	10/59 (17.0)	0.21	5/21 (23.8)	5/38 (13.2)	0.18
	≥5%	10/39 (25.6)		4/11 (36.4)	6/28 (21.4)	0.39
3 months	<5%	6/37 (16.2)	0.322	2/11 (18.2)	4/26 (15.4)	0.93
	≥5%	13/58 (22.4)		6/19 (31.6)	7/39 (18.0)	0.19
	<10%	8/53 (15.1)	0.14	3/16 (18.8)	5/37 (13.5)	0.55
	≥10%	11/42 (26.2)		5/14 (35.7)	6/28 (21.4)	0.43

Table 6-14 - Association of change in FVC with mortality according to pre-specified thresholds stratified by IPF and non-IPF

Physiology	Relative change over 3-months	Overall disease progressors, No. (%)	P value (disease progression)	IPF disease progression, No. (%)	Non-IPF disease progression, No. (%)	P value (IPF vs. non-IPF)
28 days	<5%	12/41 (29.3)	0.009	4/11 (36.4)	8/30 (26.7)	0.41
	≥5%	14/22 (63.6)		6/6 (100.0)	8/16 (50.0)	0.04
3 months	<5%	5/25 (20.0)	0.007	0/6 (0.0)	5/19 (26.3)	0.22
	≥5%	20/37 (54.1)		9/10 (90.0)	11/27 (40.7)	0.009
	<10%	7/35 (20.0)	<0.001	1/8 (12.5)	6/27 (22.2)	0.48
	≥10%	18/27 (66.7)		8/8 (100.0)	10/19 (52.6)	0.02

Table 6-15 – Association of change in FVC with disease progression according to pre-specified thresholds stratified by IPF and non-IPF

In analyses of FVC as a continuous variable, the rate of FVC change assessed at 28 days and 3 months adjusted for age, sex, smoking status, and baseline FVC was not associated with either mortality or disease progression (Table 6-16).

Period for rate of change	Mortality			Disease progression		
	All (HR 95% CI)	IPF (HR 95% CI)	Non-IPF (HR 95% CI)	All (OR 95% CI)	IPF (OR 95% CI)	Non-IPF (OR 95% CI)
0-28d	1.01 (0.99-1.03)	1.02 (0.99-1.05)	1.00 (0.98-1.02)	1.02 (0.99-1.05)	1.35 (0.90-2.02)	1.00 (0.98-1.03)
0-3m	0.99 (0.98-1.01)	1.03 (0.98-1.08)	0.98 (0.95-1.02)	1.04 (0.99-1.07)	-	0.98 (0.95-1.02)

Table 6-16 - Summary estimates for disease outcomes according to rate of change in FVC over specified time periods, stratified by IPF and non-IPF.

Summary estimates reported for every 1% rate of decline in FVC over the given time-period. All estimates were adjusted for age, sex, smoking status, and baseline FVC

6.4 Discussion

6.4.1 Summary of findings

The key findings from this cohort of individuals with fibrotic ILD demonstrate physiological and functional (QoL scores) biomarkers have prognostic potential in ILD, regardless of IPF or non-IPF. Baseline lung function both as continuous variables, and dichotomised using frequently applied criteria, were associated with an increased risk of mortality and disease progression. Of greater significance was the association between FVC change over 3-months and mortality, with each 2.5% decline associated with a 29% increased risk of overall mortality in a fully adjusted model. Notably, a dichotomised marginal relative FVC change of 1.3% was associated with poorer outcomes, suggesting serial FVC change over three-months is a sensitive prognostic biomarker in fibrotic ILD. Serial DL_{CO} change was associated with mortality in multivariate analysis, though the strength of effect was weaker compared with FVC change, possibly attributable to its greater variability and lesser reproducibility compared with FVC[59]. Associations between baseline and serial lung function measurements with disease outcomes were largely unchanged when analyses were restricted to non-IPF participants only. Baseline 6MWD was not associated with disease outcomes, nor were demographic factors. In analyses of QoL scores as prognostic biomarkers, none of the questionnaires at baseline were conclusively associated with disease outcomes, but when categorised into stable vs. worse over three-months, the IPARC questionnaire was associated with increased mortality. For each of the prognostic biomarkers, analysis of data within the individual subgroups was not performed due to the small sample size precluding the estimation of meaningful associations.

In analyses of home spirometry in the largest prospective study of mixed fibrotic ILD, home spirometry measurements were reliable and clinically informative. Adherence to daily home spirometry in the three-month design was high despite the blinding of measurements to participants, supporting future blinded spirometry in fibrotic ILD. Home and hospital measurements were highly correlated at complementary time points, though home spirometry tended to underestimate measurements when compared with hospital spirometry. The mean difference was 0.26L at baseline, and 0.16L at three-months, with over 90% of measurements within agreement limits at both timepoints. The median CoV was 5.9% and comparable to that reported in non-blinded studies (range 3.9-8.2%)[183 422 423 425 426], with a suggestive reduction in variability over time observed which may be attributable to learning and improved technique. Longitudinal modelling was applied to evaluate disease behaviour and found a similar annualised relative decline in FVC percent predicted in non-IPF compared with IPF (7.91% vs 8.91%). An FVC decline of 5% over 28 days was associated with increased mortality and disease progression, however this association was not replicated when FVC was evaluated as a continuous variable, nor when FVC decline at three-months was examined. Therefore, the implications of these findings remain uncertain, with analysis of the complete dataset likely to help establish whether home spirometry is an earlier and more sensitive prognostic biomarker in fibrotic ILD.

6.4.2 Comparisons with existing literature

The prognostic role of lung physiology measured at either baseline or change over short time periods has been reliably established in IPF, both earlier in this thesis and in the broader scientific literature. Associations of physiology with outcomes in non-IPF fibrotic ILD

have been studied less frequently, with the ILD-GAP model perhaps the most widely accepted clinical prediction model in non-IPF ILD. The model comprises ILD subtype, patient variables (sex and age) and lung physiology (FVC and DL_{CO}) and was able to accurately estimate mortality in a large and heterogeneous cohort of fibrotic ILD[419]. The findings from this interim analysis partially corroborate the ILD-GAP model, with independent associations noted between baseline lung physiology and mortality, but not in more restricted univariate analyses of age, sex, or smoking status. Due to limited sample sizes, demographic factors were dichotomised, and adjustment for confounders including disease severity were not applied. However, the absence of association with disease outcomes for sex or smoking status is consistent with the findings of the IPD meta-analysis of clinical trial participants with IPF presented in Chapter 4.

Several studies have found short-term changes in lung function are associated with disease outcomes in IPF, but little data exists for longitudinal change in physiology specifically in non-IPF fibrotic ILD. In the present study, there was a 23% increased risk of mortality per 2.5% FVC relative decline over three-months when analyses were restricted to non-IPF ILD. This was comparable to the 15% increased risk of mortality per 2.5% relative decline estimated in the IPD meta-analysis of IPF placebo arms. Similarly, but to a lesser extent, the change in DL_{CO} was associated with an 8% increased risk of mortality per 2.5% relative decline, which was comparable to the 7% increased risk observed in IPF alone in the IPD meta-analysis. These findings suggest lung function change, particularly FVC change over three-month is an accurate determinant of prognosis regardless of fibrotic ILD subtype and replicate earlier findings from IPD meta-analysis.

Previous studies have demonstrated baseline and short-term change in 6MWD can accurately predict disease outcomes in IPF[208 408]. In this study, no association with disease progression or mortality was identified, either when 6MWD was dichotomised using the cohort median, or when considered as a continuous variable in a multivariate model. The explanation for the lack of association between 6MWD and outcomes is unclear but may be explained by the inclusion of participants with RA-ILD, who typically have restriction of mobility as part of the primary disease process which is independent to the extent of lung fibrosis. Moreover, there may be confounding by pulmonary hypertension and other cardiovascular and musculoskeletal co-morbidities that were not adjusted for in the analysis.

The IPARC and KBILD questionnaires are considered specific to IPF, whilst the others are more general health related quality of life measures. In an analysis from the PROFILE cohort, the IPARC questionnaire predicted disease progression and death[412]. Several studies have been published evaluating the minimal clinically important difference (MCID) of the KBILD questionnaire, and correlation with other physiological and QoL biomarkers, but no studies have evaluated longitudinal change in KBILD over three-month as a prognostic marker. In this study, baseline questionnaire scores stratified by the cohort median were not associated with disease outcomes but worsening scores over three-months in the IPARC questionnaire was associated with increased mortality and is worthy of more intense evaluation. These findings suggest short-term changes in IPARC may be combined with other physiological and proteomic biomarkers in future clinical prediction models, that may help accurately determine prognosis in individuals with fibrotic ILD.

Home spirometry has been studied in several studies in IPF, but fewer data exist in non-IPF ILD [183 205 422 423 425-427]. The analysis from the INUSTIS cohort was performed in participants recruited from several centres and comprises the largest cohort of mixed fibrotic ILD with a majority of non-IPF. Good agreement and inter-observer reliability between hospital and home spirometry was observed, which is consistent with published findings in IPF, and with a smaller single centre study of mixed fibrotic ILD including 27 non-IPF participants[426]. Whilst there was an association with home spirometry values modelled linearly over 28 days and mortality, this was not replicated in continuous models, nor over three-months. These findings contrast with Russell et al[183], where daily home spirometry measured over three-months in an analysis from IPF participants recruited into the PROFILE cohort was independently associated with increased mortality. In the INJUSTIS study, subgroup analyses according to IPF or non-IPF demonstrated no differences between subtypes when evaluating the prognostic role of home spirometry. The demographics of participants in the INJUSTIS and PROFILE study were comparable, with a predominance of males in their sixth or seventh decade of life, demonstrating mild-moderate FVC impairment. Although participants were blinded in the INJUSTIS study, but not in the PROFILE study analysis, this is unlikely to be of significance as the median CoV was comparable across both studies (5.9% vs. 4.96%) as was the median adherence (79% vs. 82.7%).

6.4.3 Limitations

The results presented in this chapter have several limitations that must be acknowledged, including those discussed in the previous chapter. These include missing data due to the COVID-19 pandemic, participant shielding affecting exposure to other viral agents and thus

potentially influencing disease progression, confounding from anti-fibrotic and immunomodulatory therapies, and the lack of central review of CT imaging to confirm a diagnosis. Specifically for the analyses presented in this chapter, a key limitation includes the modest sample sizes, particularly for longitudinal data. To help overcome these limitations, I categorised baseline physiology or QoL scores into high or low according to frequently used criteria or the cohort median. Additional analyses to determine optimal cut-off would be required before adopting of any biomarkers clinically. Longitudinal questionnaire data are typically analysed using the minimally clinically important difference (MCD), but this was only available for the KBILD questionnaire, and therefore for the remaining questionnaires, scores were dichotomised into stable or worse. Although these methods limit the interpretability of these biomarkers as prognostic indicators, they form the basis for further study once the complete INJUSTIS dataset is available.

Other limitations include the requirement for participants to survive at least three-months after their baseline visit to be included in analyses of longitudinal change in physiological variables. Moreover, disease progression was dependent on FVC change over 12 months, whilst the exposure variable also included FVC, though at an earlier timepoint. However, all analyses performed included associations with mortality, which was not dependent on FVC, and summary estimates remained consistent. A further limitation is the multiple statistical tests performed which increases the risk of a type 1 error, though as this was an exploratory analysis in an interim dataset, correction for multiplicity was not applied.

Results presented for home spirometry face similar limitations as above, including modest interim sample sizes and missing hospital physiology data attributable to the COVID-19 pandemic. Participants were asked to perform a single reading, instead of the typical three measurements, to minimise potential intrusiveness of multiple daily expiratory manoeuvres. Moreover, the quality of participant attempt could not be validated as the handheld device did not record flow-volume loops. However, longitudinal modelling of daily spirometry would have compensated for this limitation in analyses of short term FVC change and disease outcomes. Since earlier detection of disease outcomes remains the primary purpose of home spirometry, single non-replicated readings taken daily are likely to remain sufficient for this purpose. A further limitation was the exclusion of participants who did not possess a smartphone, which may have enriched the cohort to be more competent in the use of home technology. Whilst this is noteworthy, over half of the INJUSTIS cohort consented to home spirometry, and as technological advances continue, a greater percentage of individuals with ILD are likely to be comfortable with remote monitoring.

In comparisons of hospital and home spirometry measurements, there was not always complete alignment of timepoints at which FVC was obtained, particularly at baseline when hospital spirometry was obtained pragmatically as standard of care within an acceptable timeframe from recruitment. This may have contributed to larger discrepancies with home spirometry at this time point compared with three-month research visits. Regardless, there was good correlation between hospital and home spirometry when evaluated as a single value, supporting the use of home spirometry in the monitoring of fibrotic ILD.

In analyses of individual disease behaviour and for exploring the association with disease outcomes, a linear regression model was applied. However, there is emerging evidence that FVC change does not follow a linear decline in a proportion of individuals with IPF, and therefore nonlinear models may provide additional insights and should be considered in future analyses.

6.5 Summary

This chapter presents interim data from the ongoing INJUSTIS study and evaluates the role of demographic factors, lung physiology and QoL scores as prognostic biomarkers in fibrotic ILD. The key findings demonstrate lung function, particularly FVC change over three-months, were independently associated with poorer outcomes in fibrotic ILD, and are consistent with findings from the earlier IPD meta-analysis in IPF. However, the FVC change over three-months measured using home spirometry was not associated with mortality, and although a greater than 5% relative decline over 28 days was prognostic, further study is required to establish the role of home spirometry as a prognostic biomarker or clinical trial endpoint in fibrotic ILD. Home spirometry measurements were accurate and reliable when compared with hospital spirometry and this is likely to be particularly relevant where clinical access or trial participation is limited due to geographical barriers, individual preference, clinical service demands and future pandemics.

Chapter 7 An exploratory analysis of blood biomarkers in the INJUSTIS cohort

7.1 Introduction

The potential utility of blood biomarkers in IPF and the limitations of previous studies have been extensively described in earlier chapters. In summary, though several biomarkers have been associated with prognosis in IPF, findings have been inconsistent with poor replication. In this chapter, I perform an unbiased exploratory proteomic analysis to identify novel analytes in carefully selected IPF participants with extremes of disease recruited into the INJUSTIS study. The intention is to measure relative differences of analytes and identify patterns in progressive disease compared with stable disease. Findings from this discovery analysis will inform more focussed biomarker analysis and enable replication in the complete INJUSTIS cohort once available. The study hypothesis is centred around shared pathogenic mechanisms in fibrotic ILD irrespective of subtype, and thus biomarkers that predict progressive disease in IPF will be investigated in all participants, irrespective of ILD subtype. Analyses for novel biomarkers will be performed in this chapter, whilst simultaneously exploring specific biomarkers that have been investigated in the systematic review presented in Chapter 3.

7.1.1 Aims of study

1) To identify novel prognostic blood biomarkers in IPF using an unbiased approach
2) To validate previously described blood biomarkers in IPF
3) To use the findings to inform future biomarker analysis in fibrotic ILD

7.2 Methods

7.2.1 Participant selection

A small cohort of 24 participants with IPF were selected in an exploratory analysis from the ongoing INJUSTIS study. As sample sizes were small and analyses were not statistically powered, participants with extremes of stable or progressive disease were selected to maximise differences in biomarker quantification between the groups. 12 participants with stable disease and 12 participants with progressive disease were selected using pre-specified inclusion criteria in a hierarchical fashion.

Change in FVC over three-months using home spirometry was adopted as the primary method for selection of participants, followed by hospital spirometry where home spirometry was unavailable. The mean of readings obtained using home spirometry between day 90 and 96 were compared with mean baseline values, calculated as the average of readings obtained between day 1 and 7. Three-month change was chosen to maximise the number of participants available for inclusion, as fewer participants had baseline and 12-month data available. All participants in the stable group were selected based on an FVC increase over three-months using either hospital or home spirometry, and the majority of participants in the progressive group (11/12) were selected based on an FVC decline ≥10% over three-months. To identify the remaining participant with progressive disease, an FVC decline < 10% over three months, but ≥ 10% over 12 months using hospital spirometry was applied, though no participants were identified for inclusion. The third and final criteria for identification of progressors was participants who died from respiratory causes within 12 months. Several participants fulfilled these criteria, so inclusion was

narrowed to the participant who died and had the greatest FVC change between baseline and three-months.

Stable disease	Progressive disease
FVC increased over 3 months using home spirometry (n=5)	≥10% FVC decline over 3 months using hospital or home spirometry (n=11)
FVC increased over 3 months using hospital spirometry (n=7)	≥10% FVC decline over 12 months (but <10% over 3 months) (n=0)
	Dead within 12m (n=1)

Table 7-1 - Criteria for selecting participants for Olink biomarker analysis

7.2.2 Summary of biomarker assays

Several proteomic platforms to accurately quantify blood proteins were considered. Though enzyme-linked immunoassays (ELISA) have been a mainstay of biological research for many years, offering high specificity and sensitivity for measuring proteins in the blood, they are limited to measuring a single analyte. Measuring multiple analytes using an ELISA involves performing several assays in parallel, is time-consuming, requires greater sample volumes and increases the risk of error.

Multiple analytes can instead be measured using multiplex immunoassays, which combine assays into a single reaction volume and can be broadly classified into those requiring spatially separate assays and those that utilise bead immobilisation. Spatially separate assays are similar to an ELISA except multiple antibody pairs share a similar reaction volume. In the bead immobilisation approach also commonly termed Luminex technology, colour-coded magnetic beads coated with analyte-specific capture antibodies bind the analytes of

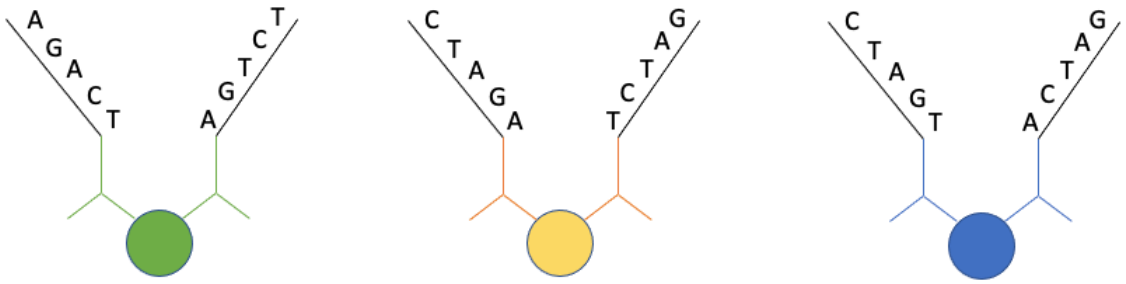
interest. Unbound materials are removed, and the samples are incubated with biotinylated detection antibodies, followed by the addition of streptavidin. Specialised equipment utilises dual laser to classify the bead and quantify the amount of analyte bound. However, multiplex immunoassays are often limited by cross-reactivity where antibodies against specific antigens bind to non-target antigens, and therefore validated antibody pairs are essential to improve specificity of readouts. To help mitigate these limitations, we used the Olink platform, which utilises novel Proximity Extension Assay (PEA) technology and is described in greater detail below.

7.2.3 Olink biomarker assay

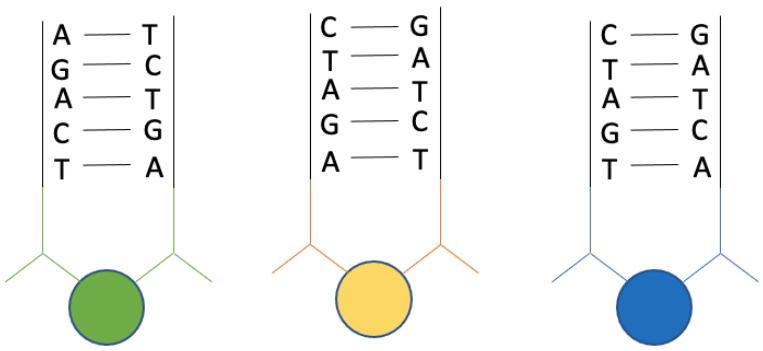
Baseline heparin plasma samples aliquoted into at least 40µL from identified participants were placed in a 96-well fully skirted PCR plate and shipped on dry ice to Olink laboratories based in Uppsala, Sweden for further processing. The Olink 1536 panel measures 1463 unique proteins across four separate panels (cardiometabolic, inflammation, neurology, and oncology) and requires just 3µL of sample. Olink panels utilise unique PEA technology that uses matched pairs of antibodies labelled with unique DNA oligonucleotides that are incubated within samples and allowed to pair-wise bind to target proteins. Once an antibody pair simultaneously binds to a target protein, the antibodies are brought into proximity, allowing their DNA oligonucleotides to hybridise, serving as a template for DNA polymerase dependent extension. This generates a double-stranded DNA “barcode” which is unique for each detected protein, and proportional to the concentration of protein biomarker present in the sample. The DNA molecule is amplified using standard PCR

techniques and readout is performed using Next Generation Sequencing (NGS), resulting in a scalable and specific method for simultaneously quantifying hundreds of proteins.

A) Immunoassay



B) Hybridisation



C) Extension

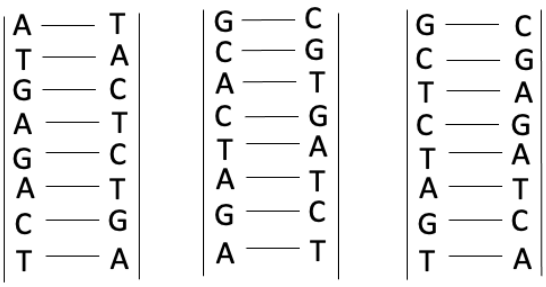


Figure 7-1 – Olink Proximity Extension Assay (PEA) technology

A) *Antibody pairs labelled with DNA oligonucleotides bind target analytes in solution, B) Oligonucleotides brought into proximity hybridise, C) DNA polymerase extends oligonucleotides to create a DNA barcode ready for readout by Next Generation Sequencing.*

7.2.4 Quality control

Analysis of data can be affected by several technical factors, and therefore quality control (QC) to monitor performance using Olink's built in system was utilised. QC was performed with the addition of internal and external controls to each sample

7.2.5 Statistical analysis

Demographic details of participants included in the analysis were presented for stable and progressive disease separately, with between-group comparisons performed using t-tests for continuous outcomes and chi-squared tests for categorical outcomes. Protein biomarkers were measured using normalised protein expression (NPX), which is an arbitrary relative quantification unit related to protein concentration and is expressed on a logarithmic 2 scale, where a difference of 1 NPX approximates to a doubling of the protein concentration. NPX values for analytes that did not pass quality control (QC) were excluded to ensure analyses were robust. The coefficient of variation (CoV) for intra-assay (variance between sample replicates on same plate) and inter-assay (variance between runs of sample replicates on different plates) precision were calculated for each panel separately using sample controls and presented in tables.

Median NPX values were estimated for pre-specified individual analytes in participants with progressive disease and compared with median values in stable disease using Wilcoxon rank sum tests, using a p value significance threshold of 0.05, and presented using boxplots. To adjust for multiplicity, a Bonferroni adjusted p value threshold of 0.0033 was applied (0.05 alpha value/15 biomarkers tested). In unbiased exploratory analyses, the difference in

biomarker NPX levels between stable disease and progressors and their associated p values calculated using Wilcoxon rank sum tests, were plotted for all included analytes using volcano plots. A nominal p value of 0.05 and an effect size of -0.5 to 0.5 on a log2 scale, equating to approximately 50% change between stable disease and progressors were arbitrarily set to identify biomarkers that may have significance. Stratification by male only gender was additionally performed for individual analyte box plots and volcano plots. Pathway analysis for proteins differentially expressed in either stable or progressive disease with a p value < 0.05 was performed using freely available online software[428].

7.3 Results

7.3.1 Baseline demographics

The baseline demographics and clinical features of included participants are shown below. Included participants were of white ethnicity, and over half in both stable and progressive groups had previously smoked. Participants with progressive disease were more likely to be younger, male, have radiological honeycombing and have poorer baseline lung function than those with stable disease. FVC relative decline over three-months as measured by both hospital and home spirometry was greater in participants with progressive disease, and greater proportions of individuals were dead at 12 months (7 vs. 0; p=0.002).

	Progressors (n=12)	**Stable (n=12)**	**P value**
Age, mean (SD)	70.49 (7.99)	75.01 (6.54)	0.144
Male, %	92%	67%	0.132
Ethnicity, White	100%	100%	N/A
Smokers (ex/current)	67%	58%	0.673
Baseline FVC, L	2.99 (0.96)	3.15 (0.92)	0.682
Baseline FVC % predicted	78.66 (18.69)	86.19 (13.9)	0.275
Baseline DLCO, %predicted	47.42 (16.95)	61.71 (22.88)	0.118
3-month FVC, % predicted	75.44 (19.14)	88.01 (13.98)	0.107
3-month FVC relative change on hospital spirometry, %	-3.85 (10.67)	3.75 (5.14)	0.05
3-month FVC relative change on home spirometry, %	-10.55 (4.50)	9.44 (15.3)	0.005
Dead before 12m, n	7	0	0.002
Presence of CT honeycombing, %	83%	50%	0.083
Baseline 6MWD, m	318 (115)	319 (116)	0.987

Table 7-2 - Demographic and baseline clinical features of included participants in Olink exploratory analysis.

Comparisons between IPF and non-IPF were performed using Fisher's test for proportions, t-test for means and Wilcoxon test for medians, with p values presented.

7.3.2 QC summary

The number of samples that passed QC for all biomarkers in an individual panel ranged from 83-96%, with a breakdown shown in the table.

Olink Panel	No. of samples that passed QC/Total no. of samples	Passed samples (%)
Explore 384 Cardiometabolic	20/24	83
Explore 384 Inflammation	20/24	83
Explore 384 Neurology	23/24	96
Explore 384 Oncology	23/24	96

Table 7-3 - Quality control summary

7.3.3 Coefficient of variation (CoV)

Linear NPX-values from control samples on each plate were used to calculate the intra- and inter- CoV, where:

intra-cov = standard deviation (control samples per plate)/mean (control samples per plate)

inter-cov = standard deviation (control samples on all plates)/mean (control samples on all plates).

The average intra-assay CoV ranged from 10-11% across the four plates.

Olink Panel	Intra-assay %CoV	Inter-assay %CoV
Explore 384 Cardiometabolic	10	10
Explore 384 Inflammation	11	11
Explore 384 Neurology	11	11
Explore 384 Oncology	10	10

Table 7-4 - Average %CoV for all assays per plate

7.3.4 Proteins detected

Of the 1463 proteins analysed, 1307 (88.8%) proteins were detected in more than 50% of the samples:

Olink Panel	No. of proteins detected/Total no. of proteins	Detected proteins, %
Explore 384 Cardiometabolic	341/369	92
Explore 384 Inflammation	332/368	90
Explore 384 Neurology	307/367	84
Explore 384 Oncology	327/368	89

Table 7-5 - Proteins detected by each panel

7.3.5 Focussed biomarker analysis

Of the 20 pre-specified biomarkers investigated in Chapter 3, measurements in participants with progressive and stable disease were available for 15 separate biomarkers, with data from the Olink platform unavailable for CA19-9, KL-6, LOXL2, MMP-1, and Periostin. Of the 15 biomarkers investigated, median measurements in progressors were higher compared with stable disease for CA-125 (NPX 0.81 vs. -0.07; p=0.009) and CCL-18 (NPX 1.5 vs. 1.1; p=0.033), though neither reached statistical significance when a bonferroni adjusted p value of 0.003 was applied. Whilst several of the remaining biomarkers appeared to be numerically different in the two groups, none reached statistical significance in between group comparisons (Figure 7-2 and 7-3). MMP-7, an epithelial biomarker shown to predict mortality and disease progression in an earlier IPD meta-analysis, was unable to differentiate stable and progressive disease in this cohort (NPX 0.72 vs. 0.78; p=0.199).

Further analyses restricted to male participants (n=19) demonstrated significantly elevated CA-125 measurements in progressors using a bonferroni adjusted p value.

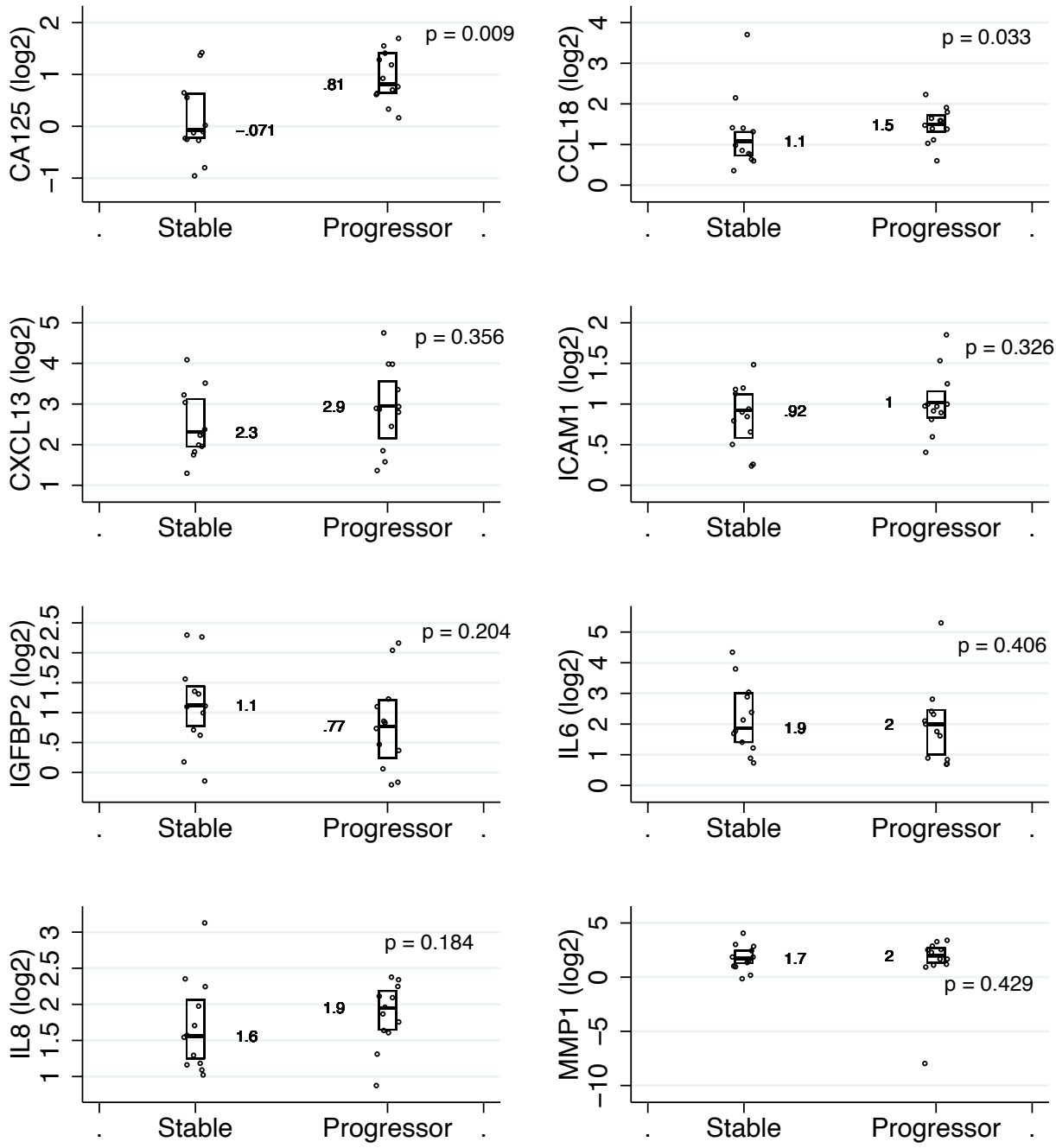


Figure 7-2 - Boxplots for biomarkers investigated 1/2

Biomarkers listed on Y-axis. Log2 NPX values shown for participants with stable and progressive disease. Wilcoxon rank sum test used for between group comparison of median values and p values presented on individual plots.

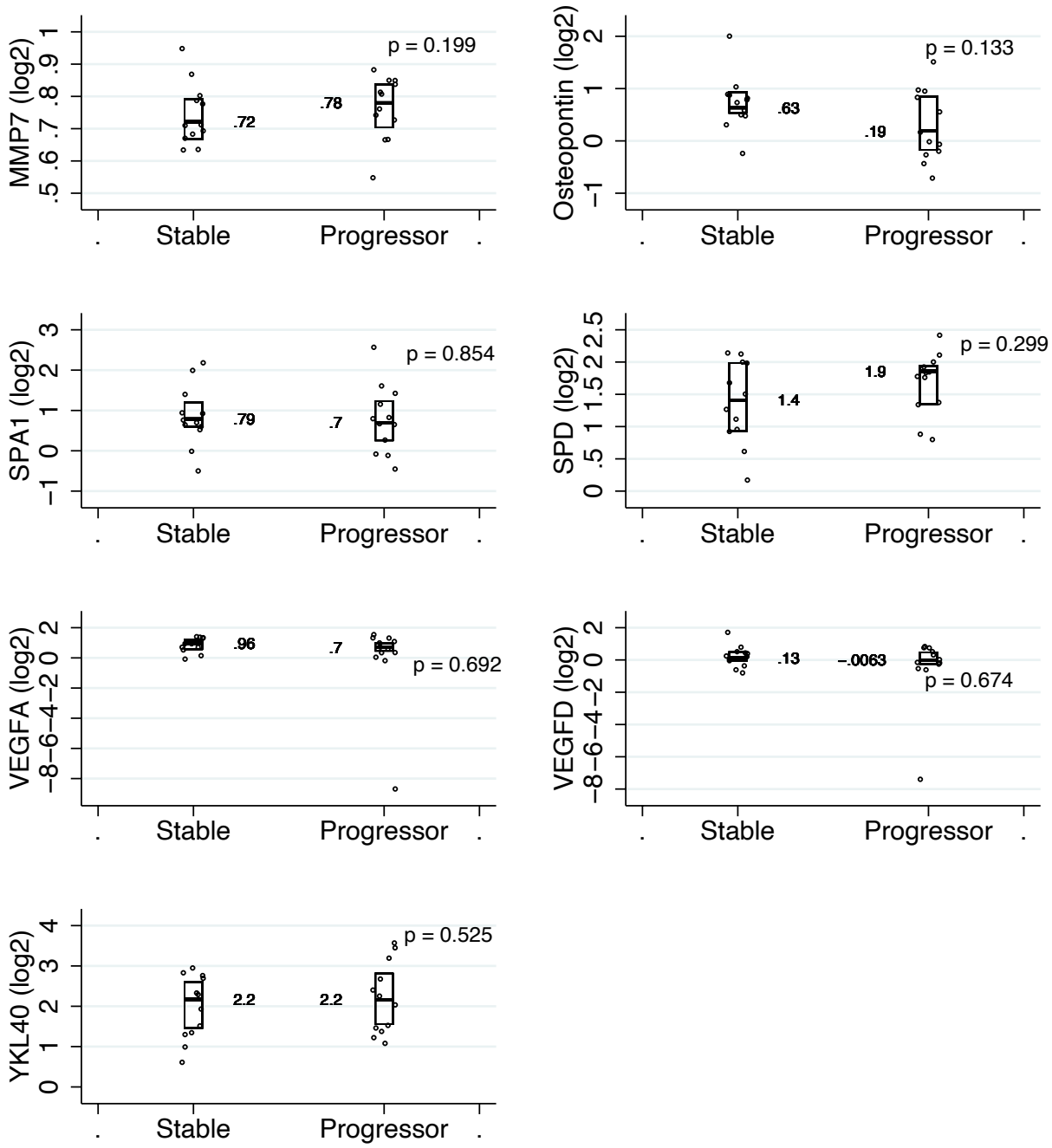


Figure 7-3 – Boxplots for biomarkers investigated 2/2

Biomarkers listed on Y-axis. Log2 NPX values shown for participants with stable and progressive disease. Wilcoxon rank sum test used for between group comparison of median values and p values presented on individual plots.

Biomarker	Stable (NPX)	Progressors (NPX)	P value
SP-A	0.79	0.7	0.854
SP-D	1.4	1.9	0.299
IGFBP2	1.1	0.77	0.204
IL6	1.9	2.0	0.406
CXCL13	2.3	2.9	0.356
IL8	1.6	1.9	0.184
ICAM-1	0.92	1.0	0.326
MMP-1	1.7	2.0	0.429
MMP-7	0.72	0.78	0.199
CA-125	-0.07	0.81	0.009
Osteopontin	0.63	0.19	0.133
VEGF-A	0.96	0.7	0.692
VEGF-D	0.13	-0.006	0.674
YKL-40	2.2	2.2	0.525
CCL-18	1.1	1.5	0.033

Table 7-6 - Summary of biomarker measurements in participants with stable and progressive disease.

Median biomarker values presented for each group on a log2 scale (NPX) and p values calculated using Wilcoxon rank sum tests

Biomarker	Stable (NPX)	Progressors (NPX)	P value
Male only (n=19)			
SP-A	0.79	0.7	0.364
SP-D	1.4	1.9	0.248
IGFBP2	1.1	0.77	0.322
IL6	1.6	1.9	0.409
CXCL13	2.3	2.9	0.409
IL8	1.6	1.9	0.216
ICAM-1	0.92	1.0	0.186
MMP-1	1.7	2.0	1.00
MMP-7	0.72	0.78	0.160
CA-125	-0.07	0.81	0.003
Osteopontin	0.63	0.19	0.409
VEGF-A	0.96	0.7	0.457
VEGF-D	0.13	-0.006	0.509
YKL-40	2.2	2.2	0.322
CCL-18	1.1	1.5	0.248

Table 7-7 - Summary of median biomarker measurements for male participants only.

7.3.6 Exploratory analysis

Exploratory analyses to identify proteins that were differentially expressed in either stable or progressive disease were performed and presented using volcano plots stratified by colour coded panels (Figure 7-4). The strongest association was observed for Mucin-16 (CA-125), whilst three other proteins including C-C motif Chemokine Ligand-19 (CCL-19), C-C motif Chemokine Ligand-24 (CCL-24) and Peptidase M20 Domain Containing 1 (PM20D1) were significantly elevated in participants with progressive disease. A total of 13 proteins (Table 7.8) were significantly elevated in stable disease, with an over-expression of inflammatory proteins observed in this cohort, compared with progressors.

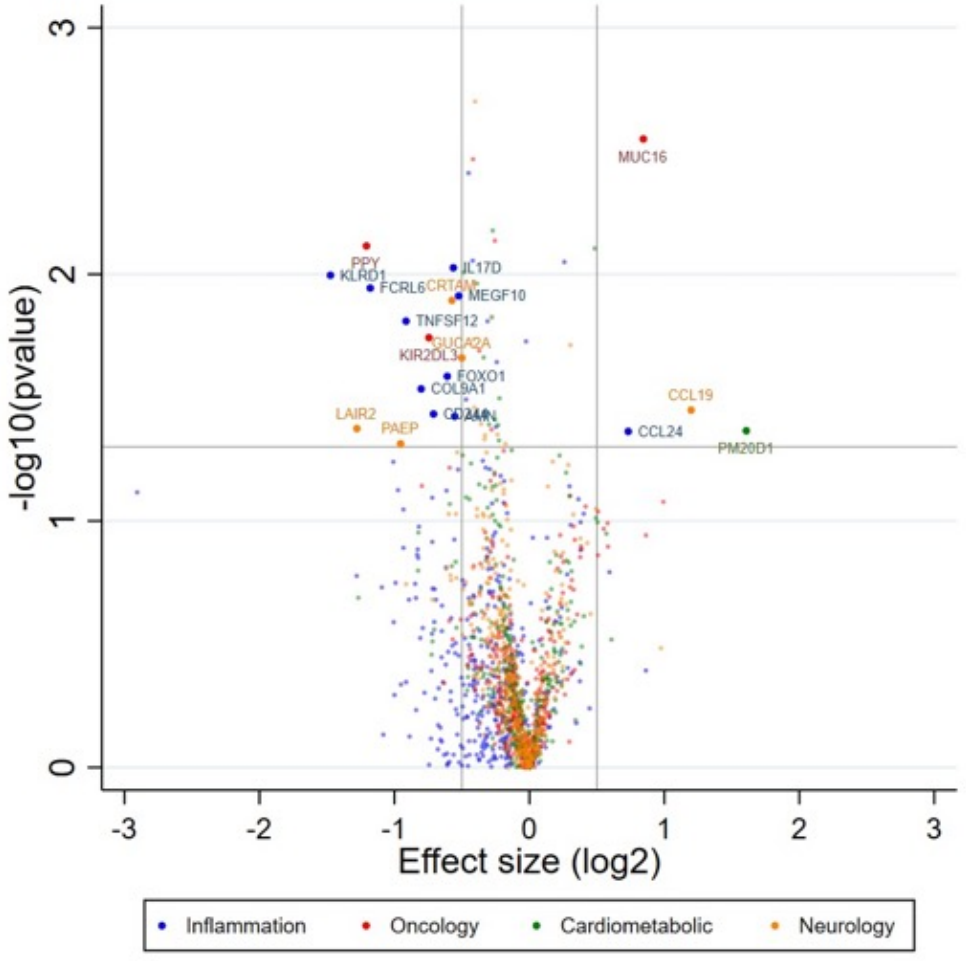


Figure 7-4 - Volcano plots for all analytes and their colour coded Olink panel.

Effect size shown on x axis using log2 scale, and p value on y axis using -log10 scale.

Analyte	Panel	Effect size (log2)	P value
Greater in progressors			
PM20D1	Cardiometabolic	1.61	0.043
CCL19	Neurology	1.20	0.035
MUC16 (CA-125)	Oncology	0.84	0.003
CCL24	Inflammation	0.73	0.043
Lower in progressors			
KLRD1	Inflammation	-1.48	0.010
LAFIR2	Neurology	-1.28	0.043
PPY	Oncology	-1.21	0.008
FCRL6	Inflammation	-1.18	0.011
PAEP	Neurology	-0.96	0.049
TNFSF12	Inflammation	-0.92	0.015
COL9A1	Inflammation	-0.80	0.029
KIR2DL3	Oncology	-0.75	0.018
CD244	Inflammation	-0.71	0.037
FOXO1	Inflammation	-0.61	0.026
CRTAM	Neurology	-0.58	0.013
IL17D	Inflammation	-0.57	0.009
MEGF10	Inflammation	-0.53	0.012

Table 7-8 - Exploratory analysis highlighting analytes with a log2 effect size > 0.5 or <-0.5 in participants with progressive disease compared with stable disease, and p value < 0.05.

Further exploratory analysis restricted to male participants (n=19) were performed to identify whether there were specific biomarkers associated with progressive disease in males only (Figure 7-5).

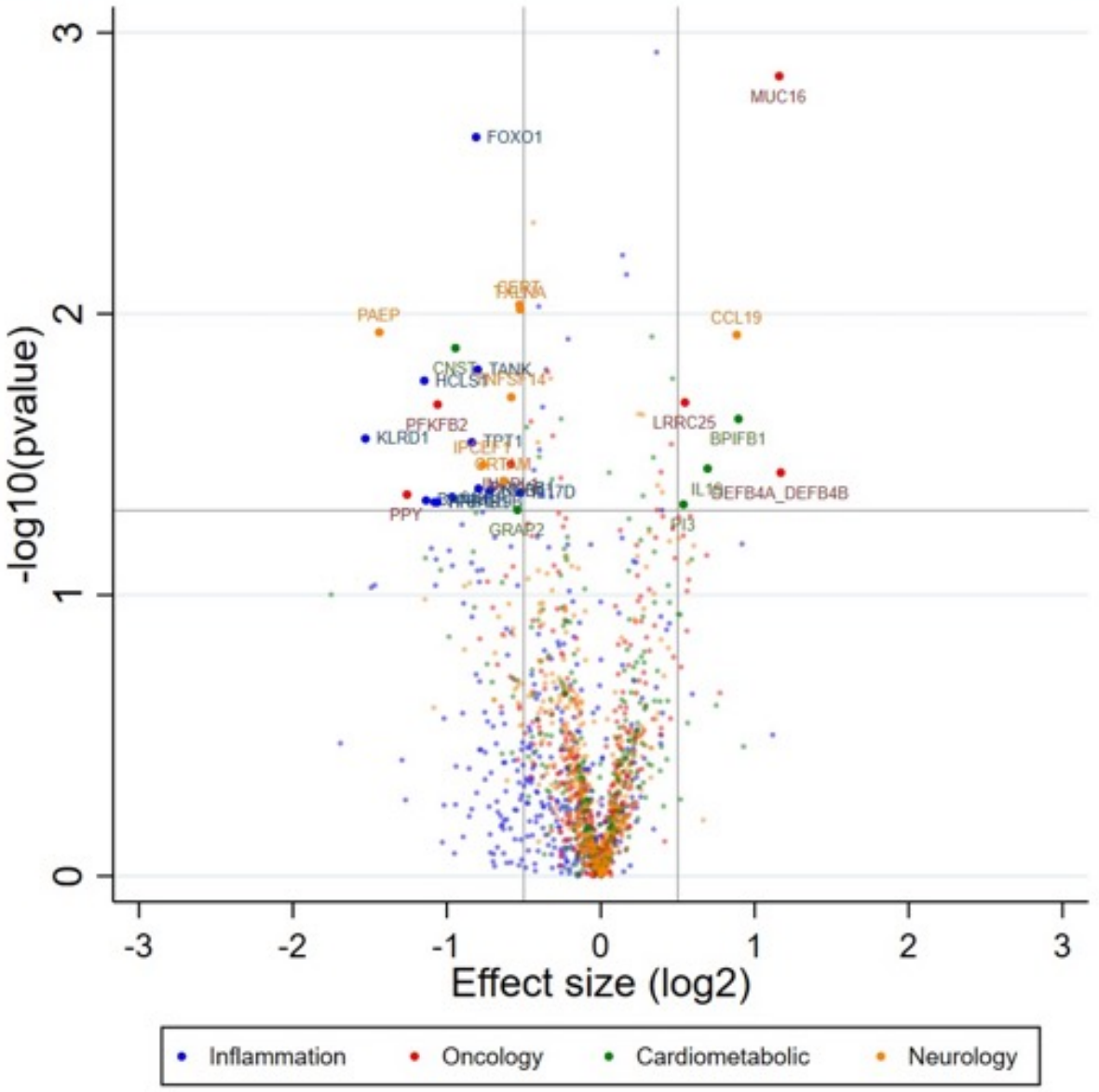


Figure 7-5 - Volcano plots for all analytes investigated and their colour coded Olink panel, in males only

Effect size shown on x axis using log2 scale, and p value on y axis using -log10 scale.

Similar to the whole cohort, MUC16 (CA-125) showed the strongest association with progressive disease, although several other blood biomarkers were differentially expressed (Table 7-9). Visualisation of the volcano plot confirmed an over-expression of inflammatory proteins in males with stable disease consistent with the analysis performed in the whole cohort.

Analyte	Panel	Effect size (log2)	P value
Greater in progressors			
DEFB4a-b	Oncology	1.17	0.037
MUC16 (CA-125)	Oncology	1.16	0.001
BPIFB1	Cardiometabolic	0.89	0.024
CCL19	Neurology	0.88	0.012
IL19	Cardiometabolic	0.69	0.035
LRRC25	Oncology	0.54	0.021
PI3	Cardiometabolic	0.53	0.048
Lower in progressors			
KLRD1	Inflammation	-1.53	0.028
PAEP	Neurology	-1.44	0.012
PPY	Oncology	-1.26	0.044
HCLS1	Inflammation	-1.15	0.017
BANK1	Inflammation	-1.14	0.046
PPP1R9B	Inflammation	-1.06	0.047
PFKFB2	Oncology	-1.06	0.021
LSP1	Inflammation	-0.97	0.045
CNST	Cardiometabolic	-0.95	0.013
TPT1	Inflammation	-0.84	0.029
FOXO1	Inflammation	-0.81	0.002
TANK	Inflammation	-0.80	0.016
PRKAB1	Inflammation	-0.80	0.042

Table 7-9 - Exploratory analysis in male participants only, highlighting analytes with a log2 effect size > 0.5 or <-0.5 in participants with progressive disease compared with stable disease, and p value < 0.05.

7.3.7 Pathway analysis

Pathway analysis for 53 proteins with a $p<0.05$ between stable and progressors, was performed to gain an insight into the underlying biology of differentially expressed proteins. There were 36 interactions between the proteins (Figure 7-6), which was significantly greater than would be expected for a random set of proteins (ten), suggesting the proteins were biologically connected.

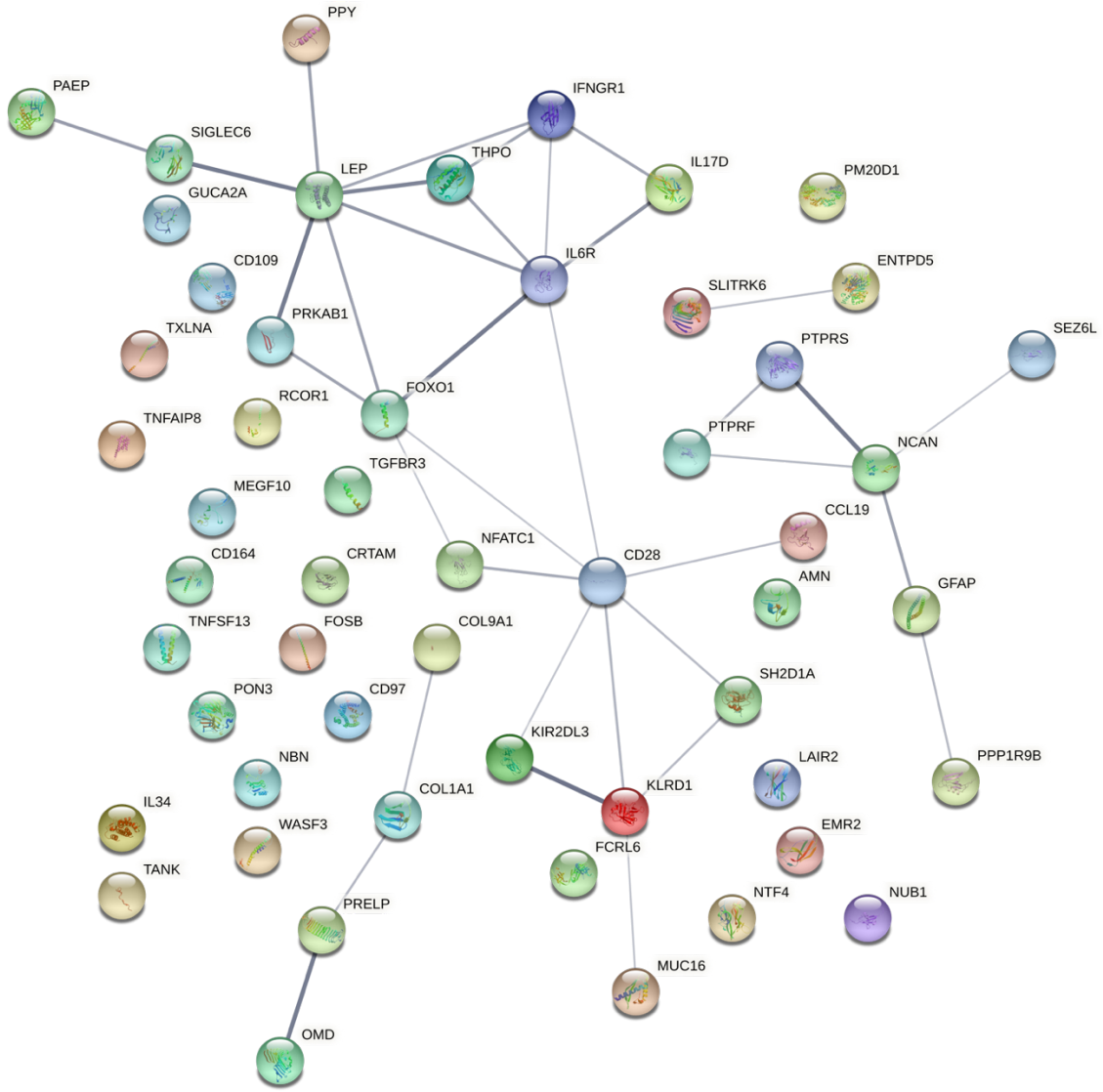


Figure 7-6 - Network analysis demonstrating interaction between included proteins.

Each node represents all the proteins produced by a single protein-coding gene locus. Edges represent protein-protein associations that are meant to be specific and meaningful. The thickness of edges represents the strength of confidence

34 biological pathways were enriched when a Benjamini-Hochberg corrected p value was applied (Table 7-10), including pathways relating to prostaglandin, and those involved in the regulation of lymphocyte and leukocyte mediated immunity.

Biological processes	observed gene count	background gene count	Strength (log10 scale)	false discovery rate
response to prostaglandin E	3	25	1.65	0.0355
regulation of lymphocyte mediated immunity	5	152	1.08	0.0378
regulation of leukocyte mediated immunity	6	209	1.03	0.0201
positive regulation of immune effector process	6	223	1	0.0251
positive regulation of cytokine production	8	461	0.81	0.0251
regulation of immune response	14	896	0.76	0.00077
positive regulation of response to external stimulus	8	511	0.76	0.0387
cytokine-mediated signalling pathway	9	678	0.69	0.0407
regulation of response to external stimulus	13	1013	0.68	0.0038
cell adhesion	12	925	0.68	0.0077
regulation of immune system process	18	1514	0.64	0.00077
positive regulation of immune system process	11	949	0.63	0.0271
response to cytokine	12	1101	0.6	0.0244
cellular response to cytokine stimulus	11	1013	0.6	0.0394
regulation of multicellular organismal development	20	2096	0.55	0.00077
immune response	15	1588	0.54	0.0133
immune system process	22	2481	0.52	0.00077
positive regulation of multicellular organismal process	16	1770	0.52	0.0122
cell surface receptor signalling pathway	20	2325	0.5	0.0023
regulation of cell differentiation	16	1874	0.5	0.0201
regulation of cell population proliferation	14	1642	0.5	0.0432
regulation of developmental process	22	2648	0.49	0.0013
regulation of multicellular organismal process	24	3227	0.44	0.0018
regulation of response to stimulus	29	4114	0.42	0.00077
regulation of signal transduction	20	3107	0.38	0.0454
regulation of cell communication	22	3514	0.36	0.0355
regulation of signalling	22	3553	0.36	0.0381
signalling	30	5239	0.33	0.0067
signal transduction	28	4876	0.33	0.0123
cell communication	30	5320	0.32	0.0077
system development	25	4426	0.32	0.0394
multicellular organism development	28	5023	0.31	0.0201
positive regulation of biological process	31	6112	0.27	0.0262
response to stimulus	36	8046	0.22	0.0394

Table 7-10 – Biological pathways implicated in exploratory biomarker analysis

The first column represents the biological process implicated by the included proteins. The observed gene count indicates the number of proteins in the network annotated with a particular term, and the background gene count represents how many proteins in total have this term assigned. Strength of association is measured using a log10 (observed/expected) and describes how large the enrichment effect is. The false discovery rate describes how significant the enrichment is using p values corrected for multiple testing using the Benjamini-Hochberg procedure.

7.4 Discussion

7.4.1 Summary of findings

There are several key findings from this biomarker analysis of 1463 proteins in 24 individuals with extremes of IPF recruited into the INJUSTIS study. In unbiased exploratory analysis, several proteins including CA-125 (MUC16), CCL-19, CCL-24 and PM20D1 were differentially overexpressed in progressive IPF, whilst 13 analytes and numerous inflammatory proteins were elevated in stable disease. Pathway analysis identified several biological processes that may be implicated in the pathogenesis of IPF, and further study is necessitated. In sensitivity analyses restricted to males, the strongest effect size was observed for CA-125, although several other biomarkers were differentially expressed, suggesting the relationship between blood biomarkers and sex requires further investigation. More focussed analysis of pre-specified biomarkers demonstrated an association with disease outcomes only for CCL-18. MMP7 which was associated with disease progression and mortality in IPD meta-analysis was unable to differentiate between stable and progressive disease.

7.4.2 CA-125

CA-125 had the smallest p value across all biomarkers for differences in concentration between stable and progressive disease. These findings are consistent with the PROFILE study, where baseline measurements, and change over three-months in CA-125 predicted both overall survival and disease progression[124]. Notably, when analyses of this cohort were restricted to male participants only, CA-125 remained the biomarker with the strongest association with progressive disease. [229] CA-125 recognises mucous-associated antigens (MUC16) and is the most widely used tumour marker for the detection and monitoring of

ovarian cancer, whilst also playing an active role in several other cancers including pancreatic and breast cancer[429]. Disappointingly, the targeting of CA-125 with antibodies for the management of ovarian cancer has failed to demonstrate benefit in randomised trials.[430] In normal lung, CA-125 is understood to be secreted in small quantities by the bronchial epithelium, with increased secretion relative to disease severity observed in IPF[124]. In a recent study, CA-125 was overexpressed in the lung tissue of individuals with IPF compared with healthy controls and localised to fibroblasts and alveolar type 2 cells[431]. When MUC16 was repressed, there was an attenuation of TGF-β1 induced lung fibroblast proliferation, suggesting direct or indirect targeting of MUC16 could be a potential drug target in IPF. Other than the PROFILE study, no other published studies have evaluated the role of CA-125 in individuals with IPF, but there are emerging data suggesting CA-125 may be important in other fibrotic ILDs[432]. In a retrospective analysis of 80 participants with SSc-ILD, baseline CA-125 levels inversely correlated with FVC, and predicted disease progression over two years of follow up[433]. These findings suggest CA-125 may be a biomarker of progressive fibrosis irrespective of ILD subtype, and analysis of the complete INJUSTIS dataset is eagerly awaited to explore this hypothesis further.

7.4.3 Other biomarkers of progressive disease

Exploratory analysis identified several proteins that were enriched in individuals according to whether they had stable or progressive disease. Several enriched proteins were inflammatory in origin, and visual inspection of volcano plots demonstrated the majority of proteins that were at least 50% higher in stable disease compared with progressors, belonged to the inflammatory panel. When inspection was restricted to proteins enriched in

stable disease that were statistically significant, 11/13 were of inflammatory origin. Moreover, of the three proteins (other than CA-125) that were significantly elevated in progressors, two were inflammatory: CCL-19 and CCL-24. CCL-19 and CCL-24 are signalling proteins known as chemokines that typically lead to dendritic and T-cell migration, and induce transition of fibroblasts to myofibroblasts[434]. In experimental models, elevated CCL-24 levels have been demonstrated in hepatic, dermal and pulmonary fibrosis, and monoclonal antibodies targeted against CCL-24 have been shown to significantly attenuate fibrosis[435 436]. Further work is needed to elucidate the precise mechanisms of these possible biomarkers.

To contextualise and understand the findings of this exploratory analysis, a pathway analysis was performed using all proteins that were differentially expressed with statistical significance. There was significant enrichment of 34 biological pathways, many of which related to immunological processes. Other than carrying prognostic potential, a key function of blood biomarkers is they enable the study of diseases at a biological and molecular level. The identification of pathways implicated in progressive disease, irrespective of ILD aetiology will enable researchers to focus attention to understanding shared signalling pathways that are likely to be important in the pathogenesis of disease. A greater understanding of these pathways has the potential to ultimately lead to the development of therapeutics that can target these biological pathways. Simultaneously, proteins that represent these biological processes may serve as prognostic biomarkers, especially if they are stable and easy to measure. The findings of this exploratory analysis highlight the

potential of blood biomarkers, and further proteomic analysis will be performed in the complete INJUSTIS cohort to identify mechanistic pathways shared across fibrotic ILDs.

7.4.4 Limitations

The main limitation in this exploratory blood biomarker analysis relates to its small sample size, thereby limiting statistical power to detect differences in biomarker concentrations between stable and progressive disease. To mitigate this limitation, participants with extremes of IPF disease were selected to maximise difference in biomarker levels, and analyses are presented as exploratory only. For the same reason, adjustments for multiplicity were not applied, and findings will be replicated in the completed dataset. A further limitation is related to the method for selecting participants, which was typically performed according to three-month change in FVC in nearly all included participants (23/24). It remains unclear whether FVC follows a linear decline in IPF, and therefore on an individual level, three-month FVC change may not accurately correspond with disease progression. However, there were 7/12 deaths in the progressors, and 0/12 in the stable, suggesting three-month FVC change was related to disease outcomes in this cohort. A further limitation is possible confounding by anti-fibrotic therapies, though the numbers of individuals receiving anti-fibrotics were small (3/12 in progressors; 2/12 in stable). Reassuringly, this study was able to replicate data obtained for CA-125 in the PROFILE study, where nearly all participants were anti-fibrotic naïve. In crude comparisons, mean CA-125 measurements were lower in progressors receiving anti-fibrotic therapies compared with those not receiving anti-fibrotics (0.76 vs. 1.02), suggesting CA-125 levels may be lowered in response to anti-fibrotics. Future work should determine whether CA-125 and other

biomarkers have prognostic potential in individuals who are receiving anti-fibrotic therapies, and whether they represent theranostic biomarkers of treatment response. A further limitation relates to the stratification of analytes into four panels (inflammatory, cardiometabolic, neurology and oncology) by Olink. The accuracy of this analyte collation has not been verified, and it remains plausible that proteins may have been miscategorised. However, this was mitigated by pathway analyses which are unbiased and not influenced by Olink panel classifications. Other limitations include the collection of blood samples over 18 months across several sites in the UK with plasma storage time likely to vary substantially between centres and thus increase the risk of protein degradation. However, this is unlikely to be a source of considerable variability as all samples were collected, processed, and stored according to a protocol, and each site received training on managing samples.

7.5 Summary

This chapter presents the results of an unbiased blood biomarker analysis in 24 individuals with extremes of IPF, which will guide biomarker analytic strategy in the larger INJUSITS cohort. These findings also offer further support for the role of CA-125 as a prognostic biomarker in IPF and support the role of blood biomarkers in identifying biological pathways and molecular endotypes that are likely to be associated with pulmonary fibrosis. Biological pathways can be studied to understand disease pathogenesis, whilst also offering potential as therapeutic targets. In this cohort, several inflammatory biological processes were implicated, and further research is required to understand their significance. Exploratory protein analyses will be performed in the complete INJUSTIS cohort to both replicate these findings and explore possible commonalities and differences across fibrotic ILD subtypes.

Chapter 8 Final Discussion

8.1 Summary of thesis aims

This thesis has focused on understanding progressive fibrotic lung diseases, and explored the role of biomarkers as diagnostic, prognostic, theranostic, and endotypic markers. To summarise, specific aims for this thesis included:

1) To evaluate the role of serum proteins as biomarkers in pulmonary fibrosis
2) To determine the role of blood biomarkers as therapeutic targets
3) To describe the baseline features and longitudinal disease behaviours of a cohort with mixed fibrotic ILD
4) To assess the role of demographics and physiological variables as biomarkers of clinical progression in pulmonary fibrosis
5) To perform an exploratory analyses of blood biomarkers to identify novel analytes and their biological pathways associated with disease progression

8.2 Summary of findings

In this thesis I have evaluated several biomarkers and demonstrated their potential role in the management of individuals with fibrotic lung diseases. To further understand the potential strengths and limitations of blood biomarkers as prognostic tools and as therapeutic targets, I began (Chapter 2) by specifically appraising the existing blood biomarker research in IPF. Since IPF represents the prototypic progressive fibrotic ILD, the intention was to identify blood biomarkers with prognostic potential in IPF that may also help identify key pathogenic pathways. Such prognostic biomarkers and disease pathways can then be later characterised and studied in other fibrotic ILDs to explore whether there are shared disease pathways irrespective of ILD aetiology. Therefore, I performed a systematic review of prognostic blood biomarkers in IPF, with IPD meta-analysis for MMP-7 studies specifically. In the narrative review, a total of 15 blood biomarkers were included, and several biomarkers measured at baseline were associated with an increased risk of mortality or disease progression, though replication of effects across studies was weak. In general, across all the included biomarkers, three-month change in biomarkers did not predict mortality, except CA19-9 in the PROFILE study suggesting a duration of three-months may be too short to track blood biomarker change in relation to disease progression and mortality.

Importantly, several limitations with included studies were identified, including the use of data-dependent biomarker thresholds to maximise effect sizes, the omission of power-calculations, modest sample sizes, and summary estimates that were frequently unadjusted for important confounders known to influence the association between biomarkers and

outcomes. Due to these limitations, alongside weak replication of effects across a limited number of studies, it was concluded that based on existing evidence, there remains insufficient evidence to recommend any of the included blood biomarkers for clinical implementation as prognostic markers, though several biomarkers showed potential. There were sufficient data available for the epithelial biomarker, MMP7, to enable IPD meta-analysis and overcome several of these limitations.

IPD meta-analysis supported the role of MMP-7 as a prognostic biomarker in IPF, with every standard deviation increase in baseline MMP-7 associated with an estimated 23% increased risk of mortality and 27% increased likelihood of disease progression. Consistent with observations from studies in other blood biomarkers, three-month change in MMP-7 did not predict disease outcomes. A review of the literature to understand the role of MMP-7 in other fibrotic diseases confirmed several possible associations with elevated MMP-7 levels in other pulmonary and non-pulmonary fibrotic diseases, supporting a common final pathway shared across organs and fibrotic diseases[290 291].

In the following chapter, I performed a systematic review and meta-analysis of interleukin inhibitors for the treatment of COVID-19 associated viral pneumonitis, with a view to understanding the role of blood biomarkers as therapeutic targets. Whilst COVID-19 was never the intended focus of this thesis, this work was conducted amidst a global pandemic, which alongside many tragic consequences, created several opportunities for research. Whilst this review supported the use of tocilizumab, an IL-6 inhibitor, for the treatment of COVID-19, there were insufficient data to assess detailed trends in IL-6 levels, nor to

evaluate clinical outcomes according to pre-treatment IL-6 levels. Where sufficient data were reported, high variability in IL-6 was observed, highlighting possible limitations of serum IL-6 as a prognostic biomarker. Importantly, in several studies, tocilizumab did not alter outcomes, whilst in other studies, was associated with increased mortality, suggesting a therapeutic "window of opportunity". IL-6 has several physiological roles in humans particularly in regulating the acute phase response which may explain why inhibition may be deleterious in some circumstances. These findings help contextualise the potential role of blood biomarkers as therapeutic targets and emphasise a detailed understanding of cellular biology alongside pathogenesis of disease before blood biomarkers can be targeted therapeutically. These findings also highlight the importance of smaller mechanistic studies for the discovery and validation of blood biomarkers.

Having considered the role of blood biomarkers, the next chapter (Chapter 4) sought to determine the role of physiological markers in IPF, both as prognostic markers and as potential surrogate endpoints in future interventional clinical trials. Meta-analysis of IPD from ten placebo arms of IPF interventional trials identified several key findings. Baseline FVC, DL_{CO} and 6MWD were independently associated with mortality, whereas FVC and $DL_{CO,}$ but not 6MWD were associated with disease progression. When the association between demographics and outcomes was explored, only age was an independent predictor of mortality, with a 4% increased risk of death per year increase. Though these findings relating to baseline physiology and demographic factors have been demonstrated previously, studies have been associated with several limitations including retrospective designs and modest sample sizes. This study helps validate these findings in the largest

cohort of IPF participants pooled from ten interventional clinical trials performed worldwide, whilst utilising robust methodology and provides reliable interpretations of effect size.

Of greater novelty was the association between three-month change in physiology and poorer outcomes. For every 2.5% relative decline in FVC there was an associated 14% increased risk of mortality and 29% increased likelihood of disease progression. When FVC decline over three-months was dichotomised according to the cohort median (2.3%), notable differences in FVC decline over twelve-months were observed between groups suggesting the three months decline in lung function is highly predictive of twelve-month change. Whilst short-term change in DL_{CO} and 6MWD showed an association with outcomes, the effect size was lower compared with change in FVC. A 7% increased risk of mortality for every 2.5% relative decline in DL_{CO}, and 9% increased risk of mortality per 20m decline in 6MWD was observed. Previous studies have demonstrated change in physiology over 24 weeks can accurately predict poor outcomes in IPF, but this is the first study to establish the prognostic significance of a change in physiology over a shorter period of 12-weeks. Optimal thresholds for three-month change in FVC for predicting disease outcomes with the greatest sensitivity and specificity were identified using ROC analysis, with a 3% and 5.7% relative decline most strongly predictive of disease progression and mortality respectively. Collectively these findings suggest three-month changes in physiology, particularly FVC, which were previously regarded as evidence of clinically stable disease may have major implications for understanding short term prognosis and may permit enrichment into clinical trials based on short term disease behaviour.

Findings for three-month change in FVC and disease outcomes were replicated in trial treatment arms, supporting the prognostic significance of three-month FVC change irrespective of anti-fibrotic treatment. A 20% greater risk of overall mortality per 2.5% relative FVC decline, and 46% greater odds of disease progression was estimated. Notably, comparing FVC change between placebo and corresponding treatment arms, a treatment benefit from anti-fibrotics could be observed at the early three-month time point. Such findings have the potential to impact future shortened clinical trials in IPF by utilising FVC change over three months as a surrogate endpoint.

Having demonstrated the role of demographic, blood, and physiological biomarkers in IPF, the next part of this thesis sought to determine the natural disease behaviour in non-IPF fibrotic ILD, whilst also studying the prognostic significance of the identified biomarkers in this cohort. An interim analysis of 191 participants from the ongoing INJUSTIS study was performed in Chapter 5. Analysis of baseline characteristics suggested both IPF and non-IPF groups were well matched, with the majority of participants male, in their seventh decade of life, ethnically white, and with a previous smoking history. Baseline lung function, total six-minute walk distance and QoL questionnaire scores were comparable between both groups. In longitudinal analysis, greater declines in lung function and 6MWD were observed in IPF compared with non-IPF, but differences were not significant. Similarly, compared with non-IPF, a greater proportion of individuals with IPF had evidence of disease progression (58.6% vs 37.1%) and mortality (14.3% vs 10.2%), though differences were not statistically significant. These findings are unsurprising and must be contextualised with the understanding that not all individuals with non-IPF fibrotic ILD have progressive fibrotic

phenotypes, and therefore a subgroup will have more indolent disease courses relative to IPF. These findings also reiterate the importance of objective biomarkers measured earlier in the disease course to help characterise patients according to likely disease trajectories.

In the following chapter (Chapter 6), I utilised the INJUSTIS cohort to assess the role of prognostic biomarkers across fibrotic ILDs and examine whether biomarkers associated with poorer outcomes in IPF were also able to predict a progressive fibrotic phenotype in other ILDs. Consistent with findings demonstrated in IPF (Chapter 4), baseline FVC and DL_{CO} were associated with an increased risk of mortality, both when dichotomised using frequently used criteria, and when analysed as continuous variables in multivariate models. Notably, change in FVC over three-months strongly predicted mortality, with each 2.5% decline associated with a 29% increased risk of overall mortality in the whole INJUSTIS cohort, and a 23% increased risk of mortality in individuals with non-IPF ILD alone. The association between DL_{CO} change over three-months and outcomes was modest in comparison, with each 2.5% decline associated with an 8% increased risk of mortality, suggesting DL_{CO} change over three-months on its own may be less useful as a prognostic biomarker.

QoL scores from five questionnaires and their association with outcomes were evaluated, and no questionnaires measured at baseline were conclusively associated with mortality or disease progression. However, when changes in QoL scores over three-months were dichotomised into stable or worse, the IPARC questionnaire was associated with increased mortality. This suggests the change in IPARC over three-months may be a sensitive marker of disease progression, particularly when combined with other biomarkers in composite

models. Analyses evaluating baseline demographics, or six-minute walk distance demonstrated no association with disease outcomes. For all biomarkers assessed, analysis of data within the individual ILD subgroups could not be performed due to small sample sizes precluding meaningful conclusions.

In the second part of this chapter, the role of home spirometry in fibrotic ILD, both as an alternative to hospital spirometry, and as a potential trial endpoint was assessed. Comparisons between hospital and home spirometry were performed at baseline and three-months. At both time-points, home spirometry underestimated FVC in comparison with hospital spirometry, but there was high correlation between the two measurements, and over 90% of home spirometry values were within the agreement limits of hospital values. The underestimation of FVC using home spirometry has been previously observed in IPF[183], but is of modest clinical relevance, as serial FVC change is likely to be more clinically insightful than individual measurements at specified timepoints. Comparisons between hospital and home spirometry were replicated when analyses were restricted to non-IPF participants only, supporting the use of home spirometry as an alternative to hospital spirometry in fibrotic ILD.

Longitudinal modelling was performed to identify whether home spirometry measurements represent an earlier biomarker of increased mortality. Over three-months, the overall median adherence to daily spirometry was 79%, despite the blinding of measurements, supporting future blinded spirometry in fibrotic ILD. The CoV was slightly higher in the larger non-IPF cohort compared with IPF (6.59% vs. 4.38%) but was comparable to previous non-

blinded studies in IPF[422 423]. Earlier findings presented in this thesis support the change in FVC over three-months measured using hospital spirometry as an accurate predictor of mortality and disease progression in both IPF and non-IPF fibrotic ILD. When a similar timeframe was applied to home spirometry using linear regression, change in FVC was unable to predict mortality. Although, an FVC decline greater than 5% over 28 days was associated with increased mortality, findings could not be replicated when the rate of FVC change was treated as a continuous variable and adjusted for potential confounders. These findings lay doubt on the additional prognostic significance of home spirometry, and thus the utilisation of home spirometry as an early-phase clinical trial endpoint in fibrotic ILD. Analysis of the complete INJUSTIS cohort will provide further clarification.

In the final chapter (Chapter 7), blood biomarkers that were identified in the systematic review (Chapter 2) were studied in a cohort of 24 individuals with extremes of IPF. CA-125 was strongly overexpressed in individuals with progressive disease compared with stable disease, validating the findings of the PROFILE study. Emerging data suggest CA-125 may be overexpressed in individuals with other fibrotic diseases and thus may represent a biomarker of progressive fibrosis irrespective of ILD subtype[432]. Notably, MMP7 which was associated with disease progression and mortality in IPD meta-analysis was unable to differentiate between stable and progressive disease.

Alongside their prognostic and theranostic role, it can be argued the principal benefit of blood biomarkers in fibrotic ILD is to identify biological pathways representative of progressive fibrosis, which can be studied to both understand pathogenesis and target

therapeutically. An unbiased exploratory analysis of nearly 1500 proteins was performed which identified several biomarkers, particularly of inflammatory origin, that were differentially expressed in stable vs. progressive disease. Pathway analysis identified several biological pathways that were significantly enriched, many of which related to immunological processes. The findings of this exploratory analysis highlight the potential of blood biomarkers in identifying molecular endotypes associated with progressive fibrosis, and further proteomic analysis will be performed in the complete INJUSTIS cohort to identify mechanistic pathways shared across fibrotic ILDs.

Taken collectively, the data presented in this thesis strongly support an important role for clinical biomarkers in fibrotic ILD. Firstly, the findings confirm there are several biomarkers that offer prognostic potential in IPF including blood proteins, physiological variables, and baseline demographic factors. It is likely a combination of these biomarkers alongside existing known prognostic factors such as radiological scores, genetic signals, and patient reported symptoms, assessed in composite models, will lead to significant improvements in our ability to accurately predict disease related outcomes. This will enable the identification of individuals with progressive disease before the onset of irreversible fibrosis to enable prognostication, facilitate stratification of therapies using a personalised medicine approach, and allow stratification into clinical trials.

These findings also support the role of biomarkers both in the development of therapeutics and their testing in clinical trials. The targeting of specific prognostic blood biomarkers such as MMP-7 and CA-125, alongside enriched biological pathways in individuals with

progressive disease identified using exploratory blood biomarker analysis, is likely to offer therapeutic benefit and further research is needed. Once therapeutics are developed, biomarkers offer several potential benefits in clinical trials. Other than their role in identifying progressors to enable enrichment of clinical trial populations, this research supports the use of three-month change in FVC as an earlier trial endpoint. This has the potential to revolutionise clinical trials and will help fast-track the development and testing of novel therapeutics. Moreover, whilst not specifically explored in this thesis, it is probable blood biomarkers representing pathways targeted by novel therapeutics will offer additional information as study endpoints in interventional trials.

Whilst the INJUSTIS study evaluating fibrotic ILDs other than IPF is incomplete, analysis of interim data demonstrates a substantial proportion of individuals with a progressive fibrotic phenotype, who share baseline characteristics with non-progressors, thus highlighting the importance of accurate and validated biomarkers in pulmonary fibrosis. The change in QoL questionnaire scores and lung physiology over three-months showed promise as prognostic markers, though validation is required. Whilst the blood biomarkers explored in this thesis could not be investigated in the INJUSTIS cohort due to ongoing recruitment, an eventual understanding of molecular endotypes shared across fibrotic ILDs will offer further pathogenic, diagnostic, prognostic and theranostic insights and has the potential to reclassify ILD management according to disease behaviours rather than aetiology alone.

8.3 Clinical implications

The findings from this thesis validate the role of MMP-7 and CA-125 as prognostic blood biomarkers in IPF, and they should be considered for clinical implementation as prognostic tools. Whilst CA-125 assays are routinely available in biochemistry laboratories, further work to standardise MMP-7 measurements is required to enable wider application. ROC analysis to determine optimal MMP-7 and CA-125 thresholds for predicting disease progression and mortality with the greatest sensitivity and specificity should be performed. Blood biomarkers should be combined with existing prognostic markers including symptoms, radiology, and lung function, to identify individuals at greatest risk for poorer outcomes, which will ultimately enable clinicians to tailor further management supporting a precision medicine approach. Moreover, the change in physiology over three-months is likely to be clinically informative, particularly in composite models with other prognostic biomarkers. Whilst small variations in FVC may reflect test variability, the pre-test probability for disease progression in fibrotic ILD is high, and thus marginal declines may be clinically important and worthy of more intense evaluation.

Findings from this thesis also support the feasibility of remote lung function monitoring using home spirometry in fibrotic ILDs other than IPF. Remote monitoring has several clinical implications including the potential to enable early detection of rapidly declining FVC suggestive of infection or acute exacerbation, monitoring response to therapies and empowering individuals with their own health. Moreover, remote monitoring is likely to be particularly relevant where clinical access or trial participation is limited due to geographical barriers, individual preference, clinical service demands or future pandemics.

8.4 Future research

The work presented in this thesis forms the basis for further research of biomarkers in pulmonary fibrosis and identifies several priorities which are summarised below.

i) *What are future MMP-7 research priorities in fibrotic ILD?*

Future research should standardise MMP-7 assays to enable study comparisons and the adoption of MMP-7 as a clinical biomarker. Longitudinal studies should aim to identify optimal MMP-7 thresholds for predicting disease progression and mortality using ROC analysis. MMP-7 change following initiation of anti-fibrotic therapy may represent a biomarker of treatment response and predict an earlier response to pharmacotherapy than more conventional methods. Further research should examine the relationship between anti-fibrotic therapy and MMP-7. Moreover, the potential role of MMP-7 as a therapeutic target requires greater understanding and this should be prioritised for future research. The utility of blood biomarkers showing potential in IPF should ultimately be explored in well-defined individuals with non-IPF fibrotic ILD, where there are likely to be mechanistic similarities and common fibrotic pathways.

ii) Are there further blood biomarkers that offer prognostic insights in fibrotic ILD?

The exploratory analyses of nearly 1500 proteins in a small cohort of individuals with IPF described in Chapter 7 suggests there may be several prognostic proteomic biomarkers that are yet to be characterised. Further exploratory and unbiased analyses in large prospective cohorts using an approach consistent with that adopted in GWAS studies is likely to identify

further analytes with prognostic potential. Serum biomarker analysis in the final INJUSTIS cohort will be performed to validate previous findings but also to identify novel analytes. Future biomarkers studies should include detailed analysis plans, robust sample size calculations, the use of discovery and validation cohorts to replicate findings, adjustment of potential confounders, and the standardisation of biomarker assays to enable comparisons across studies. Results from individual studies should be pooled using IPD meta-analysis to increase sample sizes and thus offer additional power to detect novel analytes. Furthermore, biomarkers representing various pathogenic pathways should be combined in future studies to increase our understanding of IPF pathogenesis and assess whether combinations of biomarkers increase the specificity and sensitivity for predicting disease outcomes.

iii) Are there differences by sex for blood biomarkers in fibrotic ILD?

This thesis has largely combined male and female participants when assessing the association between blood biomarkers and disease outcomes. Intriguingly, when exploratory biomarker analyses described in chapter 7 were restricted to male participants, there were several differences in biomarkers compared with the whole cohort. Since numbers were small, reliable inferences could not be made, but these findings require further study. Since IPF and other fibrotic ILDs show a strong association with male sex, future work should explore differences in blood biomarkers according to sex, by either stratification or analysis of interactions with sex.

iv) Are there blood biomarkers that can predict acute exacerbations?

Acute exacerbations are a devastating consequence of fibrotic ILD, and are associated with extremely poor survival[156]. Whilst several studies have attempted to identify biomarkers that can that reliably predict acute exacerbations, findings have been inconsistent and unsatisfactory. The identification of a blood biomarker that recognises disease phenotypes that have a high-risk for acute exacerbations, will form the basis to understand pathogenesis, and enable the development of targeted therapies that may contribute to improved outcomes. In the final INJUSTIS cohort, serum from individuals who experienced an exacerbation or respiratory-related hospitalisation will be studied to ascertain whether there is a specific molecular signature.

v) Do blood biomarkers offer theranostic potential?

An important potential benefit of blood biomarkers in lung fibrosis is the ability to predict an earlier response to the initiation of pharmacotherapy compared with existing methods that rely on longitudinal changes in lung physiology or radiology. Since the majority of blood biomarker studies have been performed in anti-fibrotic naïve cohorts, it is unknown whether previously identified biomarkers such as CA-125 and MMP-7 are able to predict response to anti-fibrotic therapy. Further studies to establish the role of blood biomarkers as therapeutic biomarkers are needed. In addition, few studies in IPF have evaluated the change in biomarkers to monitor disease activity once anti-fibrotics are commenced. In a post-hoc analysis of the INMARK trial, nintedanib reduced blood concentrations of biomarkers associated with collagen synthesis and epithelial injury as early as four weeks[437-

[439]. The identification of short-term changes in blood biomarkers that represent a meaningful therapeutic response once anti-fibrotics are commenced will help validate their role in individual patients and as early-phase clinical trial endpoints.

vi) Can blood biomarkers be therapeutically targeted in fibrotic ILD?

A key aim of blood biomarker discovery other than the prognostication of disease, is to enable development of targeted therapeutics. In several other conditions, including COVID-19 as presented in Chapter 2, the targeting of blood biomarkers with therapies has shown tremendous success. Whilst previous therapies targeted against MMPs and CA-125 have shown disappointing results in various cancers, there is now greater biological understanding to suggest a more biomarker specific and lung targeted approach may be worth exploring further. There is likely to be a window of opportunity where biomarker suppression may outweigh any potential deleterious effects. As novel biomarkers and therapies are identified, a detailed understanding of blood biomarkers, biological pathways, and the pharmacology of the therapy under investigation will be required to accurately assess this crucial subject.

vii) Is there a common molecular endotype across fibrotic ILDs?

A key priority of this thesis was to establish disease behaviour and the role of biomarkers in fibrotic ILDs other than IPF. Several similarities with IPF have been demonstrated including

shared risk factors, radiological similarities, genetic risk factors, and similarities in disease behaviour. Whilst this thesis has been unable to include blood biomarker analysis in non-IPF fibrotic ILD, there are highly likely to be mechanistic similarities and shared biological pathways that represent a progressive fibrotic phenotype. The use of blood biomarkers to identify molecular endotypes holds great potential in fibrotic ILD, and alongside the identification of novel analytes, the study of MMP-7 and CA-125 analysis will be performed once recruitment to the INJUSTIS study is incomplete. The identification of molecular endotypes has the potential to alter the current classification which is based on disease aetiology rather than disease behaviours and help establish the role of these biomarkers as prognosticators and therapeutic targets across fibrotic diseases.

viii) An analysis of the complete INJUSTIS cohort

As described the INJUSTIS cohorts forms the largest prospective dataset in mixed fibrotic ILD. Analysis of the final INUSTIS cohort will include several additional analyses to understand natural disease behaviours and characterise the role of biomarkers in non-IPF fibrotic ILD. Alongside performing all the analyses described in this thesis in larger sample sizes with more complete follow up data, there are several other objectives which have been described in Chapter 5. To summarise, these will include an epidemiological and survival analysis to understand the natural history in each of the fibrotic ILDs, an exploration of the association between environment exposures and disease outcomes, the identification of minimal clinically important differences in questionnaire scores over three-months that represent disease progression, and an investigation of gene expression profiles which affect disease progression.

8.5 Impact of COVID-19

8.5.1 Impact on planned research

The impact of the COVID-19 pandemic on the research carried out as part of this thesis cannot be underestimated. During the peak of the pandemic, I was redeployed to the NHS frontline to help with the COVID-19 response, which led to significant disruption of my research for several months. The INJUSTIS study was a particular casualty of COVID-19, with several challenges faced in the previous 18 months. Prior to the pandemic, excellent progress was being made with 178/250 participants recruited and projections to recruit the remaining participants ahead of the study completion date. This would have enabled the analysis of a complete dataset to explore the thesis hypothesis that there are shared pathogenic pathways across fibrotic ILDs. For several reasons including staff shortages due to shielding and isolation, competing demand from high priority COVID-19 studies, and study participants shielding, the INJUSTIS study was paused for over 12 months for both new and follow up visits. This has led to a substantial delay in study completion but has also resulted in several missed follow up and lung function visits. Although recruitment has since slowly recommenced, several hospital sites have been unable to resume due to ongoing challenges in their research departments attributable to COVID-19.

Since data from the INJUSTIS study was intended to form the basis of my original thesis, I have had to significant adapt my research. Therefore, the data presented in this thesis from the INJUSTIS study is preliminary and from interim analyses only, and further analysis will be performed on the complete dataset once the remaining participants have completed their follow-up. Despite the challenges, COVID-19 created several research opportunities, and I

was able to get involved in several projects and collaborations that otherwise would have been unlikely. To ensure a meaningful contribution to the field of biomarkers in progressive fibrosis, I performed several meta-analyses as these were compatible with remote working during the pandemic when access to patients was difficult, and the INJUSTIS study was on hold.

8.5.2 Impact on personal life

Alongside the impact of COVID-19 on my research, the pandemic has been incredibly challenging from a personal and health perspective. Unfortunately, I contracted COVID-19 and developed viral pneumonitis necessitating hospital admission, and although I made an eventual complete recovery, I was affected by breathlessness and fatigue for several weeks. Moreover, since my parents have been shielding, their care duties have fallen upon me. Although this has been a pleasure, it has undoubtedly been an additional demand on my time and health. Sadly, I have lost several extended family members to COVID-19, including two of my dad's brothers and one of my wife's uncles. My local community were particularly affected by the pandemic, and I took it upon myself to educate underserved communities using social media messaging, webinars, radio, and TV shows. Alongside the impact of acute illness and family bereavement, I have been through a house move and subsequent extensive renovation which overspilled to the start of the pandemic. Significant delays in the renovation works due to COVID-19 meant living in temporary hospital accommodation with my family, where I had no access to broadband internet for several months, making remote working and home schooling even more challenging! Although tough, these experiences have enabled me to adapt as a researcher and grow into a better-rounded individual.

8.6 Conclusion

In this thesis I have explored the role of biomarkers in progressive fibrotic ILD. These analyses have: confirmed the role of MMP-7 as a prognostic biomarker in IPF using robust IPD meta-analysis; demonstrated the success of a biomarker-targeted therapy for treating severe acute respiratory syndrome; demonstrated baseline and three-month change in lung physiological variables, particularly FVC can be used as prognostic biomarkers and clinical trial endpoints; highlighted natural disease behaviours in fibrotic ILDs; identified the role of physiology including home spirometry in non-IPF ILD; validated CA-125 as a biomarker of disease progression in an independent IPF cohort; demonstrated the potential of blood biomarkers to identify biological pathways associated with progressive fibrosis. This work forms the basis of future study into the role of biomarkers in progressive pulmonary fibrosis.

Chapter 9 References

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Chapter 10 Appendix

10.1 Systematic reviews search strategy

Interleukin inhibitors in COVID-19 search strategy

1. Respiratory Distress Syndrome, Adult/

2. SARS Virus/

3. Severe Acute Respiratory Syndrome/

4. severe acute respiratory distress syndrome*.mp.

5. Coronavirus Infections/

6. Coronavirus/

7. coronav*.mp.

8. covid*.mp.

9. SARS.mp.

10. Middle East Respiratory Syndrome Coronavirus/

11. MERS.mp.

12. anakinra.mp.

13. kineret.mp.

14. tocilizumab.mp.

15. altizumab.mp.

16. actemra.mp.

17. roactemra.mp.

18. sarilumab.mp.

19. kevzara.mp.

20. siltuximab.mp.

21. sylvant.mp.

22. Interleukin 1 Receptor Antagonist Protein/

23. anti-IL6.mp.

24. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11

25. 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23

26. 24 and 25

Blood biomarker SR search strategy

Participants	Intervention	Intervention	Outcomes
1. idiopathic pulmonary fibros*.mp.	12. Mucin-1/	45. Chitinase-3-Like Protein 1/ or Chitinase-3-like protein 1.mp.	78. prognosis.sh.
2. pulmonary fibros*.mp.	13. KL-6.mp.	46. IGFBP-2.mp. or Insulin-Like Growth Factor Binding Protein 2/	79. diagnosed.tw.
3. Pulmonary Fibrosis/ or Idiopathic Pulmonary Fibrosis/	14. krebs von den lungen-6.mp.	47. Insulin like growth factor binding protein 2.mp.	80. cohort:.mp.
4. cryptogenic fibrosing alveolitis.mp.	15. SP-A.mp.	48. ICAM-1.mp. or Intercellular Adhesion Molecule-1/	81. predictor:.tw.
5. usual interstitial pneumonia*.mp.	16. Pulmonary Surfactant-Associated Protein A/	49. VEGF.mp. or Vascular Endothelial Growth Factor A/	82. death.tw.
6. Fibrosing alveolitis.mp.	17. Pulmonary Surfactant-Associated Protein D/	50. HSP70 HEAT-SHOCK PROTEINS/ or HSP70.mp.	83. exp models, statistical/
7. Idiopathic Interstitial Pneumonia*.mp.	18. Pulmonary Surfactants/	51. LEPTIN/ or Leptin.mp.	84. disease progression.sh.
8. Interstitial pneumonia*.mp.	19. SP-D.mp.	52. CXCL13.mp. [mp=title, abstract, original title, name of substance	85. disease progression.mp.
9. Idiopathic interstitial lung disease.mp.	20. surfactant protein*.mp.	53. Chemokine CXCL13/ or C-X-C motif chemokine 13.mp.	
10. Chronic interstitial pneumonia*.mp.	21. CA-125 Antigen/ or CA125.mp.	54. Forced Vital Capacity.mp. or Vital Capacity/	
	22. cancer antigen 125.mp.	55. FVC.mp.	
	23. mucin 16.mp.	56. Forced Expiratory Volume/ or FEV1.mp.	
	24. CA-19-9 Antigen/ or CA19-9.mp.	57. forced expiratory volume.mp.	
	25. cancer antigen 19-9.mp.	58. 6-minute walk.mp.	
	26. carbohydrate antigen 19-9.mp.	59. Six-minute walk.mp.	
	27. Matrix Metalloproteinase 1/ or MMP-1.mp.	60. Walk Test/	
	28. Matrix Metalloproteinase 7/ or MMP-7.mp.	61. walk test.mp.	
	29. matrix metalloproteinase.mp. or Matrix Metalloproteinases/	62. 6MWT.mp.	
	30. LOXL2.mp.	63. 6MWD.mp.	
	31. Protein-Lysine 6-Oxidase/	64. Pulmonary diffusing capacity.mp. or Pulmonary Diffusing Capacity/	
	32. protein-lysine 6-oxidase.mp.	65. Diffusion capacity for carbon monoxide.mp.	
	33. periostin.mp.	66. DLCO.mp.	
	34. Osteoblast-specific factor 2.mp.	67. Transfer factor.mp. or Transfer Factor/	
	35. Epitopes/ or Neoepitope*.mp.	68. Gas transfer.mp.	
	36. Chemokines, CC/ or CCL18.mp.	69. TLCO.mp.	
	37. Chemokine CCL18.mp.	70. KCO.mp.	
	38. Chemokines, CC/ or CC-chemokine ligand 18.mp.	71. PHYSIOLOGY/	
	39. IL-8.mp. or Interleukin-8/	72. Physiolog*.mp.	
	40. Interleukin-8.mp.	73. SPIROMETRY/	
	41. CXCL8.mp.	74. spiromet*.mp.	
	42. Chemokine ligand 8.mp.	75. biomarkers.mp. or BIOMARKERS/	
	43. Chitinase-3-Like Protein 1/ or YKL-40.mp.	76. ((Serum or clinical or immun* or lab or laboratory or biochemical or biological) and marker*).mp.	
	44. CHI3L1.mp.		

Physiology biomarker SR – search strategy

1. idiopathic pulmonary fibros*.mp.

2. pulmonary fibros*.mp.

3. Pulmonary Fibrosis/ or Idiopathic Pulmonary Fibrosis/

4. cryptogenic fibrosing alveolitis.mp.

5. usual interstitial pneumonia*.mp.

6. Fibrosing alveolitis.mp.

7. Idiopathic Interstitial Pneumonia*.mp.

8. Interstitial pneumonia*.mp.

9. Idiopathic interstitial lung disease.mp.

10. Chronic interstitial pneumonia*.mp.

11. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10

12. Forced Vital Capacity.mp. or Vital Capacity/

13. FVC.mp.

14. Forced Expiratory Volume/ or FEV1.mp.

15. forced expiratory volume.mp.

16. 6 minute walk.mp.

17. Six minute walk.mp.

18. Walk Test/

19. walk test.mp.

20. 6MWT.mp.

21. 6MWD.mp.

22. Pulmonary diffusing capacity.mp. or Pulmonary Diffusing Capacity/

23. Diffusion capacity for carbon monoxide.mp.

24. DLCO.mp.

25. Transfer factor.mp. or Transfer Factor/

26. Gas transfer.mp.

27. TLCO.mp.

28. KCO.mp.

29. PHYSIOLOGY/

30. Physiolog*.mp.

31. SPIROMETRY/

32. spiromet*.mp.

33. 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32

34. 11 and 33

35. limit 34 to humans

36.. limit 35 to english language

37.. limit 36 to (clinical trial, all or randomized controlled trial)

10.2 Email sent to authors for IPD

MMP7 SR

We would be very grateful for your assistance in undertaking a robust meta-analysis. The team at University of Nottingham (UK), led by Prof Gisli Jenkins, are conducting a systematic review and meta-analysis of blood biomarkers in IPF. The protocol for the study can be found on PROSPERO: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=120402

As part of the review, we will conduct a meta-analysis of the association of MMP-7 levels with mortality in IPF. We have chosen this biomarker because there is sufficient published data to make it feasible and useful.

To assist with this, we would be extremely grateful if you could kindly provide us with individual patient data from your highly relevant study entitled "…" published in …

We also note significant heterogeneity in disease progression definitions across individual studies, and therefore hope to meta-analyse MMP-7 level associations with a shared definition based on FVC and mortality and would also appreciate data to assist with this. We appreciate the inconvenience such requests entail, and we would like to make the process as smooth as possible, we will of course acknowledge all support.

The attached excel spreadsheet highlights the anonymised data we are seeking for each patient, where available:

- MMP-7 level (baseline and 3-months)
- Assay method (type of assay used)
- Sample type (serum or plasma)
- Age
- Gender (M or F)
- Follow up time (days)
- Dead or alive at end
- Time to death (days)
- Baseline FVC (% predicted)
- 3-month FVC (% predicted)
- 12-month FVC (% predicted)
- Smoking (ever or never)

Thank you for your help, we look forward to communicating with you further.

Physiology SR

We would be very grateful for your assistance in undertaking a robust meta-analysis on lung function in patients with IPF.

The team led by Professor Gisli Jenkins (University of Nottingham, UK), are conducting a systematic review and individual patient data meta-analysis of clinical trial placebo arms. As part of the review, we are hoping to explore whether baseline and 3 month change in physiological biomarkers (FVC, DLCO, 6MWD) predict relevant outcomes such as disease progression and mortality in antifibrotic-naïve IPF patients. The protocol for the study can be found on PROSPERO: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=164935

To assist with this, we would be extremely grateful if you could kindly provide us with **placebo** arm individual patient data from your highly relevant study entitled "…" published in "…" We appreciate requests for patient data can be an inconvenience and stretch precious resource, so we hope to make the process as simple as possible. We will acknowledge all support.

We would be very grateful if you could populate the attached spreadsheet, which details the anonymised data we are seeking for each patient:

- Patient pseudoID
- Age at consent
- Height (cm)
- Ethnicity
- Gender (M or F)
- Smoking (ever or never)
- Follow up time (days)
- Dead or alive at end
- Time to death (days)
- Baseline, 3- and 12-month FVC (L)
- Baseline, 3- and 12-month FVC (% predicted)
- Baseline, 3- and 12-month DLCO (ml/min/mmHg)
- Baseline, 3- and 12-month DLCO (% predicted)
- Baseline, 3- and 12-month 6 min walk distance (m)

If data for these specific time points (3 and 12 months) are not available, we would value the closest data.

It would also be helpful if you could highlight the method used to calculate the FVC % predicted, and also whether a strategy has been applied to any missing data e.g. imputation.

Thank you for your help, we look forward to further communications with you.

10.3 Summary of study results in blood biomarker SR

Author (year)	Sample size	Follow up (months)	Effect size (Variance)	Level of adjustment	Effect size reported for
SP-A					
Kinder (2009)	82	36	HR 3.27 (95% CI 1.49-7.17)	a,b,c,d,e,g	per bio SD
Doubkova (2016)	18	NR	Not significant (NR)	x	bio > or < median (98.1ng/mL)
Hamai (2016)	65	31	HR 1.01 (95% CI 0.99-1.02)	x	continuous
SP-D					
Kinder (2009)	82	36	HR 2.04 (95% CI 0.99-4.22)	a,b,c,d,e,g	per bio SD
Collard (2010)	67	NR	OR 1.23 (95% CI 0.36-4.21)	"Bivariate" - NR	log change in bio
Doubkova (2016)	18	NR	Not significant (NR)	x	bio > or < median (623.1ng/mL)
Hamai (2016)	65	31	HR 1.00 (95% CI 0.99-1.002)	x	continuous
Maher (2017) - *Validation*	206	36	HR 2.72 (95% CI 1.65-4.48)	x	bio > or < 38.7ng/mL
CCL-18					
Prasse (2009)	72	24	HR 7.98 (95% CI 2.49-25.51)	a,b,c,d,e	bio > or < 150ng/mL
Hamai (2016)	65	31	HR 1.007 (95% CI 0.99-1.01)	X	continuous
Neighbors (2018) – *Test*	123	12	OR 4.4 (95% CI 1.13-17.15)	x	bio ≥ or < median
Neighbors (2018) – *Replication*	237	12	OR 3.37 (95% CI 1.17-9.67)	x	bio ≥ or < median
CXCL-13					
Guo (2020)	126	60	HR 1.03 (95% CI 1.02-1.06)	a	bio > or < 62pg/mL

Vuga (2014)	95	>24	HR 14.9 (95% CI 1.1-197.2)	a,b,d,e	bio > or < highest quartile
Neighbors (2018) – *Test*	123	12	OR 2.95 (95% CI 0.76-11.46)	x	bio ≥ or < median
Neighbors (2018) – *Replication*	237	12	OR 6.17 (95% CI 1.75-21.8)	x	bio ≥ or < median
KL-6					
Collard (2010)	67	NR	OR 0.41 (95% CI 0.06-2.93)	"Bivariate" - NR	bio log change
Hamai (2016)	65	31	HR 1.001 (95% CI 1.00-1.002)	a,b,c	continuous
Guo (2020)	126	60	HR 1.83 (95% CI 1.01-3.69)	a	bio > or < 800U/mL
IL-8					
Richards (2012) – *Derivation*	140	22	HR 2.4 (95% CI 1.2-4.79)	a,b,d	bio > or < 0.0029
Richards (2012) – *Validation*	101	17	HR 2.3 (95% CI 0.94-5.64)	a,b,d	bio > or < 0.0097
Papiris (2018)	41	12	OR 1.067 (95% CI 1.01-1.12)	x	per increase of 1pg/mL
CA19-9					
Maher (2017) – *Validation*	206	36	HR 2.95 (95% CI 1.82-4.78)	x	bio > or < 22 U/mL
CA-125					
Maher (2017) – *Validation*	206	36	HR 3.01 (95% CI 1.64-5.54)	x	bio > or < 12 U/mL
LOXL2					
Chien (2014) – *ARTEMIS*	69	24	HR 1.87 (95% CI 0.28-12.45)	d,e,f,h	bio > or ≤ 800pg/mL
Chien (2014) – *GAP*	104	24	HR 2.28 (95% CI 1.18-4.38)	b	bio > or ≤ 700pg/mL
Periostin					
Okamoto (2011)	77	36	Not significant (NR)	x	NR

Neighbors (2018) - *Test*	123	12	OR 3.05 (95% CI 0.79-11.88)	x	bio ≥ or < median
Neighbors (2018) – *Replication*	237	12	OR 1.91 (95% CI 0.72-5.05)	x	bio ≥ or < median
YKL-40					
Neighbors (2018) – *Test*	123	12	OR 1.77 (95% CI 0.53-5.92)	x	bio ≥ or < median
Neighbors (2018) – *Replication*	237	12	OR 2.7 (95% CI 0.94-7.75)	x	bio ≥ or < median
ICAM-1					
Richards (2012) - *Derivation*	140	22	HR 2.6 (95% CI 1.43-4.73)	a,b,d	bio > or < 202.5ng/mL
Richards (2012) – *Validation*	101	17	HR 2.8 (95% CI 1.36-5.76)	a,b,d	bio > or < 300ng/mL
ECM neoepitopes					
Jenkins (2015) –*Discovery* ***BGM***	55	26	HR 1.17 (95% CI 0.53-2.58)	x	two-fold increase in bio value
Jenkins (2015) –*Validation* ***BGM***	134	21	HR 1.34 (95% CI 0.92-1.97)	x	two-fold increase in bio value
Jenkins (2015) –*Discovery* ***C1M***	55	26	HR 1.21 (95% CI 0.66-2.22)	x	two-fold increase in bio value
Jenkins (2015) –*Validation* ***C1M***	134	21	HR 1.62 (95% CI 1.14-2.31)	x	two-fold increase in bio value
Jenkins (2015) –*Discovery* ***C3A***	55	26	HR 1.34 (95% CI 0.95-1.88)	x	two-fold increase in bio value
Jenkins (2015) –*Validation* ***C3A***	134	21	HR 1.91 (95% CI 1.06-3.46)	x	two-fold increase in bio value
Jenkins (2015) –*Discovery* ***C3M***	55	26	HR 2.18 (95% CI 0.95-5.00)	x	two-fold increase in bio value
Jenkins (2015) –*Validation* ***C3M***	134	21	HR 1.56 (95% CI 0.94-2.59)	x	two-fold increase in bio value
Jenkins (2015) –*Discovery* ***C5M***	55	26	HR 1.66 (95% CI 0.95-2.91)	x	two-fold increase in bio value
Jenkins (2015) –*Validation* ***C5M***	134	21	HR 1.07 (95% CI 0.66-1.72)	x	two-fold increase in bio value
Jenkins (2015) –*Discovery* ***C6M***	55	26	HR 1.49 (95% CI 0.86-2.56)	x	two-fold increase in bio value

Jenkins (2015) –*Validation* ***C6M***	134	21	HR 1.39 (95% CI 0.93-2.06)	x	two-fold increase in bio value
Jenkins (2015) –*Discovery* ***CRPM***	55	26	HR 3.74 (95% CI 1.46-9.58)	x	two-fold increase in bio value
Jenkins (2015) –*Validation* ***CRPM***	134	21	HR 1.87 (95% CI 0.98-3.56)	x	two-fold increase in bio value
Jenkins (2015) – *Discovery* ***ELM***	55	26	HR 0.96 (95% CI 0.48-1.92)	x	two-fold increase in bio value
Jenkins (2015) – *Discovery* ***ELM2***	55	26	HR 0.96 (95% CI 0.75-1.24)	x	two-fold increase in bio value
Jenkins (2015) – *Discovery* ***P3NP***	55	26	HR 1.48 (95% CI 0.67-3.27)	x	two-fold increase in bio value
Jenkins (2015) – *Discovery* ***VICM***	55	26	HR 1.11 (95% CI 0.83-1.49)	x	two-fold increase in bio value
Collagen synthesis peptides					
Organ (2019) **P1NP**	145	34	HR 0.81 (95% CI 0.6-1.11)	d,e	two-fold increase in bio value
Organ (2019) **PRO-C3**	145	34	HR 1.2 (95% CI 0.74-1.93)	d,e	two-fold increase in bio value
Hoyer (2020) **PRO-C3**	184	36	HR 2.32 (95% CI 1.33-4.04)	a	continuous
Organ (2019) **PRO-C6**	145	34	HR 1.11 (95% CI 0.57-2.16)	d,e	two-fold increase in bio value
Hoyer (2020) **PRO-C6**	184	36	HR 2.18 (95% CI 0.74-4.35)	a	continuous
Organ (2019) **P1NP:C1M**	145	34	HR 0.77 (95% CI 0.6-0.99)	d,e	two-fold increase in bio value
Organ (2019) **PRO-C3:C3M**	145	34	HR 1.17 (95% CI 0.77-1.79)	d,e	two-fold increase in bio value
Organ (2019) **PRO-C6:C6M**	145	34	HR 0.86 (95% CI 0.59-1.26)	d,e	two-fold increase in bio value
Hoyer (2020) **PRO-C6**	184	36	HR 1.8 (95% CI 0.74-4.35)	a	continuous

Table 10-1 - Studies reporting mortality outcomes.

x=no adjustments, a=age, b=gender, c=smoking, d=FVC e=DLCO, f= 6MWT, g=race, h=medication

Author (year)	Sample size	Follow up (months)	Effect size (Variance)	Level of adjustment	Effect size reported for
SP-D					
Maher (2017) - *Discovery*	106	36	HR 1.01 (95% CI 0.97-1.06)	x	rising vs stable bio over 3 months
Maher (2017) – *Validation*	206	36	HR 0.99 (95% CI 0.59-1.67)	a,b,c,d	rising vs stable bio over 3 months
CA19-9					
Maher (2017) - *Discovery*	106	36	HR 1.02 (95% CI 1.00-1.05)	X	rising vs stable bio over 3 months
Maher (2017) – *Validation*	206	36	HR 1.39 (95% CI 0.79-2.46)	a,b,c,d	rising vs stable bio over 3 months
CA-125					
Maher (2017) - *Discovery*	106	36	HR 1.77 (95% CI 1.39-2.26)	x	rising vs stable bio over 3 months
Maher (2017) – *Validation*	206	36	HR 2.39 (95% CI 1.4-4.08)	a,b,c,d	rising vs stable bio over 3 months
ICAM-1					
Maher (2017) - *Discovery*	106	36	HR 1.002 (95% CI 0.99-1.01)	x	rising vs stable bio over 3 months
IGFBP-2					
Maher (2017) - *Discovery*	106	36	HR 1.02 (95% CI 1.002-1.03)	x	rising vs stable bio over 3 months
IL-8					
Maher (2017) - *Discovery*	106	36	HR 1.02 (95% CI 0.98-1.07)	x	rising vs stable bio over 3 months
ECM neoepitopes					
Jenkins (2015) –*Validation* ***BGM***	134	21	HR 1.07 (95% CI 1.00-1.15)	a,c,d,e	rising vs stable bio over 3 months

Organ (2019) **BGM**	145	34	HR 1.41 (95% CI 0.8-2.47)	a,b,c	rising vs stable bio over 3 months
Jenkins (2015) –*Validation **C1M***	134	21	HR 1.01 (95% CI 1.00-1.02)	a,c,d,e	rising vs stable bio over 3 months
Organ (2019) **C1M**	145	34	HR 1.84 (95% CI 1.03-3.27)	a,b,c	rising vs stable bio over 3 months
Jenkins (2015) –*Validation **C3A***	134	21	HR 1.05 (95% CI 1.01-1.1)	a,c,d,e	rising vs stable bio over 3 months
Jenkins (2015) –*Validation **C3M***	134	21	HR 1.1 (95% CI 1.04-1.17)	a,c,d,e	rising vs stable bio over 3 months
Organ (2019) - **C3M**	145	34	HR 2.44 (95% CI 1.39-4.31)	a,b,c	rising vs stable bio over 3 months
Jenkins (2015) –*Validation **C5M***	134	21	HR 1.00 (95% CI 1.00-1.00)	a,c,d,e	rising vs stable bio over 3 months
Jenkins (2015) –*Validation **C6M***	134	21	HR 1.04 (95% CI 1.01-1.08)	a,c,d,e	rising vs stable bio over 3 months
Organ (2019) **C6M**	145	34	HR 2.19 (95% CI 1.25-3.82)	a,b,c	rising vs stable bio over 3 months
Jenkins (2015) –*Validation **CRPM***	134	21	HR 1.33 (95% CI 1.1-1.6)	a,c,d,e	rising vs stable bio over 3 months
Organ (2019) **CRPM**	145	34	HR 2.13 (95% CI 1.21-3.75)	a,b,c	rising vs stable bio over 3 months
Jenkins 2015) – *Validation **VICM***	55	26	HR 1.01 (95% CI 0.99-1.03)	a,c,d,e	rising vs stable bio over 3 months
Collagen synthesis peptides					
Organ (2019) **P1NP**	145	34	HR 0.76 (95% CI 0.44-1.3)	a,b,c	rising vs stable bio over 3 months
Organ (2019) **PRO-C3**	145	34	HR 1.62 (95% CI 0.95-2.79)	a,b,c	rising vs stable bio over 3 months
Organ (2019) **PRO-C6**	145	34	HR 1.14 (95% CI 0.67-1.93)	a,b,c	rising vs stable bio over 3 months
Organ (2019) **P1NP:C1M**	145	34	HR 0.73 (95% CI 0.41-1.29)	a,b,c	rising ratio levels
Organ (2019) **PRO-C3:C3M**	145	34	HR 0.83 (95% CI 0.49-1.43)	a,b,c	rising ratio levels
Organ (2019) **PRO-C6:C6M**	145	34	HR 0.55 (95% CI 0.32-0.95)	a,b,c	rising ratio levels

Table 10-2 - Studies reporting short term biomarkers change and their association with mortality. x=no adjustments, a=age, b=gender, c=smoking, d=FVC e=DLCO, f= 6MWT, g=race, h=medication

Author (year)	Sample size	Outcome timepoint (months)	Disease progression definition	Effect size (Variance)	Level of adjustment	Effect size reported for
SP-A						
Raghu (2018)	130	12	FVC decrease ≥10% predicted or DL_{CO} decrease > 15% or lung transplantation or death	AUROC 0.61 (90% CI 0.52-0.7)	NR	NR
SP-D						
Collard (2010)	67	NR	Acute exacerbation	361ng/mL vs 294ng/mL (p=0.01)	x	median bio in event and non-event
Maher (2017) *Discovery*	104	12	All-cause mortality or FVC decline ≥ 10%	GR 1.35 (95% CI 1.1-1.649)	x	bio level in progressive vs. stable
Maher (2017) *Validation*	204	12	All-cause mortality or FVC decline ≥ 10%	GR 1.35 (95% CI 1.12-1.62)	x	bio level in progressive vs. stable
Raghu (2018)	130	12	FVC decrease ≥10% predicted or DL_{CO} decrease > 15% or lung transplantation or death	AUROC 0.62 (90% CI 0.53-0.7)	NR	NR
CCL-18						
Prasse (2009)	67	24	FVC decline ≥ 10% predicted or death	OR 6.75 (95% CI 2.52-18.1)	x	bio < or > 150ng/mL
Ohshimo (2014)	77	36	Acute exacerbation	HR 2.92 (95% CI 0.76-11.4)	x	bio > or < 212ng/mL
Neighbors (2018) *Test*	123	12	FVC ≥10% absolute decline, 50m decline in 6MWT or death	HR 1.64 (95% CI 1.04-2.83)	x	'high' vs 'low' bio
Neighbors (2018) *Replication*	237	12	FVC ≥10% absolute decline, 50m decline in 6MWT or death	HR 1.32 (95% CI 0.76-2.13)	x	'high' vs 'low' bio
Raghu (2018)	130	12	FVC decrease ≥10% predicted or DL_{CO} decrease > 15% or lung transplantation or death	AUROC 0.62 (90% CI 0.54-0.71)	NR	bio > or < 150ng/mL
CXCL-13						
Neighbors (2018) *Test*	123	12	FVC ≥10% absolute decline, 50m decline in 6MWT or death	HR 1.23 (95% CI 0.89-1.69)	x	'high' vs 'low' bio
Neighbors (2018) *Replication*	237	12	FVC ≥10% absolute decline, 50m decline in 6MWT or death	Not significant (NR)	x	'high' vs 'low' bio

KL-6						
Collard (2010)	67	NR	Acute exacerbation	1791 U/mL vs 895 U/mL (p=0.003)	x	median bio in event and non-event
Ohshimo (2014)	77	36	Acute exacerbation	HR 11.8 (95% CI 1.43-97.8)	a,b,c,h	bio > or < 1300U/mL
Jiang (2018)	20	12	FVC decline ≥ 10% or DL_{CO} decline ≥ 15%, or death	OR 1.00 (95% CI 1.00-1.00)	x	continuous bio
Raghu (2018)	130	12	FVC decrease ≥10% predicted or DL_{CO} decrease > 15% or lung transplantation or death	AUROC 0.6 (90% CI 0.51-0.68)	NR	NR
IL-8						
Richards (2012) *Derivation*	140	12	FVC relative decline ≥ 10%	HR 2.00 (95% CI 1.22-3.28)	a,b,d	bio > or < 0.0092ng/mL
Richards (2012) *Validation*	101	12	FVC relative decline ≥ 10%	HR 1.2 (95% CI 0.5-2.85)	a,b,d	bio > or < 0.0092ng/mL
Maher (2017) *Discovery*	104	12	All-cause mortality or FVC decline ≥ 10%	GR 1.51 (95% CI 1.12-2.023)	x	bio level in progressive vs. stable
CA19-9						
Maher (2017) *Discovery*	104	12	All-cause mortality or FVC decline ≥ 10%	GR 3.12 (95% CI 1.7-5.7)	x	bio level in progressive vs. stable
Maher (2017) *Validation*	204	12	All-cause mortality or FVC decline ≥ 10%	GR 2.42 (95% CI 1.6-3.65)	x	bio level in progressive vs. stable
CA125						
Maher (2017) *Discovery*	104	12	All-cause mortality or FVC decline ≥ 10%	Not significant (NR)	x	bio level in progressive vs. stable
Maher (2017) *Validation*	204	12	All-cause mortality or FVC decline ≥ 10%	GR 1.26 (95% CI 1.05-1.51)	x	bio level in progressive vs. stable
LOXL2						
Chien (2014) *ARTEMIS*	69	24	Mortality, hospitalisation or lung function decline (FVC≥10% & DL_{CO}≥5%, or DL_{CO} ≥ 15% and FVC≥5%)	HR 5.41 (95% CI 1.65-17.73)	d,e,f,h	bio > or ≤ 800pg/mL

Chien (2014) *GAP*	70	24	Mortality, hospitalisation or lung function decline (FVC≥10% & DL_{CO}≥5%, or DL_{CO} ≥ 15% and FVC≥5%)	HR 1.78 (95% CI 1.01-3.11)	x	bio > or ≤ 700pg/mL
Periostin						
Naik (2012)	50	11	Death, acute exacerbation, transplantation, relative FVC decline ≥ 10% or DL_{CO} > 15%	HR 1.47 (95% CI 1.03-2.1)	a,b,c,d,e	per bio SD
Neighbors (2018) *Test*	123	12	FVC ≥10% absolute decline, 50m decline in 6MWT or death	HR 2.08 (95% CI 1.24-3.47)	x	'high' vs 'low' bio
Neighbors (2018) *Replication*	237	12	FVC ≥10% absolute decline, 50m decline in 6MWT or death	HR 1.75 (95% CI 0.87-2.84)	x	'high' vs 'low' bio
Raghu (2018)	130	12	FVC decrease ≥10% predicted or DL_{CO} decrease > 15% or lung transplantation or death	AUROC 0.6 (90% CI 0.51-0.69)	NR	NR
YKL-40						
Neighbors (2018) *Test*	123	12	FVC ≥10% absolute decline, 50m decline in 6MWT or death	HR 1.39 (95% CI 0.79-2.41)	x	'high' vs 'low' bio
Neighbors (2018) *Replication*	237	12	FVC ≥10% absolute decline, 50m decline in 6MWT or death	Not significant (NR)	x	'high' vs 'low' bio
Raghu (2018)	130	12	FVC decrease ≥10% predicted or DL_{CO} decrease > 15% or lung transplantation or death	AUROC 0.58 (90% CI 0.49-0.67)	NR	NR
ICAM-1						
Richards (2012) *Derivation*	140	12	FVC relative decline ≥ 10%	HR 1.6 (95% CI 1.00-2.56)	a,b,d	bio > or < 202.5ng/mL
Richards (2012) *Validation*	101	12	FVC relative decline ≥ 10%	HR 2.2 (95% CI 1.21-4.01)	a,b,d	bio > or < 262ng/mL
Maher (2017) *Discovery*	104	12	All-cause mortality or FVC decline ≥ 10%	GR 1.29 (95% CI 1.02-1.65)	x	bio level in progressive vs. stable
Raghu 2018	130	12	FVC decrease ≥10% predicted or DL_{CO} decrease > 15% or lung transplantation or death	AUROC 0.65 (90% CI 0.56-0.73)	NR	NR
ECM neoepitopes						
Jenkins (2015) *D+V cohort* **BGM**	186	12	All-cause mortality or FVC decline ≥ 10%	Not significant (NR)	x	bio level in progressive vs. stable
Jenkins (2015) *D+V cohort* **C1M**	186	12	All-cause mortality or FVC decline ≥ 10%	Not significant (NR)	x	bio level in progressive vs. stable

Jenkins (2015) *D+V cohort* **C3M**	186	12	All-cause mortality or FVC decline ≥ 10%	P=0.011 (NR)	x	bio level in progressive vs. stable
Jenkins (2015) *D+V cohort* **C5M**	186	12	All-cause mortality or FVC decline ≥ 10%	Not significant (NR)	x	bio level in progressive vs. stable
Jenkins (2015) *D+V cohort* **C6M**	186	12	All-cause mortality or FVC decline ≥ 10%	P=0.013 (NR	x	bio level in progressive vs. stable
Jenkins (2015) *D+V cohort* **CRPM**	186	12	All-cause mortality or FVC decline ≥ 10%	P=0.014 (NR)	x	bio level in progressive vs. stable
Jenkins (2015) *D+V cohort* **VICM**	186	12	All-cause mortality or FVC decline ≥ 10%	P=0.033 (NR)	x	bio level in progressive vs. stable
Jenkins (2015) *D+V cohort* **C3A**	186	12	All-cause mortality or FVC decline ≥ 10%	P=0.003 (NR)	x	bio level in progressive vs. stable
Jenkins (2015) *Discovery only* **P3NP**	186	12	All-cause mortality or FVC decline ≥ 10%	P=0.63 (NR)	x	bio level in progressive vs. stable
Jenkins (2015) *Discovery only* **ELM**	186	12	All-cause mortality or FVC decline ≥ 10%	P=0.55 (NR)	x	bio level in progressive vs. stable
Jenkins (2015) *Discovery only* **ELM2**	186	12	All-cause mortality or FVC decline ≥ 10%	P=0.42 (NR)	x	bio level in progressive vs. stable
Hoyer (2020) **PROC3**	184	6	All-cause mortality or FVC decline ≥ 10%	P=0.005 (NR)	NR	NR
Hoyer (2020) **PROC6**	184	6	All-cause mortality or FVC decline ≥ 10%	P=0.031 (NR)	NR	NR

Table 10-3 - Studies reporting disease progression outcomes including definition of disease progression outcome used and effect sizes reported.

x=no adjustments, a=age, b=gender, c=smoking, d=FVC e=DLCO, f= 6MWT, g=race, h=medication, NR=not reported

Author (year)	Sample size	FVC change measured at (months)	Effect size (Variance)	Level of adjustment	Effect size reported for
SP-A					
Doubkova (2016)	18	NR	155.8 ng/mL vs 87.15 ng/mL; p=0.01	x	baseline bio in PFT "improvement" vs "stabilisation"
SP-D					
Doubkova (2016)	18	NR	861.4ng/mL vs. 802.8ng/mL; p=0.76	x	baseline bio in PFT "improvement" vs "stabilisation"
Kennedy (2015)	13	6	r= -0.64 (95% CI -0.89 to -0.08)	x	baseline bio correlation with %pred FVC change
Ohta (2017)	60	6-12	r= 0.09 (p>0.05)	x	baseline bio correlation with %pred FVC change
CCL-18					
Neighbors (2018) – *Test*	123	12	-3.1% (p=0.03)	x	%pred FVC change in baseline bio ≥ or < median (411.5ng/mL)
Neighbors (2018) – *Replication*	237	12	-3.6% (p=0.004)	x	%pred FVC change in baseline bio ≥ or < median (458.6ng/mL)
Prasse (2009)	67	6	r=0.54 (p<0.0001)	x	baseline bio correlation with %pred FVC change
CXCL-13					
Guo (2020)	126	12	r= 0.56 (p<0.001)	x	baseline bio correlation with %pred FVC change
Neighbors (2018) – *Test*	123	12	-3.2% (p=0.06)	x	%pred FVC change in baseline bio ≥ or < median (87.9ng/mL)
Neighbors (2018) – *Replication*	237	12	-3.7% (p=0.05)	x	%pred FVC change in baseline bio ≥ or < median (88.7ng/mL)
KL-6					
Guo (2020)	126	12	r= 0.71 (p<0.001)	x	baseline bio correlation with %pred FVC change
Ohta (2017)	60	6-12	r= 0.09 (p>0.05)	x	baseline bio correlation with %pred FVC change
Okamoto (2011)	26	6	Not significant (NR)	x	baseline bio correlation with %pred FVC change

Periostin					
Neighbors (2018) – *Test*	123	12	-3.6% (p<0.001)	x	%pred FVC change in baseline bio ≥ or < median (67.8ng/mL)
Neighbors (2018) – *Replication*	237	12	-2.5% (p=0.19)	x	%pred FVC change in baseline bio ≥ or < median (65.4ng/mL)
Ohta (2017)	60	6-12	r= -0.43 (p<0.01)	x	baseline bio correlation with %pred FVC change
Okamoto (2011)	26	6	r= -0.50 (p<0.01)	x	baseline bio correlation with %pred FVC change
YKL-40					
Neighbors (2018) – *Test*	123	12	-2.4% (p=0.04)	x	%pred FVC change in baseline bio ≥ or < median (100.3ng/mL)
Neighbors (2018) – *Replication*	237	12	-1.5% (p=0.70)	x	%pred FVC change in baseline bio ≥ or < median (109.5ng/mL)

Table 10-4 - Studies reporting association with baseline biomarkers and change in forced vital capacity (FVC).

Author (year)	Sample size	Timepoint of outcome (months)	Disease progression definition	Effect size (Variance)	Level of adjustment	Effect size reported for
SP-D						
Maher et al (2017) *Discovery*	106	12	All-cause mortality or FVC decline ≥ 10%	p=0.029	x	rising vs stable bio over 3 months
Maher et al (2017) *Validation*	206	12	All-cause mortality or FVC decline ≥ 10%	Not significant (NR)	x	rising vs stable bio over 3 months
CXCL-13						
Vuga et al (2014)	95	>24	Respiratory failure	HR 7.2 (95% CI 1.3-40.0)	x	bio "increase greatest vs. less increased" (time not specified)
CA19-9						
Maher et al (2017) *Discovery*	106	12	All-cause mortality or FVC decline ≥ 10%	p<0.001	x	rising vs stable bio over 3 months
Maher et al (2017) *Validation*	206	12	All-cause mortality or FVC decline ≥ 10%	Not significant (NR)	x	rising vs stable bio over 3 months
CA125						
Maher et al (2017) *Discovery*	106	12	All-cause mortality or FVC decline ≥ 10%	p=0.041	x	rising vs stable bio over 3 months
Maher et al (2017) *Validation*	206	12	All-cause mortality or FVC decline ≥ 10%	p=0.0028	x	rising vs stable bio over 3 months
KL-6						
Jiang et al (2018)	20	12	FVC decline ≥ 10%, DL_{CO} decline ≥ 15% or death	OR 3.61 (95% CI 1.05-6.22)	a,b,c,d,e	Change in KL-6 (not otherwise specified)

Table 10-5 - Studies reporting short term biomarkers change and their association with disease progression.

x=no adjustments, a=age, b=gender, c=smoking, d=FVC e=DLCO, f= 6MWT, g=race, h=medication, NR=not reported

10.4 Summary of study results in COVID-19 SR

Author	Outcomes
ANAKINRA	
Balkhair	IMV occurred in 31% in the anakinra group and 75% in the control (p < 0.001). Death occurred in 29% vs. 46% in the control (p = 0.082).
Huet	IMV or death in anakinra group vs control HR 0.22; 95% CI 0.1-0.49. For death alone: HR 0·30; 95% CI 0·12–0·71. Decrease in CRP vs control group.
Kooistra	No difference between anakinra and control group in time on IMV (23 vs 17 days; p=0.79), length of ICU stay (24 days vs 17; p=0.59), 28 day mortality (19% vs 18%; p=087)
*Kyriazopoulou	severe respiratory failure lower in anakinra treated group (22.3% vs 59.2%), and lower 30-day mortality (aHR 0.49, 95%CI 0.25-0.97).
Cauchois	Fewer no. days with oxygen < 3L/min in anakinra group vs control at day 20 (p<0.05). No. of days without IMV similar. Rapid reduction of CRP with anakinra vs. controls (p<0.001)
Cavalli	Control: Survival at 21 days of 56%. Mechanical ventilation-free survival 50%. Tocilizumab high dose: Survival of 90% at 21 days (p=0.009 vs control group). IMV-free survival 72% (p=0.15)
# Narain	No effect on mortality (aHR 0.79; 95% CI 0.44-1.42)
SARILUMAB	
Benucci	87% discharged within 14 days.
Della-Torre	Survival similar in both groups (HR 0.36; 95% CI 0.08-1.68). In treatment group, median time to death higher (19 vs. 4 days; p=0.006), median time to CRP normalisation lower (6 vs. 12 days; p<0.0001). Median time to clinical improvement, discharge and IMV free survival similar. Median time to clinical improvement shorter in individuals with a baseline PaO2/FiO2 >100mgHg (7 vs 28 days; HR 0.18; 95% CI 0.02-0.26)
* Gordon	Mean aOR for survival was 2.01 (95%CI 1.18-2.71). Compared with control, median aOR for organ support-free days was 1.76 (95%CI 1.17-2.91). Sarilumab associated with improved time to ICU discharge (aHR 1.64; 95%CI 1.21-2.45), improved time to hospital discharge (aHR 1.6; 95%CI 1.17-2.40), improved ordinal scale outcomes at day 14 (aOR 1.86; 95%CI 1.22-2.91).
Gremese	83% (89.7% in medical wards and 64.3% in ICU) improved on therapy. Overall mortality of 5.7%
Sinha	10.9% of patients died. Mortality was lower in patients with FiO2 < 0.45 (HR 0.24; 95% CI 0.08-0.74)

SILTUXIMAB	
* Gritti	30-day mortality lower in treatment arm (HR 0.46; 95% CI 0.22-0.97). 53% recovered and were discharged.
TOCILIZUMAB	
Albertini	respiratory rate at d14 lower in treated (21.5 vs 25.5 breaths/min: 95% CI -7.5 to -0.4). No difference in requirement for intubation. Significant fall in CRP in treated patients on d7 (p=0.04)
Antony	8.8% of patients died and 11.3% required mechanical ventilation. CRP levels reduced post therapy, whereas IL-6 increased
Campins	32.4% of patients were admitted to intensive care, 13.8% died. No difference in median CRP and IL-6 between survivors and dead
* Carvalho	Tocilizumab not associated with mortality (HR 3.97; 95% CI 0.28-5.72), or positive cultures (OR 1.73; 95% CI 0.22-13.82)
Dastan	14% required IMV, remaining patients showed clinical improvement. By d28, 16.7% of patients died
* Gordon	aOR for survival was 1.64 (95%CI 1.14-2.35). Compared with control, aOR for organ support-free days was 1.64 (95%CI 1.25-2.14). Tocilizumab associated with improved time to ICU discharge (aHR 1.42; 95%CI 1.18-1.70), improved time to hospital discharge (aHR 1.41; 95%CI 1.18-1.70), improved ordinal scale outcomes at day 14 (aOR 1.83; 95%CI 1.40-2.41).
Hermine	At day 14, fewer patients died or needed ventilation compared with controls (aHR 0.58; 90% CI 0.30-1.09). At day 28, mortality was similar in both groups (aHR 0.92; 95%CI 0.33-2.53)
Malekzadeh	By day 14, 4.7% (4/86) of severe patients and 50% (20/40) of critical patients died. By the end, 7% (6/86) of severe patients and 60% (24/40) of critical patients died.
Mikulska	14-day mortality was 13.8% vs. 21.8% in control group. Mortality at study end lower in treatment group (HR 0.48; 95% CI 0.23-0.99)
Morena	Over a median follow up of 34 days, 67% of patients showed an improvement in clinical severity. Overall mortality rate was 27%
Perrone	Pre-specified expected lethality rates defined as 20% and 35% at 14 and 30 days respectively. Lethality rates were 18.4% (95% CI 13.6-24.0, p=0.52) and 22.4% (95% CI 17.2-28.3, p<0.001) at 14 and 30 days. In tocilizumab group alone, lethality rates were 15.6% and 20%.
Perrone	In the validation cohort, lethality rates were lower than the null hypothesis both at 14 and 30 days (10.9% and 20.0%)
* Rosas, I.	No improvement at day 28 (p=0.36), or mortality. Ordinal scale values similar (OR 1.19; 95% CI 0.81-1.76). Median time to hospital discharge shorter with tocilizumab than placebo (20 and 28 days; HR 1.35 95% CI 1.02-1.79). Median duration of ICU stay shorter with tocilizumab (9.8 and 15.5 respectively, p=0.045). Median time to improvement from baseline in 2 or more categories on ordinal scale was 14 days (12-17) in tocilizumab arm and 18 (15-28) days in placebo (p=0.08). Incidence of IMV was 27.9% in tocilizumab arm and 36.7% in placebo (p=0.14)

Roumier	Tocilizumab reduced requirement for IMV (aHR 0.58; 95% CI 0.36-0.94). No difference in mortality (aHR 0.68; 95% CI 0.31-1.75)
Salama	IMV or death at day 28 was lower in tocilizumab group (aHR 0.56; 95% CI 0.33 - 0.97). Mortality similar in both groups (10.4% vs 8.6%).
Salvarani	28% in the tocilizumab and 27% in SOC groups showed clinical worsening within 14 days (RR, 1.05; 95% CI, 0.59-1.86). Mortality at 14 days and at 30 days was comparable in the 2 groups
* Sanchez-Montalva	Mortality at 7 days was 26.8%. ARDS developed in 54.9%
Sciascia	Tocilizumab associated with increased survival (HR 2.2; 95% CI 1.3-6.7). Overall mortality was 11%
Stone	HR for intubation or death compared with placebo was 0.83;95% CI, 0.38 to 1.81. At 14 days, 18.0% in tocilizumab and 14.9% in of placebo had disease progression. At 14 days, 24.6% of tocilizumab group and 21.2% of placebo were receiving supplemental oxygen.
Strohbehn	At 24 hours, 75% of tocilizumab vs 34.1% of control were afebrile (p=0.001). 86.2% of tocilizumab vs. 14.3% control achieved CRP decrease of at least 25% (p<0.001). Median time to recovery was 3 days (IQR 2-5)
Toniati	At 10 days 77% of patients improved or stabilised and 23% worsened. Mortality was 20%
Biran	Exposure to tocilizumab was associated with lower hospital mortality (HR 0.64; 95% CI 0.47-0.87). In subgroup analyses, tocilizumab associated with decreased hospital mortality in those with a CRP≥150mg/L (HR 0.48;95% CI 0.3-0.77), but not in those with CRP>150mg/L (HR 0.92;95% CI 0.57-1.48).
Canziani	30-day mortality unaffected (aHR 0.82; 95% CI 0.42-1.58). Between days 6 and 30, HR 0.41 (95% CI 0.17-0.96) for tocilizumab vs controls. Tocilizumab associated with lower risk of IMV (HR 0.36; 95% CI 0.16-0.83). No effect on thrombotic events, bleeding, infection
Capra	Tocilizumab associated with reduced risk of mortality (HR 0.035; 95% CI 0.004-0.347)
Chillmuri	Tocilizumab associated with lower composite endpoint of IMV or death (aHR 0.29; 95% CI 0.16-0.54)
De Rossi	Tocilizumab associated with reduced mortality (aHR 0.057; 95% CI 0.017-0.187). Survival rate or mean time to discharge did not differ between two administration (IV and SC) routes.
Eimer	No difference in all-cause mortality at 30 days (HR 0.52; 95% CI 0.19-1.39. In tocilizumab group, significantly more ventilator free days. Freedom from IMV was achieved earlier and in a higher proportion of patients (HR 2.83; 95% CI 1.48-5.4). Length of hospital stay shorter in tocilizumab group
Fisher	No difference in mortality associated with tocilizumab (OR 1.04, 95% C.I. 0.27 – 3.75)
Galvan Roman	patients with high IL-6 not treated with TCZ showed high 139 mortality (HR: 4.6; p=0.003), as well as those with low IL-6 treated with tocilizumab (HR: 3.6; p=0.016).

* Garcia	Tocilizumab associated with fewer ICU admissions (10.3% vs. 27.6%; p=0.005) and need for IMV (0 vs 13.8%, OR 0.03, 95% CI 0.007-0.1)
Gokhale	Tocilizumab associated with reduced mortality (HR 0.616;95% CI 0.38-0.99)
Guaraldi	Tocilizumab use associated with reduced risk of death (7% vs. 20%; aHR 0.38; 95% CI 0.17-0.83) and composite outcome of IMV or death (aHR 0.61;95% CI 0.4-0.92).
Guisado-Vasco	Increased mortality with tocilizumab (aOR 2·4, 95% CI, 1·13 - 5·11)
Gupta	Patients treated with tocilizumab had a lower risk of death compared with those not treated with tocilizumab (HR, 0.71; 95% CI, 0.56-0.92)
Hill	Tocilizumab not associated with lower risk of mortality (aHR 0.57; 95% CI 0.21-1.52) or a difference in clinical improvement (aHR 0.92; 95% CI 0.38-2.22)
Holt	In multivariate analysis, tocilizumab administration had no effect on mortality (OR 0.32; 95% CI 0.02-3.69)
Ip	Tocilizumab associated with reduced mortality within the ICU setting (aHR 0.76; 95% CI 0.57-1.00)
Kewan	Median time to clinical improvement in tocilizumab vs. no tocilizumab was 6.5 days (IQR 4-9) vs. 7 days (IQR 5-10) among all patients (HR 1.14; 95% CI 0.55-2.38). Shorter median length of hospital stay with tocilizumab. The median duration of vasopressor support and IMV were 2 days (IQR: 1·75 – 4·25 days) vs. 5 days (IQR: 4 – 8 days), p = 0.039, and 7 days (IQR: 4 – 14 days) vs. 10 days (IQR: 5 – 15 days) in tocilizumab vs. no tocilizumab cohorts, p = 0.11
Kimmig	Tocilizumab was associated with higher risk of mortality (35.2% vs 19.3%, p=0.02)
Klopfenstein	Death and/or ICU admissions higher in tocilizumab cohort vs control (72% vs 25%; p=0.002). No difference in death alone (25% vs 48%; p=0.0066)
Lewis	Tocilizumab associated with improved survival (aHR 0.24; 95% CI 0.18-0.33). Similar time to hospital discharge (aHR 0.86; 95% CI 0.78-1.17)
Martinez-Sanz	In patients with CRP>150mg/L, tocilizumab associated with decreased risk of death (aHR 0.34; 95% CI 0.16-0.72) and ICU admission or death (aHR 0.38; 95% CI 0.19-0.81), but not in those with CRP <150mg/L. For all patients, tocilizumab not associated with risk of death (HR 0.77; 95% CI 0.48-1.22)
# Narain	No effect on mortality (aHR 0.79; 95% CI 0.47-1.32)
Nasa	mortality at day 7 and 28 was significantly lower in the tocilizumab group (p = 0.007 and p = 0.001 respectively).
Patel	CRP improved in all tocilizumab patients. No difference in mortality with tocilizumab but more patients discharged compared with controls (55% vs 24%)
* Petrak	No difference between tocilizumab and mortality (aOR 0.83; 95%CI 0.34-1.98). However early therapy was associated with reduced mortality (aOR 0.15; 95%CI 0.04-0.5)
Pettit	Mortality rate higher in tocilizumab cohort (39% vs 23%; p=0.03).
Potere	Tocilizumab associated with reduction in CRP over three days. None of the tocilizumab patients had disease progression (requirement of oxygen or mechanical ventilation) whereas progression occurred in 50% of control group

*Ramaswamy	Mortality lower in tocilizumab group (HR 0.25; 95% CI 0.07-0.9)
Rodriguez-Bano	Tocilizumab associated with reduced risk of death (aHR 0.12; 95% CI 0.02-0.56) and reduced risk of composite outcome of intubation or death (aHR 0.32; 95% CI 0.15-0.67)
Rojas-Marte	Similar mortality in both groups (52% vs 61%; p=0.09)
Roomi	No difference in hospital mortality (aOR 0.28; 95% CI 0.05-1.4), IMV (aOR 1.2;95% CI 0.49-2.9) and hospital discharge (aOR 0.78;95% CI 0.28-2.1). Reduction in CRP levels on day 7 compared with control (21% vs 56%; OR 0.21; 95% CI 0.08-0.55
Rosas, J.	Mortality was 20% in tocilizumab group and 35% in control group. Admission to ICU was 65% in tocilizumab and 0% in control
Rossi	Tocilizumab associated with reduced mortality (aHR 0.42; 95% CI 0.22-0.82), and reduced composite of mortality or IMV (aHR 0.34; 95% CI 0.22-0.52)
Rossotti	Tocilizumab associated with reduced mortality (unadjusted HR 0.49; 95% CI 0.26-0.95), but longer hospital stay (HR 1.66; 95% CI 1.09-2.52)
Ruiz-Antoran	Mortality lower in patients treated with tocilizumab than controls (16.8% vs. 31.5%, aHR 0.74; 95%CI 0.62-0.89)
Somers	Tocilizumab associated with lower risk of death (aHR 0.55; 95% CI 0.33-0.9)
Tian	Mortality lower in tocilizumab group (aHR 0.47; 95%CI 0.25-0.9)
Tsai	No difference in mortality between two groups (OR 1.0;95% CI 0.465-2.151)
* Wadud	Lower mortality in tocilizumab group (38.6% vs. 52%; p<0.001)
Zheng	Increased mortality in tocilizumab group, but significant reduction in CRP level at 1 week

Table 10-6 – Outcomes of included studies.

*aHR, adjusted hazard ratio; CI, confidence interval; CRP, C-reactive protein; ICU, intensive care unit; IL6, interleukin-6; IMV, invasive mechanical ventilation; IV, intravenous; N/R, not reported; OR odds ratio; SC, subcutaneous; -, not available; * non-peer-reviewed preprint study #, study investigating both anakinra and tocilizumab*

10.5 Ethical approval

Health Research Authority

East Midlands - Nottingham 1 Research Ethics Committee

The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

04 October 2018

Dr Fasihul Khan
Clinical Research Fellow
Nottingham University Hospitals
Clinical Sciences Building
Nottingham City Hospital
Hucknall Road
NG5 1PB

Dear Dr Khan

Study title:	**It's Not JUST Idiopathic Pulmonary FibrosiS Study**
REC reference:	**18/EM/0139**
Protocol number:	**18014**
Amendment number:	**1.0**
Amendment date:	**16 August 2018**
IRAS project ID:	**237010**

The above amendment was reviewed 25 September 2018 by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Discussion

The sub-committee asked the researcher to clarify the following in the protocol: "Clinical decisions will not be made on spirometry results, and patients will be asked to seek medical advice in case of clinical deterioration, as they would normally". The researcher were asked if this mean that if their reading/s are of concern, it is the patient's responsibility to seek help for this and that the information will not be passed to the appropriate healthcare professional? And if so, would a participant know what a potentially concerning reading would be and who/how to report this?
The researcher responded saying that clinical decisions will not be made on daily spirometry results for a number of reasons which include the patients are being blinded to the spirometry results. The blinded results will be forwarded to us from PatientMPower in tranches. The patient will therefore not know what their spirometry results at home are, and the researchers will not know the patients spirometry results in real time. For this and other reasons the results will therefore not be used to inform any clinical decisions.
If a patient feels more breathless or has a change in their cough or other symptoms relating to their lung disease in between their usual 3 monthly hospital clinic and spirometry visits they should contact their usual healthcare provider in line with current UK practice.
Management decisions will only be made on standard clinical information in accordance with UK practice and not on blinded research measurements.
The sub-committee were satisfied with the response.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP) [AmendmentForm_ReadyForSubmission.pdf]	1.0	16 August 2018
Other [Appendix 1 - Spiro Device Datasheet.pdf]	1.0	16 August 2018
Participant consent form [Injustis ICF Final v1.2.doc]	1.2	20 August 2018
Participant information sheet (PIS) [INJUSTIS Spiro PIS Final v1.0.doc]	1.0	16 August 2018
Participant information sheet (PIS) [INJUSTIS PIS Final v1.2.doc]	1.2	20 August 2018
Referee's report or other scientific critique report [INJUSTIS Protocol Final v1.2.docx]	1.2	16 August 2018
Research protocol or project proposal [INJUSTIS Protocol Final v1.2 tracked.docx]	1.2	16 August 2018

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

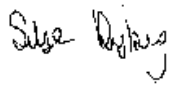
NHS
Health Research Authority

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our Research Ethics Committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

18/EM/0139:	**Please quote this number on all correspondence**

Yours sincerely



Professor Cris Constantinescu
Chair

E-mail: NRESCommittee.EastMidlands-Nottingham1@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Miss Kelly Alvey, Nottingham University Hospitals*
Dr Fasihul Khan, Nottingham University Hospitals

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Professor Gisli Jenkins
Clinical Sciences Building
City Hospital, Hucknall Road
Nottingham
NG5 1PB

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

02 July 2018

Dear Professor Jenkins

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**It's Not JUST Idiopathic Pulmonary FibrosiS Study**
IRAS project ID:	**237010**
Protocol number:	**18014**
REC reference:	**18/EM/0139**
Sponsor	**University of Nottingham**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

Following the arranging of capacity and capability, participating NHS organisations should **formally confirm** their capacity and capability to undertake the study. How this will be confirmed is detailed in the "*summary of assessment*" section towards the end of this letter.

You should provide, if you have not already done so, detailed instructions to each organisation as to how you will notify them that research activities may commence at site following their confirmation of capacity and capability (e.g. provision by you of a 'green light' email, formal notification following a site initiation visit, activities may commence immediately following confirmation by participating organisation, etc.).

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?
The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?
You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: Ms Angela Shone
Email: sponsor@nottingham.ac.uk

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **237010**. Please quote this on all correspondence.

Yours sincerely

Thomas Fairman
HRA Assessor

IRAS project ID	237010

Email: hra.approval@nhs.net

Copy to: *Ms Angela Shone, University of Nottingham, (Sponsor Contact)*
Miss Kelly Alvey, Nottingham University Hospitals, (Lead NHS R&D Contact)

IRAS project ID	237010

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
Contract/Study Agreement template [Injustis Contract]		
Copies of advertisement materials for research participants [Social Media Template]		
Covering letter on headed paper [Cover Letter Updates]		26 April 2018
Covering letter on headed paper [Covering Letter to REC]		
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [UoN Insurance]		25 July 2017
GP/consultant information sheets or letters [GP Letter]	1.1	11 June 2018
HRA Schedule of Events [NUH]	1.0	01 May 2018
HRA Schedule of Events [Other Sites]	1.0	01 May 2018
HRA Statement of Activities [NUH]	1.0	01 May 2018
HRA Statement of Activities [Other Sites]	1.0	01 May 2018
IRAS Application Form [IRAS_Form_22062018]		22 June 2018
IRAS Application Form [IRAS_Form_30042018]		30 April 2018
Letter from sponsor [Injustis Sponsor Letter]		13 April 2018
Letters of invitation to participant [Injustis Patient Letter]	1.0	13 April 2018
Other [NUH Bronch Info]		
Participant consent form [ICF]	1.1	11 June 2018
Participant information sheet (PIS) [PIS]	1.1	11 June 2018
Research protocol or project proposal [Protocol]	1.1	11 June 2018
Summary CV for Chief Investigator (CI) [GJ CV]		
Summary CV for student [FK CV]		
Validated questionnaire [EQ-5D-5L Questionnaire]		
Validated questionnaire [Cough Questionnaire]		
Validated questionnaire [SPARC Questionnaire]		
Validated questionnaire [SGR Questionnaire]		
Validated questionnaire [KBILD Questionnaire]		
Validated questionnaire [MRC Questionnaire]		

IRAS project ID	237010

Summary of assessment

The following information provides assurance to you, the sponsor and the NHS in England and Wales that the study, as assessed for HRA and HCRW Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England and Wales to assist in assessing, arranging and confirming capacity and capability.

Assessment criteria

Section	Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	There are two site types participating in the study, Nottingham University Hospitals Trust (NUH) and 'Other' Sites. The sponsor has submitted a Statement of Activities for each site type but does not intend to use these as the contract between themselves and study sites. The sponsor proposes to instead their own template agreement.
4.2	Insurance/indemnity arrangements assessed	Yes	Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical defence organisation covers the activities expected of them for this research study
4.3	Financial arrangements assessed	Yes	External study funding has been secured from the NIHR. No study funding will be provided to sites, as detailed at Schedule 1 of the relevant Statement of Activities documents.

Section	Assessment Criteria	Compliant with Standards	Comments
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	No comments
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
There are two site types participating in the study, Nottingham University Hospitals Trust (NUH) and 'Other' Sites. All sites will undertake the same study activities except that participants may consent to a research specific bronchoscopy which will only be conducted at NUH. The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England and Wales in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. Where applicable, the local LCRN contact should also be copied into this correspondence. If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England and Wales which are not provided in IRAS or on the HRA or HCRW websites, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net, or HCRW at Research-permissions@wales.nhs.uk. We will

work with these organisations to achieve a consistent approach to information provision.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and the minimum expectations for education, training and experience that PIs should meet (where applicable).
A Principal Investigator should be appointed at all participating study sites. GCP training is not a generic training expectation, in line with the HRA/HCRW/MHRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken
As a non-commercial study undertaken by local staff, it is unlikely that letters of access or honorary research contracts will be applicable, except where local network staff employed by another Trust (or University) are involved (and then it is likely that arrangements are already in place). Where arrangements are not already in place, network staff (or similar) undertaking any of the research activities listed in A18 or A19 of the IRAS form (except for administration of questionnaires or surveys), would be expected to obtain an honorary research contract from one NHS organisation (if university employed), followed by Letters of Access for subsequent organisations. This would be on the basis of a Research Passport (if university employed) or an NHS to NHS confirmation of pre-engagement checks letter (if NHS employed). These should confirm enhanced DBS checks, including appropriate barred list checks, and occupational health clearance. For research team members only administering questionnaires or surveys, a Letter of Access based on standard DBS checks and occupational health clearance would be appropriate.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England to aid study set-up.
The applicant has indicated that they do intend to apply for inclusion on the NIHR CRN Portfolio.

University of Nottingham
UK | CHINA | MALAYSIA

Nottingham Respiratory Research Unit
• Asthma • COPD • ILD • Lung Infection •

Nottingham University Hospitals NHS
NHS Trust

Participant Information Sheet
(Version 1.4 27th July 2020)

IRAS Project ID: 237010

Title of Study: It's Not JUST Idiopathic Pulmonary FibrosiS Study

Name of Chief Investigator: Professor Gisli Jenkins

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?

Fibrotic lung conditions result in scarring of the lung tissue. This causes shortness of breath and cough and has an enormous impact on people's quality of life. We currently know that progressive lung scarring may be caused by a few conditions like Rheumatoid Arthritis and previous exposure to asbestos, birds (pigeons, parrots and budgies particularly) and moulds. In cases where no cause is found, we sometimes refer to this type of lung scarring as Idiopathic Pulmonary Fibrosis (IPF). However, regardless of whether a cause is found, or not, we do not have a cure.

The aim of these studies is to understand whether patients with progressive pulmonary fibrosis, regardless of the cause, have shared reasons for their disease progression. We will do this by assessing whether the genetic, cellular and chemical signals in the blood of people with IPF and other lung scarring conditions are similar or different. We also need to understand how these conditions change over time. Researchers wish to study the genetic signatures in samples and hope it will lead to a better understanding of why people develop lung fibrosis regardless of its cause and how the disease and its symptoms progress.

Why have I been invited?

You are being invited to take part because your doctor has given you a diagnosis of fibrotic lung disease. Specifically, we are asking patients who have been told they have RA-UIP, Asbestosis, Chronic Hypersensitivity Pneumonitis and Unclassifiable Fibrotic Lung Disease or Idiopathic Pulmonary Fibrosis to help us with the study. We are inviting 250 participants like you to take part.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you

decide to take part, you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

What will happen to me if I take part?

If, after reading this information sheet and talking to a member of the research team, you would like to take part in this study, we will ask you to sign a consent form.

This study will last for 2 years and during this time you will need to have various tests/procedures and will need to attend the hospital that recruited you to the study for study visits at specific times (an initial visit and then again 3 months, 12 months and 24 months later, four visits in total).

The following test results taken as part of your routine NHS care will be used by researchers for the purposes of this study:

• CT Chest Scan findings
• Lung Function test results
• Bronchoscopy if clinically indicated: We will ask for consent to obtain additional research samples.
• Blood Test results

The following tests/procedures will be performed as part of the research study:

• Questionnaires: You will be asked to fill out 5 short questionnaires (Leicester Cough Questionnaire, The Kings Brief Interstitial Lung Disease Questionnaire, The Medical Research Council Dyspnoea Scale, EQ-5D-5L and the IPARC score) These forms ask questions about your lung fibrosis and how it effects your daily life.
• Blood Test: We would like to take a 40ml sample (the equivalent to 3 tablespoons) of blood at each of the four visits (initial visit, and then 3, 12 and 24 months). Samples for analysis of serum, plasma, blood cells, RNA and genetic analysis (optional) will be taken.
• Walking Test: we would like to assess how far you can walk in 6 minutes. We will ask you to walk from one end of the corridor to the other at your own pace, trying to cover as much ground as possible. You can stop and rest at any time. After six minutes, you will be asked to stop and the distance you have walked will be measured and any symptoms you had during the test are written down.
• Bronchoscopy (Optional): If bronchoscopy is not clinically indicated, we will request some patients to undergo this test as part of research. It will be done in accordance with endoscopy guidelines and additional information (leaflet) will be given. You can withdraw your consent at any time and can choose to not undergo this test for research purposes. You can however, still take part in the main study.
• Hand Held Spirometry : You will be provided with a small hand held spirometer to blow into (to measure breathing tests). This device connects with a smartphone application 'app' (called "patientMpower app") which will download the breathing test information onto your smartphone.We will give you full training on how to use this.

We want you to use the home spirometer for the first three months of the study every day and then at the same time as your follow ups at 3 months, 12 months and 24 months (the week before and the week after these appointments)

You will be asked to do one home spirometry breathing test (sitting down) ideally at about the same time of day.

During the first three months you will not be able to see the results of the breathing tests done at home, but after the initial three-month period and your second study visit your results will be unblended and you will be able to see them. The device is yours to keep.

The following table gives an overview of the various tests done as part of routine care and as part of the research study for those who have been asked to take part.

Tests Done As Part of Routine Care:	Tests Done as Part of Research:
Lung Function Tests at initial visit, 3, 12 and 24 months	Questionnaires at all visits
Blood Test to look for any underlying causes of lung fibrosis	Blood test (40ml at all visits) Walking Test at all visits
Bronchoscopy if clinically indicated (only for patients whose chest scans have shown enlarged lymph nodes)	Daily Spirometry at home (with hand-held spirometer) for 3 months and then for two weeks (one before and one after) follow up appointments Bronchoscopy as part of research (optional)

Expenses and payments

Travel expenses will be offered for any additional visits incurred as a result of participation. Participants will not be paid to participate in the study.

What are the possible disadvantages and risks of taking part?

As with all tests/procedures some people experience side effects, some of which are detailed below:

• Blood Tests: Occasionally some people feel faint during a blood test. If this occurs please tell the person doing the test, as you should immediately lie down to prevent fainting. Sometimes after donating blood, a bruise develops where the needle was inserted.
• Walking Test: Changes in blood pressure and heart rate, dizziness, chest pain and very rarely fainting can happen. You may feel increasingly short of breath. This is because we are testing your body's ability to withstand exercise. There will be a chair available at both ends of the corridor in case you must rest and you can stop at any time.
• Bronchoscopy: This test has its' risk outlined in the hospital Bronchoscopy booklet, which will be given in accordance with endoscopy guidelines. You will be given minimum of 24hours to ask any queries prior to obtaining consent.
• Home Spirometry: The spirometer is not known to have any adverse effects. As with normal spirometry tests, breathing out forcefully can sometimes make you feel faint, and we would recommend you undertake the breathing test sitting down. Similarly breathing tests can make you cough.

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from this study may help us to gain a greater understanding of why people do or do not develop Lung Fibrosis.

What happens when the research study stops?

We do not expect to end the study early. However, if research does unexpectedly end then we would always let you know that the study has stopped. When the study does come to an end, after you have participated for the 2-year period, your care will go back to the same as before with your normal follow up visits to your doctor.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers' contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting

NUH NHS Trust, c/o PALS
Freepost NEA 14614
Nottingham NG7 1BR
Tel: 0800 183 02 04
Email: pals@nuh.nhs.uk

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against the University of Nottingham but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in the study be kept confidential?

We will follow ethical and legal practice and all information about you will be handled in confidence.

If you join the study, we will use information collected from you and your medical records during the course of the research. This information will be kept strictly confidential, stored in a secure and locked office, and on a password protected database at the University of Nottingham. Under UK Data Protection laws the University is the Data Controller (legally responsible for the data security) and the Chief Investigator of this study (named above) is the Data Custodian (manages access to the data). This means we are responsible for looking after your information and using it properly. Your rights to access, change or move your information are limited as we need to manage your information in specific ways to comply with certain laws and for the research to be reliable and accurate. To safeguard your rights, we will use the minimum personally – identifiable information possible.

You can find out more about how we use your information and to read our privacy notice at:

https://www.nottingham.ac.uk/utilities/privacy.aspx.

The data collected for the study will be looked at and stored by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people from regulatory organisations to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

Where possible, information about you which leaves the hospital will have your name and address removed and a unique code will be used so that you cannot be recognised from it, however sometimes we need to ensure that we can recognise you to link the research data with your medical records so in these instances we will need to know your name and date of birth. We will also need this information if we need to follow up your medical records as part of the research, where we may need to ask the Government services that hold medical information about you (such as NHS Digital, the Office for National Statistics, among others) to provide this information to us. By signing the consent form you agree to the above.

Your contact information will be kept by the University of Nottingham for 10 years after the end of the study so that we are able to contact you about the findings of the study and possible follow-up studies (unless you advise us that you do not wish to be contacted). This information will be kept separately from the research data collected and only those who need to will have access to it. All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team given permission by the data custodian will have access to your personal data.

In accordance with the University of Nottingham's, the Government's and our funders' policies we may share our research data with researchers in other Universities and organisations, including those in other countries, for research in health and social care. Sharing research data is important to allow peer scrutiny, re-use (and therefore avoiding duplication of research) and to understand the bigger picture in particular areas of research. Data sharing in this way is usually anonymised (so that you could not be identified) but if we need to share identifiable information we will seek your consent for this and ensure it is secure. You will be made aware then if the data is to be shared with countries whose data protection laws differ to those of the UK and how we will protect your confidentiality.

Because of taking part in the home spirometry part of the study, your email address will be known to patientMpower Ltd. (They need this information to manage your password access, provide individualised technical support to you and be able to delete your data in case you request that at some time in the future). The patientMpower app can automatically record your geographical location if you give permission in the app. If you do this, the patientMpower app can give you information on the air quality at your location. In this case, patientMpower can know the location of your phone. However, you do not have to give this permission in the app if you don't want to.

All information (including spirometry data) recorded on the patientMpower app will be kept confidential. This information is stored in a secure cloud system which is maintained by patientMpower Ltd., Digital Depot, Dublin 8, Ireland (who developed the app). This cloud system is designed with strict security measures which meet current industry standards for health-related information. Information recorded on the patientMpower app will be viewed and analysed by named and authorised users at patientMpower Ltd and the University of Nottingham research team. The spirometry data on the patientMpower app will be transferred to the University of Nottingham. patientMpower Ltd will be acting as the Data Processor (they will process your personal information on behalf of the University) and the University will be the Data Controller (the University decides how the data is processed and takes responsibility for it). Both have safeguards in place to protect your data.

If you wish to have information deleted from the patientMpower app this can be done on request to patientMpower Ltd. You can contact them at www.info.patientmpower.com or by phone at 020 3322 4121 or using the support button within the app itself.

What will happen if I don't want to carry on with the study?

Your participation is voluntary, and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw, we will no longer collect any information about you or from you but we will keep the information about you that we have already obtained as we are not allowed to tamper with study records and this information may have already been used in some analyses and may still be used in the final study analyses. To safeguard your rights, we will use the minimum personally identifiable information possible.

Involvement of the General Practitioner/Family doctor (GP)

With your permission, we will write to your GP to notify them that you are going to take part in this study. We will also, with your permission, notify you and your GP of any clinically relevant abnormal test results that might need further action. However, abnormal test results that form part of our analysis (e.g., genetic or immunological analysis) for which the clinical implications are not yet certain will not be communicated to you or your GP.

What will happen to any samples I give?

Any tissue/blood sample you donate will be stored in a secure research facility at the University of Nottingham (Respiratory Research Unit) for as long as is required for the purposes of this study. Your sample will have your initials, date of birth and a number/code that is unique to yourself. By using these numbers, we can trace which sample belongs to you. This is important for the current study, so that we can re-test your sample if there is a problem with our tests.

We would also like to seek your consent so that any remaining samples may be stored and used in possible future research – this is optional (please indicate you agree to this on the consent form). The samples will be stored with a code unique to you and securely at the University of Nottingham under the University's Human Tissue Research Licence (no 12265).
Some of these future studies may be carried out by researchers other than current team, who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and you will not be identified in anyway. If you do not agree to this any remaining samples will be disposed of in accordance with the Human Tissue Authority's codes of practice.

Will any genetic tests be done?

Yes. It is hoped that the genetics part of the study (looking for differences in people's genes) will lead to a greater understanding of why people do or do not develop Lung Fibrosis and why some people react differently when they get it. We will be looking at genes that affect lung fibrosis.
Joining this part of the study is optional. This means you may:

• Choose not to join the genetics part of the study but still take part in the main study
• Choose not to join then change your mind before your sample is taken or at any time in the study
• Choose not to join at this time but decide to join later. If so, please talk to your study doctor.

Please be aware that the results of any genetic testing will be strictly confidential and will not be sent out to you.

If you choose after giving a sample not to take part in the genetics part of the study, we will destroy your sample within 30 days. If you choose to withdraw from the whole study after giving a sample, we will also destroy your sample within 30 days. However, if your sample is being processed we will have to wait until the laboratories have finished their tests before we can destroy it. This might take longer than 30 days. Please note, in this event, that we will not use your data for analysis.

What will happen to the results of the research study?

We intend to publish the results of this study in a medical respiratory journal. A summary of these results will also be made available on the Nottingham Respiratory Research Units website www.nrru.org.uk

Who is organising and funding the research?

This research is being organised by the University of Nottingham and is being funded by the NIHR Nottingham Biomedical Research Centre.

Who has reviewed the study?

All research in healthcare is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by East Midlands – Nottingham 1 Research Ethics Committee.

Further information and contact details

Research Officer: Lucy Howard
Tel: 0115 8231326
Email: lucy.howard@nottingham.ac.uk

Or

Dr Fasihul Khan
Tel: 0115 8231702
Email: fasihul.khan@nottingham.ac.uk

Optional Home Spirometry Participant Information Sheet
(Final Version 1.0 16th August 2018)

IRAS Project ID: 237010

Title of Study: **I**t's **N**ot **JUST I**diopathic Pulmonary Fibrosi**S** Study

Name of Chief Investigator: Professor Gisli Jenkins

We would like to invite you to take part in an optional part of our research study.

We are trying to find out if asking patients like yourself, with a Fibrotic Lung Disease, to take their own spirometry (breathing test) readings at home every day will help predict the course of lung fibrosis better than the normal testing in hospital every 3 months at clinic appointments. It is possible that patients perform better breathing tests (spirometry) when they are in their own environment and not feeling the stress of being at the hospital. Furthermore, getting multiple measurements over three months may give a more accurate assessment of lung function over three months compared with a one-off hospital visit. We also hope to see if measuring lung function every day can provide an early-warning of an exacerbation (worsening of symptoms) of patient's lung disease which may then, in the future, help guide early treatment for exacerbations.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign the optional section on the study consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

What will happen to me if I take part?

If you agree to take part, you will be provided with a small hand-held spirometer to blow into (to measure breathing tests). This device connects with a smartphone application 'app' (called "patientMpower app") which will download the breathing test information onto your smartphone. We will give you full training on how to use this.

We only want you to use the home spirometer for the first three months of the study even though the INJUSTIS study lasts for two years (with a visit when you first sign up, then again after three months, 12 months and 24 months).

You will be asked to do one home spirometry breathing test (sitting down) every day for three months, ideally at about the same time of day.

During these three months you will not be able to see the results of the breathing tests done at home. However, you will still have your usual spirometry tests, and be able to see these results, in hospital after three months as part of normal care when you see your doctor.

You can only be considered for this section of the study if you have an email address, access to internet at home and own a smartphone compatible with Apple or Android systems.

After the study is over you will be able to keep the spirometry device if you wish.

What are the possible disadvantages and risks of taking part?

The spirometer is not known to have any adverse effects. As with normal spirometry tests, breathing out forcefully can sometimes make you feel faint, and we would recommend you undertake the breathing test sitting down. Similarly breathing tests can make you cough.

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from this study may help us to see if daily handheld spirometry performed at home is better for patients than the current three-monthly testing at the hospital.

Will my taking part in the study be kept confidential?

If you choose to be part of the optional home spirometry part of the study, an outside company, patientMpower Ltd, will collect the results from your daily spirometry readings. This will mean this company will need to know your personal email address. This will allow you to have access to the 'app' and also technical support should you have an issue with the 'app' or the spirometer during the study. Your spirometry data on the patientMpower app will be linked to a unique identification number (and not to your email address). The spirometry data will be transferred to the University of Nottingham research team using this unique identification number so that they can match up the spirometry data with other information collected at the clinic visits done during the study. The patientMpower app can record your geographical location if you give permission in the app. If you do this, the patientMpower app can give you information on the air quality at your location and patientMpower can know the location of your phone. However, you don't have to give this permission if you don't want to. Strict guidelines are in place in ensure that your data will be secure and private during both transfer from mobile device and storage on a cloud server. No data from these daily readings is stored on your mobile phone device. If you were to misplace your phone or have it stolen, there would be no health-related data available to anyone else.

All data collected by patientMpower Ltd. will be transferred back to us here at the University of Nottingham. patientMpower Ltd will be acting as the Data Processor (they will process your personal information on behalf of the University) and the University will be the Data Controller (the University decides how the data is processed and takes responsibility for it). Both have safeguards in place to protect your data.

If you wish to have information deleted from the patientMpower app this can be done on request to patientMpower Ltd. You can contact them at www.info.patientmpower.com or by phone at 020 3322 4121 or using the support button within the app itself.

We can offer additional information about patientMpower Ltd. and the hand-held spirometer if requested.

What will happen if I don't want to carry on with the study?

Your participation is voluntary, and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw, we will no longer

collect any information about you or from you but we will keep the information about you that we have already obtained as we are not allowed to tamper with study records and this information may have already been used in some analyses and may still be used in the final study analyses. To safeguard your rights, we will use the minimum personally identifiable information possible.

If you decide not to take part in the daily handheld spirometry section of the INJUSTIS trial, you are still very welcome to consent to the main trial. This section is optional.

Further information and contact details

Research Officer: Lucy Howard
Tel: 0115 8231326
Email: lucy.howard@nottingham.ac.uk

OR

Dr Fasihul Khan
Tel: 0115 8231702
Email: fasihul.khan@nottingham.ac.uk

10.7 Consent form

University of Nottingham
UK | CHINA | MALAYSIA

Nottingham Respiratory Research Unit
• Asthma • COPD • ILD • Lung Infection •

Nottingham University Hospitals NHS
NHS Trust

CONSENT FORM
(Final version 1.4 27th July 2020)

Title of Study: **It's Not JUST Idiopathic Pulmonary FibrosiS Study**

IRAS Project ID: 237010

Name of Researcher: Prof Gisli Jenkins

Please initial box

1. I confirm that I have read and understand the information sheet dated - _____ version number _____ for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐

3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study for up to 10 years. I understand that my personal details will be kept confidential. ☐

4. I understand and agree that a blood sample will be taken for analysis to look for causes of lung fibrosis. ☐

5. <u>Consent for storage and use in possible future research (Optional)</u>
I agree that the samples I have given and the information gathered about me can be stored by the University of Nottingham at the Division of Respiratory Medicine, for possible use in future studies. I understand that some of these studies may be carried out by researchers other than the current team who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and I will not be identified in anyway.

Yes ☐ No ☐

6. I understand that the information held and maintained by NHS Digital and other central UK NHS bodies may be used to help contact me or provide information about my health status. ☐

7. <u>Consent for Genetic Research (Optional)</u>
I understand and agree that any samples I give may be used for genetic research in this Study and in future research aimed at why different people get Fibrotic Lung Diseases.

Yes ☐ No ☐

8. <u>Consent for Transfer of Samples to Third Parties (Optional)</u>

Yes ☐ No ☐

I also understand that part of the samples may be transferred outside the UK and to third parties, including commercial organisations for research. This would be done in an anonymised manner so that your details are kept confidential at all times.

Yes No

9. Consent for bronchoscopy (Optional) ☐ ☐

I understand and agree to having a bronchoscopy, having read the additional information leaflet outlining the risks. I agree than any samples that I give will be taken for analysis to look for specific cells in fibrotic lung diseases.

Yes No

10. Consent for daily hand-held spirometry ☐ ☐

I understand and agree to undertaking daily spirometry tests at home using the device and software provided by the study team. I understand that data collected on the software and my email address will be shared with the software provider, patientMpower Ltd.

11. I agree to my GP being informed of my participation in this study. ☐

12. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

10.8 Questionnaires

10.8.1 MRC dyspnoea scale

MRC Dyspnoea Scale

Please put a cross in the box which best applies to you.

Grade	Degree of breathlessness related to activities	
1	Not troubled by breathlessness except on strenuous exercise	☐
2	Short of breath when hurrying or walking up a slight hill	☐
3	Walks slower than most people on level ground because of breathlessness, or has to stop for breath when walking at own pace	☐
4	Stops for breath after walking about 100yds or after a few minutes on level ground	☐
5	Too breathless to leave the house, or breathless when dressing or undressing	☐

King's Brief ILD Questionnaire (K-BILD)

This questionnaire is designed to assess the impact of your lung disease on various aspects of your everyday life. Read each question carefully and answer by SELECTING the response that best applies to you. Please answer ALL questions, as honestly as you can.

1. In the last 2 weeks, I have been breathless climbing stairs or walking up an incline or hill.

1. Every time
2. Most times
3. Several Times
4. Sometimes
5. Occasionally
6. Rarely
7. Never

2. In the last 2 weeks, because of my lung condition, my chest has felt tight.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

3. In the last 2 weeks have you worried about the seriousness of your lung complaint?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

4. In the last 2 weeks have you avoided doing things that make you breathless?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

5. In the last 2 weeks have you felt in control of your lung condition?

1. None of the time
2. Hardly any of the time
3. A little of the time
4. Some of the time
5. A good bit of the time
6. Most of the time
7. All of the time

6. In the last 2 weeks, has your lung complaint made you feel fed up or down in the dumps?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

7. In the last 2 weeks, I have felt the urge to breathe, also known as 'air hunger'.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

8. In the last 2 weeks, my lung condition has made me feel anxious.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

9. In the last 2 weeks, how often have you experienced 'wheeze' or whistling sounds from your chest?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

10. In the last two weeks how much of the time have you felt your lung disease is getting worse?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

11. In the last 2 weeks has your lung condition interfered with your job or other daily tasks?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

12. In the last 2 weeks have you expected your lung complaint to get worse?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

13. In the last 2 weeks, how much has your lung condition limited you carrying things, for example, groceries?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

14. In the last 2 weeks, has your lung condition made you think more about the end of your life?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

15. Are you financially worse off because of your lung condition?

1. A significant amount
2. A large amount
3. A considerable amount
4. A reasonable amount
5. A small amount
6. Hardly at all
7. Not at all

Thank you for completing this questionnaire

10.8.3 EQ5D5L

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑

I have slight problems in walking about ❑

I have moderate problems in walking about ❑

I have severe problems in walking about ❑

I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑

I have slight problems washing or dressing myself ❑

I have moderate problems washing or dressing myself ❑

I have severe problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑

I have slight problems doing my usual activities ❑

I have moderate problems doing my usual activities ❑

I have severe problems doing my usual activities ❑

I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑

I have slight pain or discomfort ❑

I have moderate pain or discomfort ❑

I have severe pain or discomfort ❑

I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑

I am slightly anxious or depressed ❑

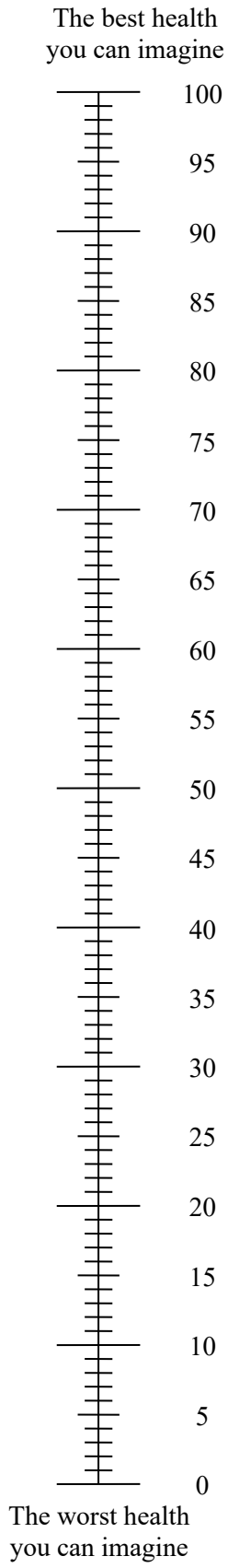
I am moderately anxious or depressed ❑

I am severely anxious or depressed ❑

I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the <u>best</u> health you can imagine.
  0 means the <u>worst</u> health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



13. **In the last 2 weeks, my cough has made me feel fed up.**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

14. **In the last 2 weeks, have you suffered from a hoarse voice as a result of your cough?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

15. **In the last 2 weeks, have you had a lot of energy?**

1. None of the time ☐
2. Hardly any of the time ☐
3. A little of the time ☐
4. Some of the time ☐
5. A good bit of the time ☐
6. Most of the time ☐
7. All of the time ☐

16. **In the last 2 weeks, have you worried that your cough may indicate a serious illness?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

17. **In the last 2 weeks, have you been concerned that other people think something is wrong with you, because of your cough?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

18. **In the last 2 weeks, my cough interrupted conversation or telephone calls.**

1. Every time ☐
2. Most times ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

19. **In the last 2 weeks, I feel that my cough has annoyed my partner, family or friends.**

1. Every time I cough ☐
2. Most times when I cough ☐
3. Several times when I cough ☐
4. Some times when I cough ☐
5. Occasionally when I cough ☐
6. Rarely ☐
7. Never ☐

Thank you for completing this questionnaire.

Designed by MEDICAL ILLUSTRATION
at LEICESTER ROYAL INFIRMARY
Birring/RESPIRATORY MEDICINE/11.02/18447VY

University Hospitals of Leicester NHS
NHS Trust

Leicester Cough Questionnaire (LCQ)

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question carefully and answer by TICKING the response that best applies to you. Please answer ALL questions, as honestly as you can.

This questionnaire will remain confidential.

Name

Date

1. In the last 2 weeks, have you had chest or stomach pains as a result of your cough?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

2. In the last 2 weeks, have you been bothered by sputum (phlegm) production when you cough?

1. Every time
2. Most times
3. Several times
4. Some times
5. Occasionally
6. Rarely
7. Never

3. In the last 2 weeks, have you been tired because of your cough?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

4. In the last 2 weeks, have you felt in control of your cough?

1. None of the time
2. Hardly any of the time
3. A little of the time
4. Some of the time
5. A good bit of the time
6. Most of the time
7. All of the time

5. How often during the last 2 weeks have you felt embarrassed by your coughing?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

6. In the last 2 weeks, my cough has made me feel anxious.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

7. In the last 2 weeks, my cough has interfered with my job, or other daily tasks.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

8. In the last 2 weeks, I felt that my cough interfered with the overall enjoyment of my life.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

9. In the last 2 weeks, exposure to paints or fumes has made me cough.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

10. In the last 2 weeks, has your cough disturbed your sleep?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

11. In the last 2 weeks, how many times a day have you had coughing bouts?

1. All the time (continuously)
2. Most times of during the day
3. Several times during the day
4. Some times during the day
5. Occasionally through the day
6. Rarely
7. None

12. In the last 2 weeks, my cough has made me feel frustrated.

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

10.8.5 IPARC

IPARC Score item	In the past month, have you been distressed or bothered by:	Not at all	A little bit	Quite a bit	Very much
1	Shortness of breath	0	1	2	3
2	Feeling weak	0	1	2	3
3	Feeling tired	0	1	2	3
4	Feeling sleepy in the day	0	1	2	3
5	Loss of appetite	0	1	2	3
6	Feeling restless and agitated	0	1	2	3
7	Uncontrolled symptoms	0	1	2	3
8	Side effects of treatment	0	1	2	3
9	Losing independence	0	1	2	3
10	Ability to carry out daily activities	0	1	2	3
11	Ability to carry out household tasks	0	1	2	3
	TOTAL				

10.9 Bronchoscopy protocol

Context:

This SOP is to be read and interpreted alongside existing NUH/British Thoracic Society bronchoscopy guidelines as well as the NUH patient information booklet and consent form.

Aim:

To detail the procedures that are to be carried out for participants having an optional bronchoscopy with a view to obtain broncho-alveolar lavage samples.

Facilities required:

Bronchoscopy suite

- Bronchoscopy must be carried out in the endoscopy department at Nottingham University's Hospitals NHS Trust with an appropriate recovery area which is monitored by a trained endoscopy nurse +/- research nurse
- An adult bronchoscope is equipped in the suite with associated software and output equipment

Resuscitation Facilities

The following must be available:

- Equipment for intubation and ambu bag
- Cardiac defibrillator and monitor
- Cardiac arrest with emergency medication

Personnel

- Staff must be appropriately trained for their job roles
- The bronchoscopy must be carried out by an appropriately trained bronchoscopist (named) who is at a level of performing this procedure unsupervised
- In absence of the named bronchoscopist, a back-up bronchoscopist should be available to cover with the same level of procedural competency as the named bronchoscopist.
- Research nurses with appropriate training will provide support to participants pre, during and post procedure.

Pre-Bronchoscopy

At booking

- If patients are on anticoagulation/anti-platelets, advise must be sought from trust guidelines:
    - Aspirin: can be continued, omitted on day of procedure
    - Clopidogrel: stopped 5 days prior to procedure
    - Prasugrel/ ticagrelor: stopped 7 days prior to procedure
    - DOACs: 48 hour prior to procedure (further information on dabigatran)
    - Further information available on NUHT trust guidelines.

- FBC and Clotting must be taken before participant's first bronchoscopy
- Participants must not (for the next 24 hours):
    - Drive a car, take public transport independently or ride a bike
    - Operate machinery or go to work
    - Make important decisions or sign any documents
    - Drink alcohol

      Hence please ensure participants are aware and that appropriate arrangement is made for alternative methods of getting home on day of bronchoscopy.

- Participants must be starved at least 6 hours prior to procedure and refrain from smoking

On day of procedure at clerking

- A recent clotting is carried out and results are normal
- Platelets >100 x 10^9/L
- Vital signs taken
- Research team should check with the participants the following (in additional to endoscopy clerking):
    - History of previous severe bronchoconstriction/ adverse reaction to bronchoscopy
    - Resting transcutaneous oxygen saturations <88%
    - Resting heart rate >130bpm pre-medication
    - Known serious ventricular arrhythmia
    - Antiplatelet, anti-coagulation use outside recommendations of trust policy

    If the patient answers 'yes' to any of the above, then the procedure should be abandoned.

- Clinical judgement should be applied as to whether bronchoscopy should proceed
- The patients' maximum allowed lidocaine dose is calculated at 8.2mg/kg
- IV access is obtained even if participants decline sedation

Bronchoscopy procedure

Premedication

- Patient identity and allergy status must be checked prior to administration of medication
- 1 set of vital signs must be done prior to pre-medication
- Lidocaine gel (2%) is introduced to the nasal passages
- 10% local anaesthetic is sprayed onto the base of tongue and oropharynx

  Total lidocaine dosage must be documented throughout the procedure and not exceed 8.2mg/kg. The procedure should be abandoned if this limit is reached.

- Sedation:
    - Midazolam 2-2.5mg intravenous (slow IV push 2mg/minute)- further doses of 0.5-1mg increments, maximum 7.5mg (for aged>70 years old:

initial dose 0.5-1mg, max dose 3.5mg) with additional Alfentanil if necessary (500micrograms/ml; 500micrograms slow IV push over 30 seconds, further 250microgram additional top up)
    - Naloxone must be available if for reversal (100-200micrograms/15 seconds and further 100mcg every 2 minutes with maximum dose 400micrograms if needed)
    - Flumazenil (200 micrograms/15 seconds, further 100micrograms every 1min up to maximum 600 micrograms)

Bronchoscopy procedure

- Patient must have pulse oximeter, automatic sphygmomanometer attached and nasal cannula with oxygen running at 2-4L/min.
- Primary suction (bronchoscope) and second suction (yanker suction) initiated
- Bronchoscope introduced trans-nasally or trans-orally (with mouth guard) and passed through to larynx.
- Lidocaine applied to anaesthetise vocal cords
- Airway intubated and lidocaine administered at carina, further lidocaine is administered in each lung.
- The left lung is observed visually first then then right lung.

Bronchoalveolar Lavage (BAL)

- Bronchial washings to be taken from the right middle lobe
- Disconnect the suction tubing from the bronchoscope and attach to lavage trap
- Connect lavage trap to suction port on bronchoscope
- Let patient know that they may cough a little
- Inject 60ml of saline slowly via the bronchoscope
- Wait 10 seconds and aspirate slowly
- Repeat injection of 60mls saline up to X 4 in total (Max instillation of 240ml; ideally no less than 100ml)
- Remove lavage trap
- BAL should be given to scientist and placed on ice immediately

Terminating bronchoscopy

The following are suggested for termination of the bronchoscopy after it has started:

- Participant withdraws consent
- The independent observer feels it is the participant's best interest
- Marked bronchospasm
- Oxygen saturations fall below 88%

Post bronchoscopy care

- All participants should be monitored for a minimum of 2 hours post procedure with monitoring from an endoscopy nurse in recovery.
- Participants must not drink for 1.5 hours dependent on level of sedation. The first sip should be witnessed by a nurse
- Participants must not (for the next 24 hours):
    - Drive a car or ride a bike
    - Operate machinery or go to work

    - Make important decisions or sign any documents
    - Drink alcohol
- Volunteers are given study member contact telephone number(s) or advised where to seek medical attention out of hours.
- Participants are reminded that they can check 'when I get home' section of the 'Bronchoscopic procedures at the endoscopy centre, City campus' for further information

Sample Processing

1) Collect BAL as above (Use 4 X 60mL saline)
2) Keep BAL cool on ice during processing
3) Pool aliquots in a single vessel
4) Remove approximately 50ml into aliquot A (minimum of at least 10-20ml)
5) 15mL aliquot B, remainder >5mL aliquot C
6) Add Phosphostop and protease inhibitor immediately to Aliquot B
7) Centrifuge all aliquots at 300g 4°C for 10 minutes
8) Remove all supernatant and freeze immediately at -80°C for exosome analysis

Aliquot A	Cytospin, cell count and differential (according to protocol below)
Aliquot B	Treat cell pellet with phosphostop and add protease inhibitor, before freezing at -80°C
Aliquot C	RNA sample – Store cell pellet in Tri-zol

Aliquot A

- After centrifugation re-suspend the cell pellet in 10mls of 2% BSA: PBS
- Perform cell count - live leukocytes, dead leukocytes and squamous cells using trypan blue exclusion. Calculate total number of cells.
- Centrifuge at 300g for 10 minutes
- Re-suspend pellet in d-PBS to give approximately $0.5x10^6$ cells/ml in accordance to cell count and prepare cytospins.

Cytospins

- Prepare a 75ul and 150ul cytospin, label with study ID, date, visit number, NRRU unique identifier
- Spin at 450rpm for 6 minutes in Shandon cytocentrifuge
- Assess which is suitable for counting and prepare another slide as back up, discarding the unsuitable one.
- Airdry slides for 15 minutes and then fix with methanol and stain with RappiDiff II solutions B & C

Aliquot B:

1) Freeze at -80°C for later cell protein analysis

For a sample from 15mL of BAL use 0.9mL phosphosafe, and 0.045mL of protease inhibitor stock
For a sample from 10mL of BAL use 0.6mL phosphosafe and 0.030mL of protease inhibitor stock

Aliquot C:

1) Lyse cells directly using 1ml TriZol for up to 5X10e6 cells (you would expect 1-2 million cells per 10 mL of BAL processed)
2) Pipette lysate up and down to mix and ensure complete lysis. Increase lysis volume if very viscous
3) Freeze at -80°C

10.10 Published manuscripts

10.10.1 A systematic review of blood biomarkers with individual participant data meta-analysis of matrix-metalloproteinase-7 in IPF – Khan et al (ERJ 2021)

EUROPEAN RESPIRATORY *journal*
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original research article

A systematic review of blood biomarkers with individual participant data meta-analysis of matrix-metalloproteinase-7 in IPF

Fasihul A. Khan, Iain Stewart, Gauri Saini, Karen A. Robinson, R. Gisli Jenkins

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A systematic review of blood biomarkers with individual participant data meta-analysis of matrix-metalloproteinase-7 in IPF

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Take home message:

Robust methodology using individual participant data meta-analysis demonstrates baseline MMP-7 levels predict overall mortality and disease progression in patients with untreated IPF independent of age, gender, smoking status and lung function.

ABSTRACT

Background

Blood derived biomarkers have been extensively described as potential prognostic markers in idiopathic pulmonary fibrosis (IPF), but studies have been limited by analyses using data-dependent thresholds, inconsistent adjustment for confounders and an array of endpoints, thus often yielding ungeneralisable results. Meta-analysis of individual participant data (IPD) is a powerful tool to overcome these limitations. Through systematic review of blood derived biomarkers, sufficient studies with measurements of Matrix Metalloproteinase-7 (MMP-7) were identified to facilitate standardised analyses of the prognostic potential of this biomarker in IPF.

Methods

Electronic databases were searched on 12th November 2020 to identify prospective studies reporting outcomes in patients with untreated IPF, stratified according to at least one pre-specified biomarker, measured at either baseline, or change over three months. Individual participant data (IPD) was sought for studies investigating MMP-7 as a prognostic factor. The primary outcome was overall mortality according to standardised MMP-7 z-scores, with a secondary outcome of disease progression in 12 months, all adjusted for age, gender, smoking and baseline FVC.

Results

IPD was available for nine studies out of twelve identified, reporting outcomes from 1664 participants. Baseline MMP-7 levels were associated with increased mortality risk (adjusted HR1.23, 95%CI 1.03;1.48, I^2=64.3%) and disease progression (adjusted OR1.27, 95%CI 1.11;1.46, I^2=5.9%). In limited studies, three-month change in MMP-7 was not associated with outcomes.

Conclusion

IPD meta-analysis demonstrated greater baseline MMP-7 levels were independently associated with an increased risk of poor outcomes in patients with untreated IPF, whilst short term changes did not reflect disease progression.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive fibrotic lung disease of unknown aetiology that affects approximately 3 million people worldwide, with a rising incidence and a median survival from diagnosis of approximately three years.[1-5] Disease trajectory is variable, ranging from slow progression to rapid loss of lung function and death.[6] The most recognised biomarker of disease progression in IPF is the change in forced vital capacity (FVC) at 12 months.[7] [8] However, lung function measurements have limitations, including test variability related to patient effort and confounding effects of comorbidities such as emphysema.[9]

Blood derived biomarkers have been extensively described as potential prognostic markers that reflect disease severity, though none have been implemented into routine clinical practice. Studies of biomarkers have been limited by small sample sizes, inconsistent methodologies including inconsistent adjustment for confounding variables, a variety of endpoints, and analysis of outcomes using data-dependent biomarker thresholds, thus often yielding inconsistent and ungeneralisable results.[10] [11]

Individual patient data (IPD) meta-analyses are considered the gold standard for collecting and synthesising evidence, offering a number of advantages over traditional aggregate methods, by enabling standardisation of analyses and outcomes, consistent adjustment for potential confounding factors and robust subgroup analyses according to patient

characteristics.[12 13] No published studies have utilised IPD to systematically synthesise the evidence for blood biomarkers in IPF. Through systematic review of blood derived biomarkers, sufficient studies with measurements of Matrix Metalloproteinase-7 (MMP-7) were identified to facilitate standardised analyses of the prognostic potential of this biomarker in IPF. Thus, we explore the association between MMP-7 measured at baseline and change over three months, and clinical endpoints including mortality and disease progression in adult patients with untreated IPF.

METHODS

The systematic review was conducted in accordance with a pre-specified protocol (PROSPERO registration number: CRD42019120402) and has been reported using PRISMA-IPD (Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Individual Participant Data) guidelines.[14]

Search strategy and study selection

Electronic database searches were carried out in MEDLINE (1946 to latest), Embase (1974 to latest), Google Scholar, the Cochrane Register of Controlled Trials and ClinicalTrials.gov, with the last search carried out on 12th November 2020. Keywords and controlled vocabulary terms for "idiopathic pulmonary fibrosis" and "biomarkers", alongside search filters for prognostic studies were applied (Figure S1).[15] Hand searches of reference lists in retrieved articles were conducted to identify further studies. Unpublished and ongoing studies were identified by searching pre-print servers including medRxiv, bioRxiv and Wellcome Open Research.

Following searches, two reviewers screened through titles and abstracts before full text review independently. Disagreements were resolved by consensus with a third reviewer.

The review included all original prospective observational studies that reported outcomes in stable or exacerbating patients aged over 18 with anti-fibrotic naïve IPF, diagnosed according to contemporaneous consensus guidelines,[16-18] stratified according to at least one pre-identified blood biomarker. Conference abstracts reporting sufficient detail were eligible for inclusion. Retrospective studies, case reports, animal studies and studies investigating non-IPF interstitial lung disease (ILD) were excluded. Language or year of publication restrictions were not applied. No minimal study sample size was specified for inclusion.

Studies reporting the following biomarkers measured at either baseline and/or trends over 3 months were eligible for review: biomarkers of epithelial dysfunction including MMP-7, Krebs von den Lungen-6 (KL-6), surfactant protein-A (SP-A), surfactant protein-D (SP-D), matrix metalloproteinase-1 (MMP-1), cancer antigen 125 (CA-125), carbohydrate antigen 19-9 (CA19-9), vascular endothelial growth factor (VEGF), insulin like growth factor binding protein 2 (IGFBP2)], biomarkers of ECM modelling [collagen synthesis peptides, neoepitopes, lysyl oxidase like 2 (LOXL2), periostin, osteopontin] and biomarkers of immune dysregulation [C-C motif chemokine ligand 18 (CCL-18), chemokine ligand 13 (CXCL13), interleukin-8 (IL-8), heat shock protein 70 (HSP70), chitinase-3-like protein 1 (YKL40), intracellular adhesion molecule 1 (ICAM-1)].

Data extraction and risk of bias assessment

IPD were sought from corresponding authors of studies investigating MMP-7 as a prognostic factor, using secure and encrypted electronic mail communication. A minimum of three reminders, each four weeks apart were sent. Data from sponsored clinical studies were requested through various online portals.[19-21] Requested data included participant demographics (age, gender, smoking status and baseline lung function), baseline and three-month MMP-7 levels and outcomes including 12-month lung function and overall mortality (Figure S2).

Where IPD were not made available, aggregate data were extracted from study publications, using a proforma and verified by a second reviewer. Data included study design, participant and biomarkers characteristics, and outcome data including sample sizes, mean values and standard deviations of biomarkers in individuals with and without the event. Time to event data were collected using adjusted hazard ratios (HR) where reported.

Risk of bias assessment was carried out independently by two reviewers using the Quality in Prognostic Studies (QUIPS) tool.[22] The QUIPS tool assesses the risk of bias across six domains: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding and statistical analysis and reporting. All studies were included in the review irrespective of their risk of bias rating. The GRADE (Grading of Recommendations, Assessment, Development and Evaluations) framework was applied to rate the overall quality of evidence for each outcome.[23]

Statistical analysis

All identified studies were included in the data synthesis, with summary tables for study characteristics. Multiple cohorts within the same study were treated as individual cohorts. The primary outcome was overall mortality. Secondary outcomes measures included change in percent predicted FVC from baseline at 12 months and disease progression defined as 10% relative decline in FVC or death within 12 months of baseline. Hazard ratios (HR) for MMP-7 levels in predicting mortality, and odds ratios (OR) for predicting disease progression, were estimated using a two-stage IPD meta-analysis with random effects and presented as forest plots. Estimates were adjusted for *a priori* confounders including age, sex, smoking history, and baseline FVC. Unadjusted analyses have been presented in the supplementary material (Figure S10). Studies with a follow up duration longer than three years were censored for survival analyses. To standardise biomarker values across studies, z scores specific to each study were calculated and analysed as exposure variables. The change in MMP-7 over three-months was calculated where available using relative percent change from baseline. Participants with missing data were excluded using listwise deletion. The I^2 statistic was used to evaluate statistical heterogeneity between studies. Meta-regression was conducted where sufficient studies were included to explore variability in heterogeneity according to: study design (cohort vs. randomised trial), single-centre studies, non-peer reviewed manuscripts, assay methods (ELISA vs. non-ELISA), and the type of blood samples used (serum vs. plasma). Publication bias was assessed using funnel plot analysis and Egger's test.[24] All statistical analyses were performed using Stata 16 (Statacorp, Texas US). Due to methodological heterogeneity, marked difference in outcome measures and insufficient studies for IPD, biomarkers other than MMP-7 have been described narratively and in tables.

RESULTS

Searches of the electronic databases on 12th November 2020 yielded 4930 articles, with a further 69 studies identified through preprint servers. Following the removal of duplicates, screening and full text review, 29 studies published worldwide between 2007 and 2020, reporting outcomes from 3950 IPF participants were included (Figure 1). A total of 12 studies reported outcomes in relation to MMP-7, of which IPD was available for nine studies (75%) reporting data from eleven individual cohorts and 1664 participants (Table 1). No issues with the integrity of IPD were identified. A further 15 blood biomarkers were evaluated across the included studies, with a number of studies evaluating combinations of biomarkers (Table S1).

Risk of bias assessment of the retrieved studies identified limitations and a number of possible biases (Figure 2, Table S2). For studies included in the MMP-7 meta-analysis, publication bias was not detected statistically, but visual inspection of funnel plots suggested publication bias was present for some of the outcomes assessed. (Figure S3 and S4). Most MMP-7 studies defined the study population specifically with clear inclusion/exclusion criteria. Biomarkers were measured consistently using the same sample matrices (plasma or serum) across included participants in each study, although details of assay platforms used to measure the analytes were frequently unreported. Outcome data were measured objectively and applied consistently to all study participants. Studies evaluating biomarkers other than MMP-7 had similar limitations and risks of bias. Blood biomarkers are known to be influenced by age and sex, as well as possible lifestyle factors such as smoking, which along with baseline lung

function are all confounders upon disease outcome.[25] In approximately half of all included studies, possible confounders were not measured, and there was inconsistent adjustment in estimations where accepted confounders were measured. Moreover, in a number of studies, analyses were performed using data-dependent biomarker thresholds that were inconsistent across studies.

Association between blood biomarkers and clinical outcomes

Baseline blood biomarkers that predict mortality

Ten studies evaluated the relationship between mortality and MMP-7, with IPD available for eight studies totalling 1492 participants. Meta-analysis demonstrated greater baseline MMP-7 values were associated with a 23% increased risk of overall mortality [adjusted HR (aHR) 1.23 per standard deviation (SD) increase, 95%CI 1.03;1.48, I^2=64.3%) (Figure 3A), though there was substantial statistical heterogeneity which could not be explained by variability in the factors assessed (Table S3). When mortality at 12 months was examined specifically, baseline MMP-7 levels were inconclusively associated with death (aHR 1.33 per SD increase, 95%CI 0.99;1.78, I^2=59.6%) (Figure 3B). Applying the GRADE framework, we rate the confidence in mortality estimates with moderate certainty (Table S4). Where IPD was unavailable, MMP-7 values above 5.7ng/mL were associated with increased mortality (aHR 2.18 95%CI 1.1;4.32) over a median follow up of 19 months in a study of 438 participants.[26] A further study of 57 participants found MMP-7 levels did not predict death[27] (Table S5).

The primary outcome of mortality was evaluated for a further 14 biomarkers in a total of 17 studies not assessed in IPD meta-analysis, with inconsistent and inconclusive findings (Figure 6 and Table S5). Study follow up times were inconsistent, effect sizes varied with wide confidence intervals, and estimates were often unadjusted for important covariates.

Change in biomarkers predicting mortality

Three studies totalling 498 participants explored the association between MMP-7 change over three months and mortality.[28 29] IPD meta-analysis showed no association with mortality (aHR 1.00, 95%CI 0.99;1.02,I^2=53.3%), nor when mortality was censored at 12 months (aOR 1.00, 95%CI 0.99;1.01,I^2=37.4%) (Figures S5 and S6).

Three publications from the same cohort evaluated the relationship between longitudinal biomarker measurement and mortality.[30-32] In both discovery and validation cohorts, a rise in CA-125 over three-months doubled the risk of death, but the remaining biomarkers were not predictive of mortality (Figure 6 and Table S6). A validation cohort of 145 participants demonstrated replication of rising neoepitopes degraded by matrix metalloproteinases (C1M, C3M, C6M and CRPM), but the rate of change of collagen synthesis peptides was not associated with mortality.[32]

Baseline biomarkers that predict disease progression and change in FVC

Ten studies measured MMP-7 levels as markers of disease progression, with eight studies totalling 1383 participants included in the IPD meta-analysis. Meta-analysis demonstrated baseline MMP-7 was associated with disease progression (aOR 1.27 per SD increase, 95%CI 1.11;1.46,I^2=5.9%) (Figure 4). Whilst heterogeneity was low, meta-regression identified sample assay techniques (ELISA vs. other) to be a source of heterogeneity. In subgroup analysis according to assay, the odds ratio for disease progression was estimated at 1.56 per SD increase (95%CI 1.26;1.82, I^2=0%) when restricted to studies using ELISA (Figure S7). When the relationship between baseline MMP-7 and relative change in FVC at 12 months was examined specifically in six studies of 891 participants, meta-analysis indicated that a 1 standard deviation greater baseline MMP-7 was associated with a -0.85% relative change in 12-month FVC percent predicted (95%CI -1.65; -0.05, I^2=0%) (Figure 5). We assess findings for disease progression and change in FVC outcomes with high certainty (Table S4). For studies not included in IPD meta-analysis, baseline MMP-7 values above 3.8ng/mL doubled the risk of disease progression (aHR 2.2 95%CI 1.4;3.7) over a median follow-up of 19 months in 211 participants.[33] In a further study of 57 participants, MMP-7 did not predict disease progression (Table S7).

Disease progression was evaluated for a number of other biomarkers in 19 studies that were not included in IPD meta-analysis. None were consistently predictive of disease progression, though there was significant heterogeneity in adopted definitions of disease progression, with lung function indices, mortality, transplant and acute exacerbations included in various combinations at non-unified time points (Figure 6 and Table S7, S8).

Change in biomarkers predicting disease progression

Three studies totalling 481 participants investigating the association between MMP-7 change over three months and disease progression were included in IPD meta-analysis. Change in MMP-7 over three-months was not associated with disease progression (aOR 1.00 per percent increase, 95%CI 0.99;1.01, I^2=22.5%) (Figure S8), nor with change in FVC over 12 months (effect size 0.01% increase per percent MMP-7 increase 95%CI -0.07;0.08, I^2=60.8%) (Figure S9). In a study of 211 participants not included in IPD meta-analysis, a two-fold change in MMP-7 over four months was associated with doubling the risk of disease progression.[33]

In one study, participants with progressive disease had rising concentrations of CA-125 over 3 months compared to those with stable disease, but no relationship was replicated for other biomarkers.[30] (Figure 6, Table S9)

Discussion

This systematic review of prospective studies in patients with untreated IPF identified 16 blood derived biomarkers and assessed 6 outcome variables, but there were only sufficient studies to undertake an IPD meta-analysis for MMP-7. IPD meta-analysis demonstrated baseline MMP-7 levels predicted all-cause mortality and disease progression and correlated with FVC percent predicted change over 12 months. There was a 23% greater risk of overall mortality and 27% greater risk of disease progression, per standard deviation increase in baseline MMP-7 values. An inconclusive association was observed for risk of 12-month

mortality. Notably, MMP-7 levels did not seem to change longitudinally over three months, with no association observed with any of the measured outcomes. However, a study not included in quantitative synthesis suggested that in those individuals where MMP-7 does rise, there may be an associated risk in progression[33]. Mortality outcomes were rated with moderate certainty and disease progression and change in FVC outcomes with high certainty (Table S4).

Our IPD meta-analysis represents the first time it has been possible to synthesise blood biomarker findings in IPF. The meta-analysis was focused on MMP-7 as there were sufficient studies available, however individually these had yielded inconsistent results, reported data-dependent thresholds and often not adjusted for confounding factors. IPD enabled analysis of MMP-7 levels as continuous variables transformed to z-scores to overcome assay variability, supported standardised definition of outcomes, and consistent adjustment for important covariates, which enabled robust and reliable conclusions. We performed two-stage IPD meta-analysis, which does not assess study estimate and effects simultaneously although is considered to produce unbiased estimates,[34] and enabled modelling IPD from 1492 participants across separate secure servers and portals. Analysis of heterogeneity in IPD meta-analysis indicated that assay type was a significant contributor to heterogeneity, particularly in estimates of disease progression.

There are limitations to this review. Whilst language restrictions were not applied, two articles in Japanese were excluded as they could not be translated to English to assess inclusion criteria. We included only those studies where participants were diagnosed according to international consensus guidelines, supporting the robustness and generalisability of our

findings. We excluded studies in IIPs not specific to IPF, which limits interpretation in non-IPF ILDs, although ongoing studies exploring shared mechanistic pathways will provide further insight.[35] Furthermore, by focussing on untreated IPF patients our results do not address the theranostic value of MMP-7 in relation to anti-fibrotic therapy. There was significant statistical heterogeneity in some of the outcomes, and therefore these should be interpreted with caution. We were unable to explain all the residual heterogeneity using the factors we assessed. IPD was not obtained from a limited number of suitable studies, and therefore we had to report these findings narratively.

Biomarkers of disease activity have the potential to facilitate clinical management and transform early-phase clinical trials by acting as surrogate endpoints. Dysfunctional epithelial cells contribute to fibrogenesis by secreting profibrotic mediators including matrix-metalloproteinases (MMPs),[36] responsible for degrading multiple components of extracellular matrix, activating biological mediators, and facilitating epithelial-mesenchymal transition.[37] Further research could elucidate the relationship between IPF pharmacotherapy and MMP-7, particularly to identify whether changes in MMP-7 levels may represent a biomarker of therapeutic response. From a clinical perspective, MMP-7 should be considered for implementation as a prognostic tool at the point of diagnosis, especially where lung function testing is cumbersome or unavailable.

Due to heterogeneity in study designs and reported outcomes, there were insufficient data for quantitative analysis in non-MMP-7 studies. Whilst many biomarkers showed an association with mortality in single studies, replication of effects across studies was weak. We highlight sources of considerable bias and variability. Studies were typically observational, of

relatively modest size with a lack of prespecified power calculations. A number of different laboratory techniques were applied to measure biomarker levels across studies, with very few studies reporting detailed assay information, particularly with regards to measures of precision, and there was inconsistency in thresholds defining positive and negative biomarker result. Short-term changes in biomarker concentrations over three-months were often not associated with specified clinical outcomes suggesting further studies are needed before such biomarkers can be adopted clinically. Further biomarker research should focus on rigorously designed longitudinal studies with discovery and validation cohorts, using validated biomarker assays and standardised endpoints. Furthermore, it is possible that combinations of biomarkers will add granularity to our understanding of pathogenesis and prognosis of IPF and further studies evaluating their utility are needed. As further studies are published, IPD meta-analysis should be considered to produce more reliable results and support generalisability.

In summary, whilst a number of other blood biomarkers have been studied for predicting prognosis, there is currently insufficient replication to enable adoption into clinical testing, with the possible exception of MMP-7. We apply robust methodology and IPD meta-analysis to demonstrate baseline MMP-7 levels predict overall mortality and disease progression in patients with untreated IPF independent of age, gender, smoking status and lung physiology. However, short term changes in MMP-7 over three-months offered limited prognostic value in the absence of an empirical threshold.

Original research

Systematic review and meta-analysis of anakinra, sarilumab, siltuximab and tocilizumab for COVID-19

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ABSTRACT

Background There is accumulating evidence for an overly activated immune response in severe COVID-19, with several studies exploring the therapeutic role of immunomodulation. Through systematic review and meta-analysis, we assess the effectiveness of specific interleukin inhibitors for the treatment of COVID-19.

Methods Electronic databases were searched on 7 January 2021 to identify studies of immunomodulatory agents (anakinra, sarilumab, siltuximab and tocilizumab) for the treatment of COVID-19. The primary outcomes were severity on an Ordinal Scale measured at day 15 from intervention and days to hospital discharge. Key secondary endpoints included overall mortality.

Results 71 studies totalling 22 058 patients were included, 6 were randomised trials. Most studies explored outcomes in patients who received tocilizumab (60/71). In prospective studies, tocilizumab was associated with improved unadjusted survival (risk ratio 0.83, 95% CI 0.72 to 0.96, I^2=0.0%), but conclusive benefit was not demonstrated for other outcomes. In retrospective studies, tocilizumab was associated with less severe outcomes on an Ordinal Scale (generalised OR 1.34, 95% CI 1.10 to 1.64, I^2=98%) and adjusted mortality risk (HR 0.52, 95% CI 0.41 to 0.66, I^2=76.6%). The mean difference in duration of hospitalisation was 0.36 days (95% CI −0.07 to 0.80, I^2=93.8%). There was substantial heterogeneity in retrospective studies, and estimates should be interpreted cautiously. Other immunomodulatory agents showed similar effects to tocilizumab, but insufficient data precluded meta-analysis by agent.

Conclusion Tocilizumab was associated with a lower relative risk of mortality in prospective studies, but effects were inconclusive for other outcomes. Current evidence for the efficacy of anakinra, siltuximab or sarilumab in COVID-19 is insufficient, with further studies urgently needed for conclusive findings.

PROSPERO registration number CRD42020176375.

Key messages

What is the key question?
► Are specific interleukin inhibitors efficacious and safe for the treatment of COVID-19?

What is the bottom line?
► Immunomodulatory therapies, particularly tocilizumab show promise as therapies for patients with severe COVID-19, but there is an urgent need for further randomised controlled trials to define the role of this treatment.

Why read on?
► Understanding evidence-based treatments for COVID-19 will ensure patients are optimally managed, thereby reducing associated morbidity and mortality.

INTRODUCTION

The novel SARS-CoV-2 was first identified in Wuhan, China, in December 2019.[1] Since then, COVID-19 has been declared a global pandemic by the WHO and continues to spread at an exponential rate with over two million deaths reported worldwide.[2 3]

The clinical manifestations of COVID-19 tend to be heterogenous ranging from asymptomatic infection to acute respiratory disease syndrome, multiorgan failure and death. Mechanisms underlying severe disease are incompletely understood, but accumulating evidence points towards a dysregulated and excessive host immune response referred to as cytokine storm syndrome.[4] During this state of immunological hyperactivation, increased circulating levels of proinflammatory cytokines including interleukin (IL)-1 and IL-6 have been demonstrated and are associated with adverse clinical outcomes.[5–7] Suppression of proinflammatory cytokines in COVID-19 may therefore be a potential therapeutic strategy.[8]

SARS-CoV-2 shares a number of genetic and clinical similarities with other zoonotic coronaviruses, including SARS-CoV and Middle East respiratory syndrome (MERS).[9 10] There are also reports of elevated proinflammatory cytokines in patients with SARS and MERS,[11 12] suggesting overlapping therapeutic targets in the management of SARS, MERS and COVID-19.

Several clinical studies evaluating the role of immunomodulatory agents in COVID-19 have been published recently. Through systematic review and critical appraisal of the literature, we assess the effectiveness and safety of specific IL-1 (anakinra) and IL-6 (tocilizumab, siltuximab, sarilumab) inhibitors for the treatment of COVID-19, drawing on the literature from previous similar coronavirus infections (SARS and MERS) where available. These agents already carry approval for the treatment of other rare non-infectious and autoimmune conditions, with an acceptable safety profile.

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METHODS

The systematic review was conducted in accordance with a prespecified protocol and has been reported in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[13]

Search strategy and study selection

Electronic database searches were carried out in MEDLINE (1946 to latest) and EMBASE (1974 to latest) and ongoing clinical trial registries (clinicaltrials.gov and EU Clinical Trials Register), with the last search carried out on 7 January 2021. Search terms were broad and included keywords and controlled vocabulary for patient and treatment-related terms (see online supplemental figure S1 for MEDLINE search strategy). Unpublished and ongoing studies were identified by searching preprint servers including medRxiv and bioRxiv. Searches were carried out independently by two reviewers in a standardised manner, followed by screening through titles and abstracts, before full-text review. Disagreements were resolved by consensus, with unresolved conflicts decided by a third reviewer.

The review included all original studies, evaluating the use of at least one of the following: anakinra, tocilizumab, sarilumab or siltuximab in patients aged over 18 suspected or confirmed with either COVID-19, SARS or MERS. Case reports and retrospective studies without a comparator arm were excluded due to their associated heterogeneity and inherent risk of bias. Language or year of publication restrictions were not applied. No minimal study sample size was specified for inclusion.

The planned primary outcomes were selected based on their clinical usefulness and included time to hospital discharge (days) and severity on an adapted 4-point Ordinal Scale at day 15 following intervention, with the following ratings: (i) death; (ii) requirement for invasive mechanical ventilation (IMV) or extracorporal membrane oxygenation (ECMO); (iii) hospitalised but no requirement of IMV/ECMO and (iv) not hospitalised. Secondary outcomes included overall mortality and treatment-related adverse events. For all outcomes studied, baseline was defined as the day of intervention.

Data extraction and risk of bias assessment

Data were extracted from article's text and figures using a data-extraction proforma and verified by a second reviewer. Information sought included study design, sample size, participant demographics, clinical investigation findings, intervention characteristics (name of agent, dose, route), treatment-related adverse events, requirement and duration of invasive and non-invasive ventilation, use and dosage of oxygen, duration of hospital stay, survival outcome measures and follow-up duration. Where ordinal outcomes were reported at multiple timepoints, those closest to day 15 post intervention were chosen for extraction. For ongoing trial protocols, the registration number, sample size and expected date of completion were recorded.

Risk of bias assessment was carried out independently in duplicate. Due to the heterogeneity of study designs, various quality assessment tools available through the National Institute of Health were applied.[14] The tools assess risk of bias through criterion specific to each study design, before providing an overall quality rating of good, fair or poor. Randomised studies were assessed using the Cochrane risk-of-bias tool for randomised trials (RoB2).[15] As per the review protocol, all studies were included irrespective of their risk of bias rating. Using the GRADE (Grading of Recommendations, Assessment, Development and Evaluations) approach, we rated the overall quality of evidence for each outcome as high, moderate, low or very low.[16]

Statistical analysis

All identified studies were included in the narrative summary with summary tables for characteristics. For the primary outcomes, numbers of individuals meeting each outcome on the adapted Ordinal Scale were pooled using rank-based Wilcoxon-Mann-Whitney tests with ties split evenly between positive and negative outcomes, providing a generalised OR (GenOR) with 95% CIs. The GenOR provides a measure of the likelihood that the intervention leads to a better rather than worse outcome when compared with a randomly chosen control.[17] Mean hospital duration and SD were extracted or were estimated from median and range/IQR using the Box-Cox method.[18] Mean difference in hospital stay was calculated where a control arm was reported. Where available, adjusted HRs and unadjusted mortality data were extracted for quantitative synthesis. Where data were not reported in a tabular format, values were extracted from plotted data using a digital plot analyser.[19]

Where sufficient studies were identified for a specific immunomodulator, findings were assessed using random effects meta-analysis and presented as forest plots. Meta-analyses were grouped by retrospective and prospective design and presented on the same plots with no overall estimate. The I^2 statistic was used to evaluate statistical heterogeneity. Although sample sizes were limited, we used pseudo R^2 from meta-regression to explore variability in heterogeneity owing to study design (single centre or multicentre), non-peer-reviewed manuscripts, concomitant use of steroids, route of drug administration (intravenous or

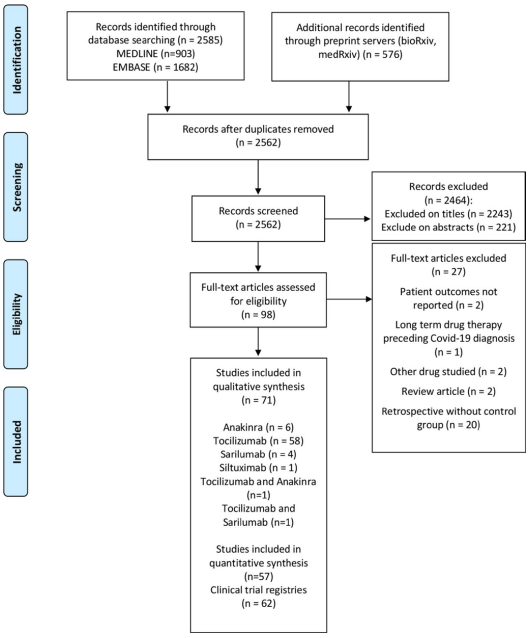

Figure 1 Flow diagram illustrates systematic search and screening strategy, including numbers meeting eligibility criteria and numbers excluded. Last search carried out on 7 January, 2021.

Table 1 Included studies with study characteristics and sample size for treatment (Tx) and control group (control) shown

Author, year	Drug	N, Tx/control	Study country	Centre	Study design	Author, year	Drug	N, Tx/control	Study country	Centre	Study design	Author, year	Drug	N, Tx/ control	Study country	Centre	Study design
Balkhair *et al*, 2020[26]	A	45/24	Oman	Single centre	Prospective with control	Roumier *et al*, 2020[32]	T	49/47	France	Single centre	Prospective with control	Kimmig *et al*, 2020[33]	T	54/57	USA	Single centre	Retrospective
Huet *et al*, 2020[29]	A	52/44	France	Single centre	Prospective with control	Salama *et al*, 2020[22]	T	249/ 128	USA	Multicentre	Double-blind RCT	Klopfenstein *et al*, 2020[34]	T	20/25	France	Single centre	Retrospective
Kooistra *et al*, 2020[35]	A	21/39	Netherlands	Multicentre	Prospective with control	Salvarani *et al*, 2020[36]	T	60/63	Italy	Multicentre	Open-label RCT	Lewis *et al*, 2020[37]	T	497/497	USA	Multicentre	Retrospective
*Kyriazopoulou *et al*, 2020[28]	A	130/130	Greece	Multicentre	Prospective	*Sanchez-Montalva *et al*, 2020[38]	T	82/0	Spain	Single centre	Prospective	Martinez-Sanz *et al*, 2020[39]	T	260/969	Spain	Multicentre	Retrospective
Cauchois *et al*, 2020[24]	A	12/10	France	Multicentre	Retrospective	Sciascia *et al*, 2020[40]	T	63/0	Italy	Multicentre	Prospective	Narain *et al*, 2020[27]	T	73/3076	USA	Multicentre	Retrospective
Cavalli *et al*, 2020[25]	A	29/16	Italy	Single centre	Retrospective	Stone *et al*, 2020[31]	T	161/ 82	USA	Multicentre	Double-blind RCT	Nasa *et al*, 2020[41]	T	22/63	India	Multicentre	Retrospective
Narain *et al*, 2020[27]	A	57/3076	USA	Multicentre	Retrospective	Strohbehn *et al*, 2020[42]	T	32/41	USA	Single centre	Phase II open label	Patel *et al*, 2020[43]	T	60/1505	USA	Single centre	Retrospective
Benucci *et al*, 2020[44]	Sa	8/0	Italy	Single centre	Prospective	Toniati *et al*, 2020[45]	T	100/0	Italy	Single centre	Prospective	*Petrak *et al*, 2020[46]	T	81/37	USA	Multicentre	Retrospective
Della-Torre *et al*, 2020[30]	Sa	28/28	Italy	Single centre	Prospective with control	Biran *et al*, 2020[47]	T	210/ 420	USA	Multicentre	Retrospective	Petit *et al*, 2020[48]	T	42/41	USA	Single centre	Retrospective
*Gordon *et al*, 2021[10]	Sa	45/397	UK	Multicentre	Adaptive RCT	Canziani *et al*, 2020[49]	T	64/64	Italy	Multicentre	Retrospective	Potere *et al*, 2020[50]	T	74/74	Italy	Single centre	Retrospective
Gremese *et al*, 2020[51]	Sa	53/0	Italy	Single centre	Prospective	Capra *et al*, 2020[52]	T	62/23	Italy	Single centre	Retrospective	*Ramaswamy *et al*, 2020[53]	T	10/10	USA	Multicentre	Retrospective
Sinha *et al*, 2020[54]	Sa	255/0	USA	Single centre	Prospective	Chilimuri *et al*, 2020[55]	T	83/ 685	USA	Single centre	Retrospective	Rodriguez-Baño *et al*, 2020[56]	T	21/65	Spain	Multicentre	Retrospective
*Gritti *et al*, 2020[21]	Si	30/30	Italy	Single centre	Prospective with control	De Rossi *et al*, 2020[57]	T	90/68	Italy	Single centre	Retrospective	Rojas-Marte *et al*, 2020[58]	T	88/344	USA	Single centre	Retrospective
Albertini *et al*, 2020[59]	T	22/22	France	Single centre	Prospective with control	Elmer *et al*, 2020[60]	T	22/22	Sweden	Single centre	Retrospective	Roomi *et al*, 2020[61]	T	96/97	USA	Single centre	Retrospective
Antony *et al*, 2020[62]	T	80/0	USA	Multicentre	Prospective	Fisher *et al*, 2020[63]	T	45/70	USA	Single centre	Retrospective	Rosas *et al*, 2020[64]	T	20/17	Spain	Single centre	Retrospective
Campins *et al*, 2020[65]	T	58/0	Spain	Single centre	Prospective	Galván-Román *et al*, 2020[66]	T	58/88	Spain	Single centre	Retrospective	Rossi *et al*, 2020[67]	T	84/84	France	Single centre	Retrospective
*Carvalho *et al*, 2020[68]	T	29/24	Brazil	Single centre	Prospective with control	*Moreno Garcia *et al*, 2020[69]	T	77/94	Spain	Single centre	Retrospective	Rossotti *et al*, 2020[70]	T	74/148	Italy	Single centre	Retrospective
Dastan *et al*, 2020[71]	T	42/0	Iran	Single centre	Prospective	Gokhale *et al*, 2020[72]	T	70/91	India	Single centre	Retrospective	Ruiz-Antorán *et al*, 2020[73]	T	268/238	Spain	Multicentre	Retrospective
*Gordon *et al*, 2021[20]	T	350/397	UK	Multicentre	Adaptive RCT	Guaraldi *et al*, 2020[74]	T	179/ 365	Italy	Multicentre	Retrospective	Somers *et al*, 2020[75]	T	78/76	USA	Single centre	Retrospective
Hermine *et al*, 2020[23]	T	63/67	France	Multicentre	Open-label RCT	Guisado-Vasco *et al*, 2020[76]	T	132/ 475	Spain	Single centre	Retrospective	Tian *et al*, 2020[77]	T	65/130	China	Multicentre	Retrospective
Malekzadeh *et al*, 2020[78]	T	126/0	Iran	Multicentre	Prospective	Gupta *et al*, 2020[79]	T	433/ 3492	USA	Multicentre	Retrospective	Tsai *et al*, 2020[80]	T	66/66	USA	Single centre	Retrospective
Mikulska *et al*, 2020[81]	T	29/66	Italy	Single centre	Prospective with control	Hill *et al*, 2020[82]	T	43/45	USA	Single centre	Retrospective	*Wadud *et al*, 2020[83]	T	84/84	USA	Single centre	Retrospective
Morena *et al*, 2020[84]	T	51/0	Italy	Single centre	Prospective	Holt *et al*, 2020[85]	T	24/30	USA	Single centre	Retrospective	Zheng *et al*, 2020[86]	T	92/89	China	Single centre	Retrospective

Continued

Table 1 Continued

Author, year	Drug	N, Tx/control	Study country	Centre	Study design
Perrone *et al*, 2020[87]	T	708/481	Italy	Multicentre	Single-arm open label and validation
*Rosas *et al*, 2020[89]	T	294/144	USA	Multicentre	Double-blind RCT

Author, year	Drug	N, Tx/control	Study country	Centre	Study design
Ip *et al*, 2020[88]	T	134/413	USA	Multicentre	Retrospective
Kewan *et al*, 2020[90]	T	28/23	USA	Single centre	Retrospective

Author, year	Drug	N, Tx/ control	Study country	Centre	Study design

*Non-peer-reviewed preprint study.
A, anakinra; RCT, randomised clinical trial; Sa, sarilumab; Si, siltuximab; T, tocilizumab.

subcutaneous) and day outcome measured. Publication bias was assessed using funnel plot analysis and Egger's test. Prospective studies without a control arm were excluded from meta-analysis and presented either in the narrative summary or in tables. All analyses were performed using Stata V.16 (StataCorp, College Station, Texas, USA).

RESULTS

Search of the electronic databases (MEDLINE and EMBASE) on 7 January 2021 yielded a total of 2585 studies, with further 576 studies identified through preprint servers. Following removal of duplicates, screening and full-text review, 71 articles published worldwide were shortlisted for inclusion (anakinra, n=6; tocilizumab, n=58; anakinra and tocilizumab, n=1; sarilumab and tocilizumab, n=1; sarilumab, n=4; siltuximab, n=1) (figure 1). Sixty-two studies were published in peer-reviewed journals, with the remaining nine identified through preprint servers. All studies were performed in patients with COVID-19, with no suitable studies identified for SARS or MERS. Overall, 29 studies were prospective in design, with 17 studies including a control group for comparison, of which 6 were randomised studies. The remaining 42 studies were retrospective studies with control arms. Included studies provided a total of 22 058 patients, of which 7328 (33%) received one of the therapies under review alongside standard of care (SOC) and 14 730 (67%) received SOC alone. Individual study characteristics for the published studies are presented in tables 1 and 2 and online supplemental tables S1 and S2.

Risk of bias assessment of the retrieved studies identified multiple limitations and highlighted a number of biases (figure 2 and online supplemental table S3). The majority of included studies defined the study population specifically with clear inclusion/exclusion criteria. Where applicable, control participants were selected from the same population. However, many studies provided insufficient detail of the interventions and outcomes being studied or reporting was inconsistent, with key design, and outcome details omitted. Statistical analysis was variably reported, with few studies providing a sample size justification. In nearly all studies, patients were on concomitant therapies, limiting the ability to discern whether a specific intervention was related to the outcome. Following a formal risk of bias assessment, 23 (32%) studies were rated as good, 37 (52%) fair and 11 (15%) poor. Publication bias, assessed by observation of funnel plots and Egger's test, was not present for any of the outcomes assessed (online supplemental figure S2).

Tocilizumab

Overall, 12 prospective studies with a control arm, eight prospective studies without a control arm, and 40 retrospective studies examining the clinical impact of tocilizumab in COVID-19 were identified. Among the prospective studies there were six randomised clinical trials (RCTs). In total, the studies reported outcomes from 20 972 patients, of whom 6563 (31%) were given tocilizumab. Criteria for eligible participants varied across the studies, with many specifying respiratory failure with laboratory evidence of hyperinflammation as a prerequisite. The dose of tocilizumab was not entirely consistent with intravenous 8 mg/kg or 400 mg the most commonly studied route and dose.

Ordinal Scale

A total of 12 studies provided outcomes on an adapted 4-point scale for 1782 patients including cases and controls (online supplemental table S4). The median time for reporting outcomes

Table 2 Treatment-related adverse events

Author, year	Therapy	Adverse effects
Balkhair *et al*, 2020[26]	Anakinra	Treatment: infection (11%), ALT rise (14%). Control: infection (18%), ALT rise (9%)
Huet *et al*, 2020[29]	Anakinra	Treatment: ALT rise (13%). Control: 9% in anakinra
Kooistra *et al*, 2020[35]	Anakinra	Treatment: secondary infection (33%). Control: secondary infection (23%)
*Kyriazopoulou *et al*, 2020[28]	Anakinra	Increased leucopenia in treatment group versus controls (8.5% vs 2.3%; p=0.05)
Cauchois *et al*, 2020[24]	Anakinra	N/R
Cavalli *et al*, 2020[25]	Anakinra	Treatment: *Staphylococcus* epidermis (14%), deranged liver enzymes (10%). Control: bacteraemia (13%), deranged liver enzymes (31%)
Narain *et al*, 2020[27]	Anakinra	N/R
Benucci *et al*, 2020[44]	Sarilumab	Nil
Della-Torre *et al*, 2020[30]	Sarilumab	Treatment: infections (21%), neutropenia (14%), liver enzyme increase (14%), thromboembolism (7%). Control: infections (18%), thromboembolism (7%)
*Gordon *et al*, 2021[20]	Sarilumab	No serious event in sarilumab group and 11 events in control
Gremese *et al*, 2020[51]	Sarilumab	Neutropenia (15%), elevated liver enzymes (11%)
Sinha *et al*, 2020[54]	Sarilumab or tocilizumab	Bacterial infection (13%)
*Gritti *et al*, 2020[31]	Siltuximab	Nil
Albertini *et al*, 2020[59]	Tocilizumab	Elevated liver enzymes (64%)
Antony *et al*, 2020[62]	Tocilizumab	N/R
Campins *et al*, 2020[65]	Tocilizumab	Nil
*Carvalho *et al*, 2020[68]	Tocilizumab	Nil
Chilimuri *et al*, 2020[55]	Tocilizumab	N/R
Dastan *et al*, 2020[71]	Tocilizumab	Transient diplopia (4.8%), Bell's palsy (2.4%)
*Gordon *et al*, 2021[20]	Tocilizumab	9 serious adverse events in tocilizumab group and 11 events in control
Hermine *et al*, 2020[23]	Tocilizumab	Treatment: serious adverse events occurred in 20 (32%). Control: 29 (43%) (p=0.21)
Lewis *et al*, 2020[37]	Tocilizumab	Increased infection rate in treatment group (aOR 4.18; 95% CI 2.72 to 6.52)
Malekzadeh *et al*, 2020[78]	Tocilizumab	Nil
Mikulska *et al*, 2020[81]	Tocilizumab	N/R
Morena *et al*, 2020[84]	Tocilizumab	Elevated liver enzymes (29%), thrombocytopenia (14%), neutropenia (6%), infections (24%)
Nasa *et al*, 2020[41]	Tocilizumab	Two patients (9.1%) developed deranged LFTs and two patients (9.1%) developed secondary sepsis
Perrone *et al*, 2020[87]	Tocilizumab	Allergic reactions (0.4%), deranged liver enzymes (10.5%)
*Petrak *et al*, 2020[46]	Tocilizumab	N/R
*Rosas *et al*, 2020[86]	Tocilizumab	66 serious infections (21%) were reported in the treatment arm and 49 (25.9%) in the placebo arm. Adverse events similar in both arms
Roumier *et al*, 2020[32]	Tocilizumab	Treatment: higher rates of neutropenia (35% vs 0%, p<0.001). Control: trend towards increased bacterial infections (22% vs 38%, p=0.089; including ventilator-acquired pneumonia: 8% vs 26%, p=0.022) and shorter time to infection (mean 18 vs 10 days, p=0.029)
Salama *et al*, 2020[22]	Tocilizumab	Serious adverse events occurred in 38 of 250 patients (15.2%) in the tocilizumab group and 25 of 127 patients (19.7%) in the placebo group
Salvarani *et al*, 2020[36]	Tocilizumab	Nil
*Sanchez-Montalva *et al*, 2020[38]	Tocilizumab	Nil
Sciascia *et al*, 2020[40]	Tocilizumab	Nil
Stone *et al*, 2020[21]	Tocilizumab	Neutropenia developed in 22 patients in the treatment group, as compared with only 1 patient in the placebo group (p=0.002), but serious infections occurred in fewer patients in the tocilizumab group (13 (8.1%)vs 14 (17.3%); p=0.03)
Strohbehn *et al*, 2020[42]	Tocilizumab	Treatment: bacterial infections (15.6%). Control: not reported
Toniati *et al*, 2020[45]	Tocilizumab	Septic shock (2%), gastrointestinal perforation (1%)
Biran *et al*, 2020[47]	Tocilizumab	Treatment: secondary bacterial infection in 17%. Control: secondary bacterial infection in 13%
Canziani *et al*, 2020[49]	Tocilizumab	HR 0.71 (95% CI 0.38 to 1.32) for infection, HR 0.89 (95% CI 0.39 to 2.06) for thrombosis, HR 1.17 (95% CI 0.47 to 2.92) for bleeding
Capra *et al*, 2020[52]	Tocilizumab	Nil
De Rossi *et al*, 2020[57]	Tocilizumab	Significant rise (from 44.3±28.3 to 103±141.3) in ALT in patients taking intravenous dose
Eimer *et al*, 2020[60]	Tocilizumab	Blood stream infection: 4 (18%) in treatment group versus 6 (27%) in control
Fisher *et al*, 2020[63]	Tocilizumab	No increased risk of secondary infection (OR 1.17; 95% CI 0.51 to 2.71)
Galván-Román *et al*, 2020[66]	Tocilizumab	N/R
*Moreno Garcia *et al*, 2020[69]	Tocilizumab	N/R
Gokhale *et al*,2020[72]	Tocilizumab	N/R
Guaraldi *et al*, 2020[74]	Tocilizumab	13% treated diagnosed with new infections versus 4% in control (p<0.0001)
Guisado-Vasco *et al*, 2020[76]	Tocilizumab	N/R
Gupta *et al*, 2020[79]	Tocilizumab	Treated and control patients experienced the following adverse events: secondary infection (140 (32.3%)vs 1085 (31.1%)), AST or ALT level elevation of more than 250 U/L (72 (16.6%)vs 452 (12.9%))
Hill *et al*, 2020[82]	Tocilizumab	In treatment group compared with control group, there was increased sepsis (21%vs16%), ALT rise (9% vs 4%) and thrombocytopenia (12% vs 4%)

Continued

Table 2 Continued

Author, year	Therapy	Adverse effects
Holt *et al*, 2020[85]	Tocilizumab	N/R
Ip *et al*, 2020[88]	Tocilizumab	N/R
Kewan *et al*, 2020[90]	Tocilizumab	Similar rates of hospital-acquired infections occurred in both cohorts (18% in treatment and 22% in control)
Kimmig *et al*, 2020[33]	Tocilizumab	Treatment associated with increased secondary bacterial (aOR 2.76; 95% CI 1.11 to 7.2) and fungal (5.6% vs 0%, p=0.112) infections
Klopfenstein *et al*, 2020[34]	Tocilizumab	N/R
Martinez-Sanz *et al*, 2020[39]	Tocilizumab	N/R
Narain *et al*, 2020[27]	Tocilizumab	N/R
Patel *et al*, 2020[43]	Tocilizumab	N/R
Pettit *et al*, 2020[48]	Tocilizumab	Overall infection rate was similar (16.2% treatment vs 17.5% control), but late onset infections occurred in more treated patients (23% vs 8%; p=0.013). In treated, 26% experienced an increase to >5 times upper limit normal of LFTs
Potere *et al*, 2020[50]	Tocilizumab	Nil
*Ramaswamy *et al*, 2020[53]	Tocilizumab	N/R
Rodríguez-Baño *et al*, 2020[56]	Tocilizumab	Secondary bacterial infection similar in both groups (treated 12.5% vs 10.3% control; p=0.57)
Rojas-Marte *et al*, 2020[58]	Tocilizumab	Bacteraemia was more common in the control group (24% vs 13%, p=0.43), while fungemia was similar for both (3% vs 4%, p=0.72)
Roomi *et al*, 2020[61]	Tocilizumab	N/R
Rosas *et al*, 2020[64]	Tocilizumab	Nil
Rossi *et al*, 2020[67]	Tocilizumab	N/R
Rossotti *et al*, 2020[70]	Tocilizumab	Infectious complication in 32.4%
Ruiz-Antorán *et al*, 2020[73]	Tocilizumab	32.6% in treated versus 30.3% in control had increase in liver enzymes. Bacteraemia in one patient (0.4%)
Somers *et al*, 2020[75]	Tocilizumab	Higher rate of superinfection in treated group (54% vs 26%; p<0.001)
Tian *et al*, 2020[77]	Tocilizumab	Deranged LFTs in 14% of tocilizumab and 14% of control group
Tsai *et al*, 2020[80]	Tocilizumab	N/R
*Wadud *et al*, 2020[83]	Tocilizumab	N/R
Zheng *et al*, 2020[86]	Tocilizumab	N/R

Adverse events for drug under study reported. Adverse events for control population reported where applicable.
*Non-peer-reviewed preprint study.
ALT, alanine transaminase; aOR, adjusted odds ratio; AST, aspartate transaminase; LFTs, liver function tests; N/R, not reported.

after treatment was 14 days (IQR 14–28). The recently available REMAP-CAP (Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia) adaptive RCT interim analysis reported a signal that tocilizumab was associated with clinical improvement at day 14 (adjusted OR (aOR) 1.83, 95% CI 1.40 to 2.41),[20] while in a separate RCT, outcomes on an ordinal severity scale did not differ between the treatment groups (HR 1.06, 95% CI 0.80 to 1.41).[21] Distinctions in statistical methodology and clinical endpoints precluded inclusion of this RCT in the specified meta-analysis. Tocilizumab was not associated with better outcomes on the Ordinal Scale in meta-analysis of the remaining prospective studies, including three RCTs (GenOR 1.09, 95% CI 0.99 to 1.19, I^2=84.3%) (figure 3). Variability in reported concomitant steroid administration had a significant contribution on the substantial heterogeneity observed (online supplemental table S5). Tocilizumab was associated with better outcomes in meta-analysis of retrospective studies, indicating a 34% greater chance of less-severe outcomes on the adapted Ordinal Scale when compared with control (GenOR 1.34, 95% CI 1.10 to 1.64, I^2=98%). However, these results should be interpreted with caution as there was severe heterogeneity which could not be explained by variability in the factors assessed.

Duration of hospitalisation

Two RCTs and nine retrospective studies reported the duration of hospitalisation for a total of 1553 survivors who received tocilizumab (figure 4). Individual RCTs comparing the duration of hospitalisation with controls identified associations of tocilizumab with a reduced hospital stay (−0.34 days, 95% CI −0.55 to −0.12)[22] and earlier hospital discharge (aHR 1.41, 95% CI 1.18 to 1.70).[20] Retrospective studies reporting the duration of hospitalisation were combined to give an overall summary estimate (20.98 days, 95% CI 16.19 to 25.78, I^2=97.1%), which was greater than the duration reported by RCTs (14.55 days, 95% CI −0.37 to 29.67, I^2=99.9%). Compared with 943 patients in retrospective studies who received SOC only, tocilizumab was not associated with a difference in the mean duration of hospital stay (0.36 days, 95% CI −0.07 to 0.80, I^2=93.8%), with variability in route of administration (intravenous or subcutaneous) associated with the severe heterogeneity in this estimate (R^2=81.64%, p<0.001).

Overall mortality

Twenty-two studies totalling 13 702 patients reported adjusted HRs for overall mortality, at a follow-up time censored at a median of 28 days (IQR 14–30). Among the studies, two were RCTs and neither reported a difference between tocilizumab and control for mortality.[21 23] When prospective tocilizumab studies were pooled, there was an emerging survival benefit, but the estimate was inconclusive (HR 0.70, 95% CI 0.44 to 1.10, I^2=0%) (figure 5). In the remaining retrospective studies, tocilizumab was associated with a 48% lower risk of adjusted mortality with substantial heterogeneity (HR 0.52, 95% CI 0.41 to 0.66, I^2=76.6%). Meta-regression identified the day of outcome measurement as a significant source of heterogeneity (R^2=99.99, p=0.08).

Risk ratios (RRs) were calculated from 42 studies, including 6 RCTs, reporting unadjusted mortality data for 15 085 patients at a median follow-up of 24 days (IQR 14–28) (figure 6).

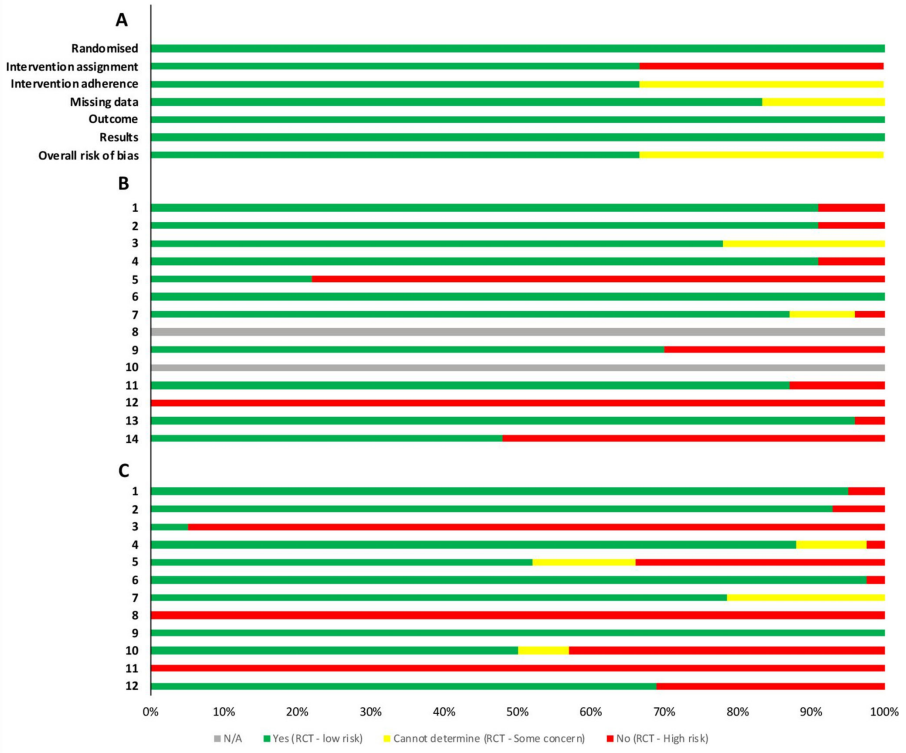

Figure 2 - Summary of risk of bias assessment. (A) Randomised clinical trials assessed using Cochrane risk of bias 2 tool (n=6). Risk of bias was assessed in six categories and scored as either low risk of bias, some concern, or high risk of bias, before an overall risk of bias was given to each study. (B) Non-randomised prospective studies (n=23). Questions numbered in the first column. 1. Was the research question or objective in this paper clearly stated? 2. Was the study population clearly specified and defined? 3. Was the participation rate of eligible persons at least 50%? 4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants? 5. Was a sample size justification, power description, or variance and effect estimates provided? 6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured? 7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed? 8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)? 9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? 10. Was the exposure(s) assessed more than once over time? 11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? 12. Were the outcome assessors blinded to the exposure status of participants? 13. Was loss to follow-up after baseline 20% or less? 14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)? (C) Summary of risk of bias assessment for retrospective studies (n=42). Questions numbered in first column. 1. Was the research question or objective in this paper clearly stated and appropriate? 2. Was the study population clearly specified and defined? 3. Did the authors include a sample size justification? 4. Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)? 5. Were the definitions, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants? 6. Were the cases clearly defined and differentiated from controls? 7. If less than 100 percent of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible? 8. Was there use of concurrent controls? 9. Were the investigators able to confirm that the exposure/risk occurred prior to the development of the condition or event that defined a participant as a case? 10. Were the measures of exposure/risk clearly defined, valid, reliable, and implemented consistently (including the same time period) across all study participants? 11. Were the assessors of exposure/risk blinded to the case or control status of participants? 12. Were key potential confounding variables measured and adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis?

Tocilizumab was associated with a 17% lower unadjusted risk of mortality compared with the control arm in prospective studies (RR 0.83, 95% CI 0.72 to 0.96, I^2=0.0%), which did not reach significance in RCTs alone (RR 0.85, 95% CI 0.71 to 1.01 I^2=0.0%) (online supplemental figure S3). Within retrospective studies, tocilizumab was associated with a 24% lower risk of

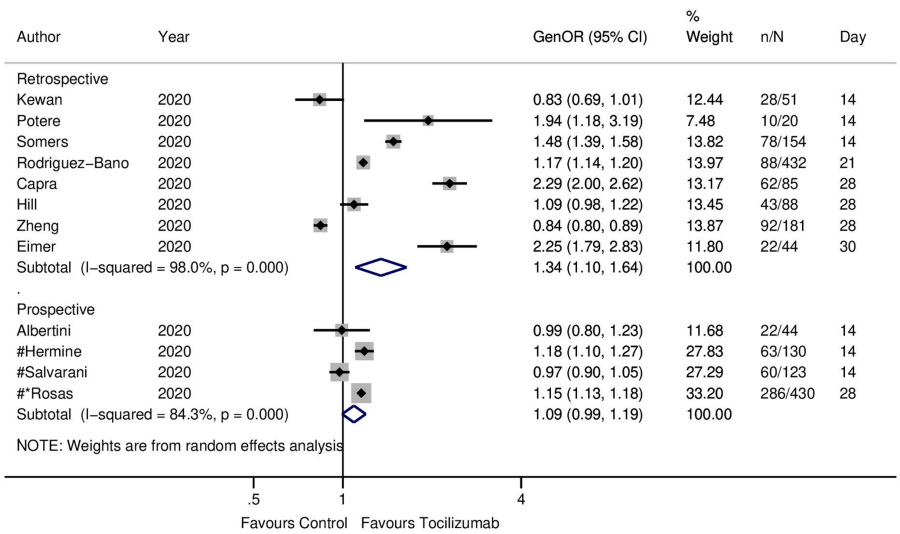

Figure 3 Tocilizumab generalised ORs for ordinal outcome forest plot. GenOR shown for each study with 95% CI and day at which ordinal outcome was recorded. Sample sizes given for patients receiving intervention (n) alongside total patients included (N) in the study. Summary estimates presented separately for prospective and retrospective studies. *Non-peer-reviewed preprint studies. #Randomised controlled trials. GenORs, generalised ORs.

mortality (RR 0.76, 95% CI 0.64 to 0.92, I^2=80.3%), although there was substantial heterogeneity which could not be explained by variability in the factors assessed. The combined case fatality (CFR) across all studies included in the meta-analysis was 21.2% (1118/5284) in the intervention arm and 31.1% (3049/9801) in the control arm. The CFR from single-arm prospective studies unable to be included in meta-analysis was 17.8% (113/634).

Other immunomodulators

Studies exploring outcomes in patients who received anakinra, sarilumab or siltuximab were not quantitatively synthesised for all outcomes, owing to differences in outcomes reported, study design and limited study numbers. Similar to studies in tocilizumab, participant criteria were inconsistent but typically included patients with respiratory failure and signs of hyperinflammation. Doses of therapeutic agents ranged from 200 to 600 mg daily for anakinra and 200–400 mg daily for sarilumab. In all studies, patients received concomitant medications including but not limited to antivirals, hydroxychloroquine and corticosteroids. Meta-analysis inclusive of all immunomodulatory agents without subanalysis is presented in online supplemental figures S4–S7.

Anakinra

Four prospective and three retrospective studies exploring outcomes in 346 patients who received anakinra and 3339 controls were retrieved. Three studies reported ordinal outcome data for both anakinra and control participants, although the outcome day varied. Anakinra was associated with improved clinical outcomes in two retrospective studies of 22 and 45 patients, respectively.[24 25] A similar association with improved clinical outcomes was reported on day 14 in a prospective study of 69 patients (GenOR 1.77, 95% CI 1.52 to 2.06).[26] Two studies reported adjusted HR for mortality with supportive results. A significant association was not observed in a retrospective study of 57 treated patients (aHR 0.79, 95% CI 0.44 to 1.42),[27] while an association was observed in a prospective study of 130 patients (aHR 0.49, 95% CI 0.26 to 0.91).[28] A significant unadjusted association was also observed in a further study of 52 patients treated with anakinra (HR 0.30, 95% CI 0.12 to 0.71).[29] RRs were calculated from four studies totalling 424 participants. In a retrospective study of 29 treated patients, anakinra improved survival (RR 0.24, 95% CI 0.07 to 0.79); associations were inconclusive when prospective studies were pooled (RR 0.70, 95% CI 0.31 to 1.58, I^2=32.8%) (online supplemental figure S8). No studies compared the duration of hospitalisation between recipients and non-recipients of anakinra.

Sarilumab

Five prospective studies exploring outcomes in 389 participants who received sarilumab were included. In the only RCT identified, sarilumab was associated with increased survival (aOR 2.01, 95% CI 1.18 to 4.71), reduced duration of hospitalisation (aHR 1.60, 95% CI 1.17 to 2.40) and improved ordinal outcomes at day 14 (aOR 1.86, 95% CI 1.22 to 2.91).[20] In a further non-randomised study of 28 participants,[30] sarilumab was not significantly associated with mortality (aHR 0.36, 95% CI 0.08 to 1.68) and comparable effects were observed among treated and non-treated patients with respect to ordinal outcomes (GenOR 1.07, 95% CI 0.90 to 1.27) and duration of hospitalisation (mean difference 0.02, 95% CI −0.51 to 0.54). The combined CFR across the five included studies was 11% (43/389) for sarilumab, while in the only study reporting control mortality data the CFR was 35.8% (142/397).

Siltuximab

A single prospective cohort study of siltuximab studying outcomes in 60 patients was identified.[31] Neither ordinal outcome data nor duration of hospitalisation were reported, but the adjusted risk of mortality was reported to be significantly

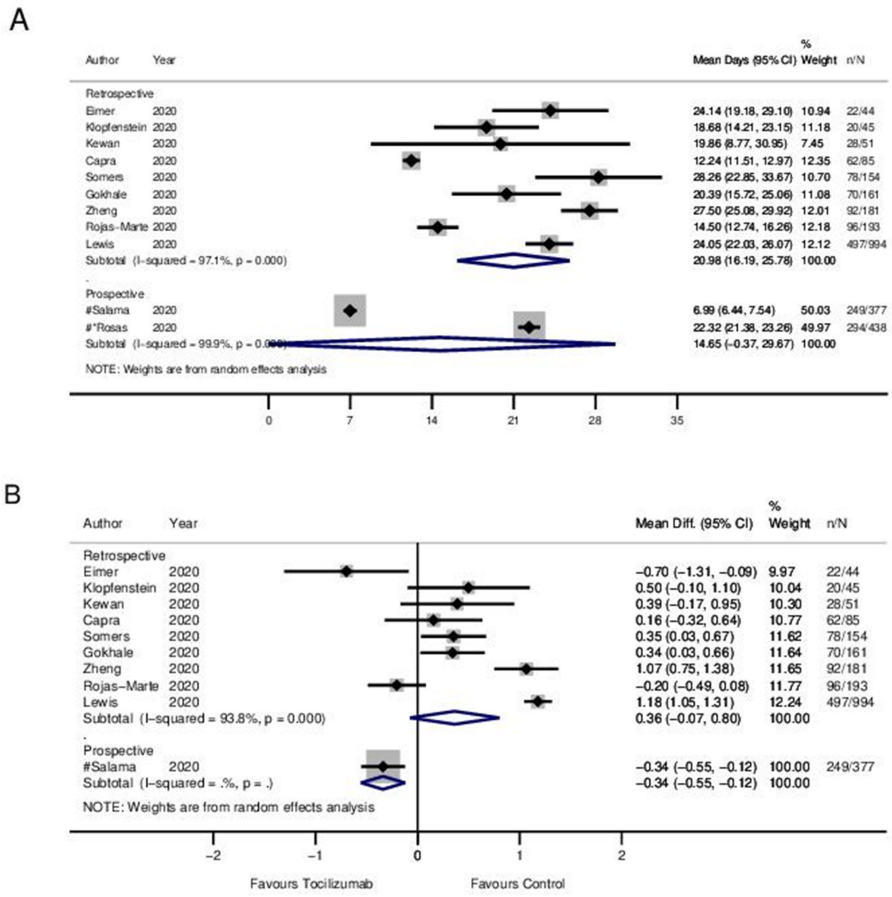

Figure 4 Tocilizumab duration of hospitalisation (days) forest plot. (A) Mean duration of hospital stay. (B) Mean difference compared with controls in duration of hospital stay. Effect sizes and associated 95% CIs presented for each study. Sample sizes given for patients receiving intervention (n) and total patients included in the study (N). Summary estimates presented separately for prospective and retrospective studies.

lower in patients who received siltuximab (aHR 0.46, 95% CI 0.22 to 0.97).

Treatment-related adverse events

Treatment-related adverse events were reported in most studies (70%) and typically included secondary bacterial infections and derangement of liver enzymes (table 2). In studies with a comparator arm exploring outcomes from patients who received anakinra or sarilumab, the frequency of treatment-related adverse events was similar in both treatment and comparator groups. Findings from studies reporting outcomes following tocilizumab administration were inconsistent. In five studies, tocilizumab recipients had an increased prevalence of secondary infections compared with controls. However, in 12 studies, tocilizumab was associated with a lower or similar rate of secondary infections compared with controls.

Clinical trials

Overall, 62 planned or in-process clinical trials (tocilizumab, 44; siltuximab, 4; sarilumab, 9; anakinra, 13) were identified through clinical registry searches, with some clinical trials exploring more than one immunomodulatory agent. Currently registered clinical trials and their estimated dates of completion are provided in online supplemental figure S9.

DISCUSSION

In this systematic review and meta-analysis, we summarised and evaluated the association between immunomodulatory agents and multiple outcomes in COVID-19. Although there was severe heterogeneity across tocilizumab studies exploring outcomes on an adapted 4-point Ordinal Scale, a beneficial effect of tocilizumab was suggested in retrospective studies compared with controls. Prospective studies followed a similar direction of association, though CIs were not conclusive. The certainty of the findings related to the adapted ordinal severity scale are assessed as moderate using GRADE (online supplemental table S6). The mean duration of hospitalisation was not altered by intervention, with low certainty of findings. Tocilizumab was associated with a survival benefit that was consistent across retrospective and prospective studies, with pooled analysis of unadjusted RRs demonstrating a 17% reduced risk of mortality in prospective

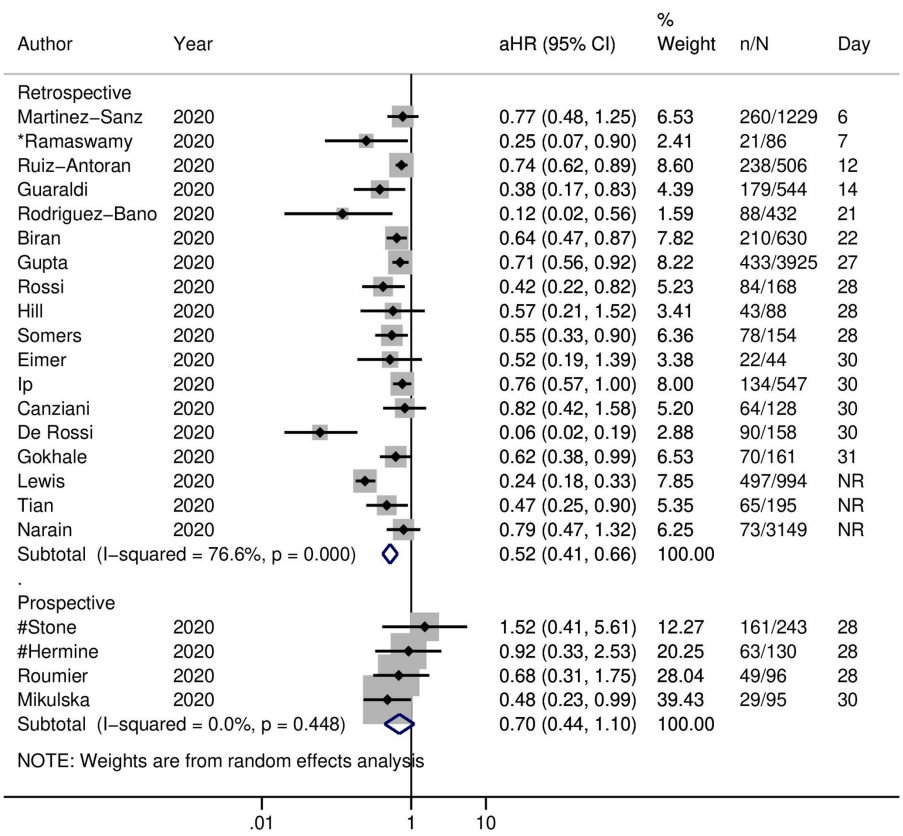

Figure 5 Tocilizumab adjusted HR for overall mortality forest plot. Adjusted HRs with associated 95% CI and day of censorship presented for each study. Sample sizes given for patients receiving intervention (n) and total patients included (N) in the study. Summary estimates presented separately for prospective and retrospective studies. *Non-peer-reviewed preprint studies. #Randomised controlled trials. aHR, adjusted HR; NR, not reported.

studies. We assess the certainty of our findings related to overall mortality as high.

Due to heterogeneity in study designs and reported outcomes, studies in patients receiving immunomodulatory agents other than tocilizumab were not quantitatively synthesised for all outcomes. In the only study reporting adjusted HRs, anakinra was associated with reduced mortality. However, pooled analysis of unadjusted ratios in non-randomised studies did not demonstrate a mortality benefit. A single sarilumab RCT demonstrated that intervention was associated with improved outcomes and reduced hospital stay. No randomised studies were identified for siltuximab. For all agents included in this review, the frequency of adverse events was similar in the treatment and control arms. Sixty-one registered clinical trials exploring immunomodulatory agents in COVID-19 were identified, of which some have been completed and published.

In this review, we highlight multiple limitations and considerable sources of interstudy heterogeneity. The majority of included studies were non-randomised cohorts of relatively modest size. Although most studies necessitated respiratory failure requiring at least basic respiratory support, participant criteria were not entirely consistent across the studies. The dosage and delivery of therapy varied across many of the non-randomised studies, and in nearly all studies patients were on concomitant medications such as antivirals, hydroxychloroquine and steroids with administration at the discretion of the treating physician, precluding causal associations of specific IL inhibitors with outcomes. Study outcomes were heterogeneous and a combination of clinical, laboratory and radiological outcomes was reported, rather than a single consistent endpoint. Furthermore, there was inconsistency in the duration of follow-up and timing of reported outcomes. Individual patient data (IPD) may have mitigated some of these limitations, but in a rapidly progressing area, seeking IPD was deemed to be unrealistic due to the associated delays. We also observed significant statistical heterogeneity as measured by I^2, and therefore the findings of our meta-analysis should be interpreted with caution. We were unable to explain all the residual heterogeneity using the factors we assessed, although concomitant steroid use, route of drug administration and the day the outcome was measured appeared to contribute within specific outcomes.

To maximise value and timeliness of our review of four specific immunomodulators, two primary endpoints and a number of secondary endpoints, we included both retrospective and preprint studies. Risk of bias was minimised by restricting analysis of non-prospective studies to those with a control group,

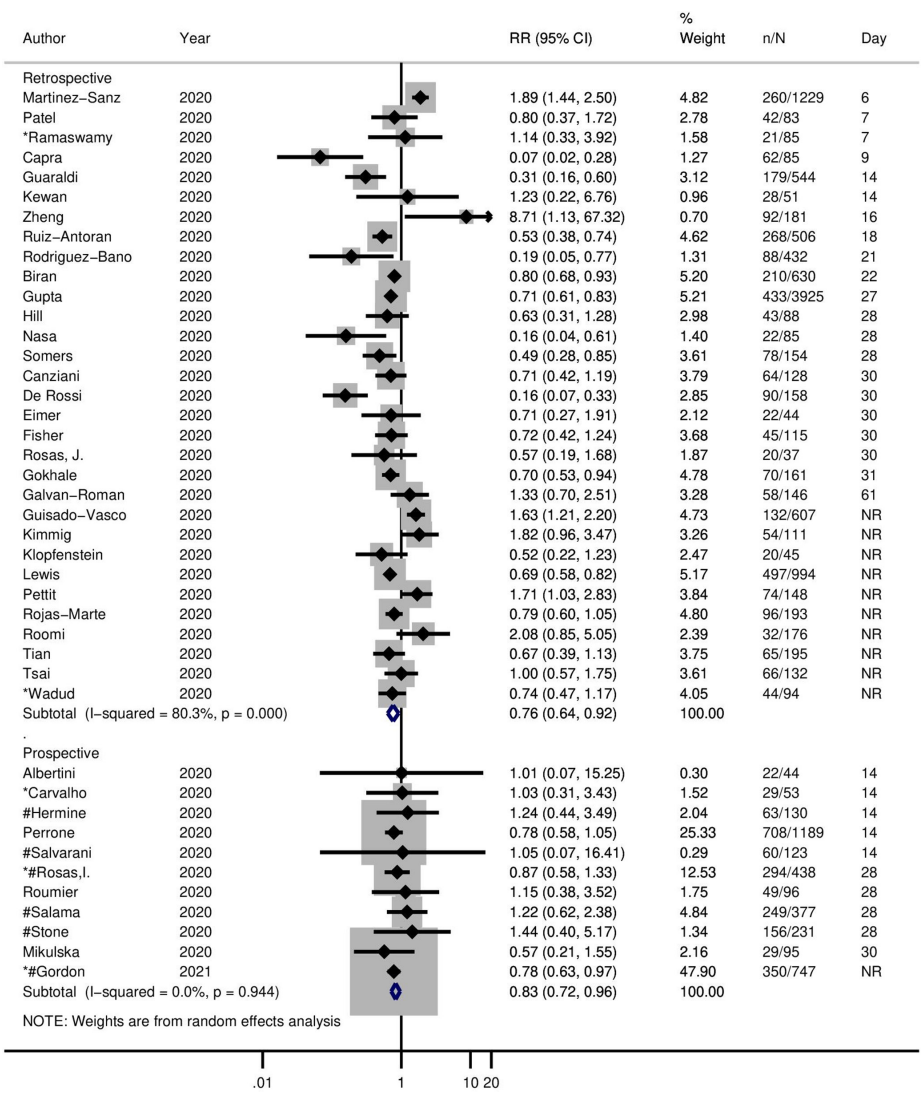

Figure 6 Tocilizumab mortality risk ratios (RRs) forest plot. RRs with associated 95% CI and day of censorship presented for each study. Sample sizes given for patients receiving intervention (n) and total patients included in the study (N). Summary estimates presented separately for prospective and retrospective studies. *Non-peer-reviewed preprint studies. #Randomised controlled trials. NR, not reported.

and caution is used to present summaries separately. We did not detect any significant publication bias in the reporting of effects. Where there was insufficient data for meta-analysis, summary outcomes were presented with qualitative synthesis to ensure the review was comprehensive. The data presented here represent findings from different countries, offering diversity in ethnic background. We were unable to identify suitable studies in SARS or MERS to comment on the generalisability of immunomodulators in other coronavirus outbreaks.

In conclusion, this systematic review provides the most up-to-date and complete evidence for a range of specific immunomodulatory therapies in the management of COVID-19. We have established that evidence for the efficacy of anakinra, siltuximab or sarilumab in COVID-19 is currently insufficient and adequately powered high-quality randomised clinical studies are urgently needed. We demonstrate through quantitative synthesis of retrospective studies that tocilizumab intervention was frequently associated with improved outcomes and reduced mortality. However, data were highly heterogeneous and must be interpreted with caution. Prospective studies demonstrated a 17% lower unadjusted risk of mortality with tocilizumab, with minimal heterogeneity and similar adjusted estimates. Further research should focus on identifying participant and disease characteristics where immunomodulatory therapy is likely to be of maximal effectiveness, while also exploring the relationship with baseline inflammatory biomarkers such as IL-6 and

C reactive protein. In summary, we demonstrate tocilizumab is associated with lower mortality in COVID-19 and other immunomodulatory therapies are worth exploring further.

Contributors FAK, IS and GJ conceived the study. FAK drafted the manuscript, performed the searches and collected the data. LF and SM verified the searches and extracted data. Analysis was performed by FAK and IS. All authors edited and approved the final version before submission.

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Competing interests GJ received grants from GlaxoSmithKline, during the conduct of the study, Astra Zeneca, Biogen and Galecto; personal fees from Boehringer Ingelheim, Daewoong, Galapagos, Heptares, Promedior and Roche; non-financial support from NuMedii and Redx; grants and personal fees from Pliant and other supports from Action for Pulmonary Fibrosis, outside the submitted work.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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10.10.3 The Its Not JUST Idiopathic Pulmonary Fibrosis Study (INJUSTIS: description of the protocol for a multicentre prospective observational cohort study identifying biomarkers of progressive fibrotic lung disease – Khan et al (BMJORR 2019)

BMJ Open Respiratory Research

The Its Not JUST Idiopathic pulmonary fibrosis Study (INJUSTIS): description of the protocol for a multicentre prospective observational cohort study identifying biomarkers of progressive fibrotic lung disease

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ABSTRACT

Introduction The Its Not JUST Idiopathic pulmonary fibrosis Study (INJUSTIS) is a multicentre, prospective, observational cohort study. The aims of this study are to identify genetic, serum and other biomarkers that may identify specific molecular mechanisms, reflecting disease endotypes that are shared among patients with progressive pulmonary fibrosis regardless of aetiology. Furthermore, it is anticipated that these biomarkers will help predict fibrotic activity that may identify patterns of disease behaviour with greater accuracy than current clinical phenotyping.

Methods and analysis 200 participants with the multidisciplinary team confirmed fibrotic lung disease (50 each of rheumatoid-interstitial lung disease (ILD), asbestosis, chronic hypersensitivity pneumonitis and unclassifiable ILD) and 50 idiopathic pulmonary fibrosis participants, recruited as positive controls, will be followed up for 2 years. Participants will have blood samples, lung function tests, quality of life questionnaires and a subgroup will be offered bronchoscopy. Participants will also be given the option of undertaking blinded home handheld spirometry for the first 3 months of the study. The primary end point will be identification of a biomarker that predicts disease progression, defined as 10% relative change in forced vital capacity (FVC) or death at 12 months.

Ethics and dissemination The trial has received ethical approval from the National Research Ethics Committee Nottingham (18/EM/0139). All participants must provide written informed consent. The trial will be overseen by the INJUSTIS steering group that will include a patient representative, and an independent chairperson. The results from this study will be submitted for publication in peer-reviewed journals and disseminated at regional and national conferences.

Trial registration number NCT03670576.

INTRODUCTION

Interstitial lung diseases (ILDs) are a group of immunoinflammatory and fibrotic diseases of the lung parenchyma. In a substantial number of patients there is progressive fibrosis of the alveoli and interstitium that leads to increasing disability and ultimately the death of patients with these diseases. Establishing the aetiology of these fibrotic lung diseases is often a clinical challenge and the relevance of aetiology to disease behaviour remains controversial. The best characterised fibrotic ILD is idiopathic pulmonary fibrosis (IPF), which has a median survival of 3 years, and 5-year survival of 25%, which is worse than most cancers.[1] Other conditions characterised by progressive pulmonary fibrosis include asbestosis, chronic hypersensitivity pneumonitis (HP), rheumatoid arthritis-associated ILD (RA-ILD), where the aetiology is assumed, and unclassifiable ILD where the clinical phenotype does not precisely reflect IPF.[2] The progression of these related conditions is also remorseless, and their genetic predisposition similar to IPF, raising the possibility of shared targetable mechanisms across disease phenotypes regardless of likely aetiology.

Recently two drugs, pirfenidone[3 4] and nintedanib,[5] have been approved for the treatment of IPF. While these drugs are described as 'anti-fibrotic', they can only be prescribed for IPF, rather than all forms of progressive fibrotic disease.[6] Therefore, patients with pulmonary fibrosis where the aetiology has been assumed, or the clinical features aren't specific for IPF, cannot receive antifibrotic therapy at the current time. However, the risk factors and molecular pathways driving fibrosis in aetiologically defined or phenotypically unusual pulmonary fibrosis may be

similar to IPF, thus potentially resulting in a large number of patients not having access to life-prolonging therapy. Our understanding of IPF has improved significantly both in terms of biomarkers[7 8] and clinical end points.[9] However, there remains a significant gap in our understanding of non-IPF ILD, with currently no approved treatments or cure.

RA-ILD, seen in 5%–10% of patients with rheumatoid arthritis remains a significant life-limiting complication with mortality in excess of 10% compared with patients without ILD. Subclinical interstitial lung abnormalities (ILAs) are seen in 30%–50% but individual risk of progression to ILD is unknown.[10] Chronic HP diagnosis rests on history of antigen exposure and radiological appearance, which often has an overlap with other ILDs. Sometimes, there is no known antigen exposure, and more recent hypotheses suggest a combination of exposure in genetically predisposed individuals.[11 12] While acute HP has a good prognosis, chronic HP is a progressive disease lacking evidence-based treatments with current therapy relying on immunosuppression. Despite improvements in radiology and the advent of multidisciplinary teams (MDTs), unclassifiable ILD remains a significant burden of ILD in clinical practice and represents between 10% and 38% of all ILDs.[13 14] These patients present a diagnostic challenge and again no evidence-based treatments are available.

Recent studies have highlighted phenotypical and molecular similarities across a range of ILDs. For example, the minor allele frequency of the MUC5B promoter single-nucleotide polymorphism (SNP), widely associated with IPF,[15] is found with increasing frequency in patients with chronic HP.[16] Short telomeres have also been associated with RA-ILD[17] and chronic HP, resulting in a prognosis similar to patients with IPF.[16] Patients with RA-ILD and usual interstitial pneumonia pattern have radiological changes that are indistinguishable from IPF and the presence of traction change and honeycombing is associated with poor outcomes regardless of aetiology.

Together, these features suggest that there may be shared mechanisms in the progression of pulmonary fibrosis common among patients with lung fibrosis due to a number of aetiologies. To explore this hypothesis, the Its Not JUST Idiopathic pulmonary fibrosis Study (INJUSTIS) will recruit a clinical cohort comprising of ILD subgroups, to explore genetic, serum and clinical biomarkers that may distinguish progressive fibrosing lung disease phenotypes regardless of aetiology. This may then eventually enable therapeutics targeting specific mechanisms of disease rather than clinical phenotypes of disease.

METHODS AND ANALYSIS

Objectives

INJUSTIS is a prospective multicentre observational cohort study that will be managed through the Nottingham Respiratory Research Unit and funded by the National Institute of Health Research (NIHR) through the Nottingham Biomedical Research Centre and an NIHR professorship (RGJ).

The primary objective is to:

- Identify biomarkers that determine progressive fibrotic lung disease irrespective of aetiology.

The secondary objectives are to:

- Identify biomarkers that predict all-cause mortality.
- Identify biomarkers that predict changes in QoL scores.
- Identify biomarkers that predict the development of disease complications (respiratory failure and acute exacerbations).
- Investigate genetic association and epigenetic modifications which affect fibrotic disease severity and progression.
- Prospectively evaluate longitudinal disease behaviour in patients with non-IPF fibrotic lung diseases with a view to developing composite clinical end points for subsequent use in intervention studies.
- Explore association of environmental exposures with disease progression and all-cause mortality.
- Investigate whether home handheld spirometry over 3 months predicts disease progression and survival.

The primary end point will be:

- Disease progression defined as relative forced vital capacity decline ≥10% or death within 12 months.

Secondary end points are:

- All-cause mortality at time of censoring.
- Number of acute exacerbations over 2 years.
- Change in Quality of Life (QoL) Questionnaire Scores from baseline to 12 weeks.
- Rate of change in biomarker activity from baseline to 12 weeks.
- Change in diffusing capacity of the lung for carbon monoxide (DLco) from baseline to 12 months.
- Change in 6 min walk distance from baseline to 12 months.
- Change in transcriptomic profiles from baseline to 12 weeks.
- Change in home handheld spirometry values from baseline to 12 weeks.

Selection of participants

Two hundred participants with recently diagnosed (within 18 months of study start date) fibrotic lung disease (50 each of rheumatoid-ILD, asbestosis, chronic HP and unclassifiable ILD) and 50 IPF participants as positive controls will be recruited from ILD clinics locally and across the UK. Only participants with MDT confirmed diagnosis of fibrotic ILD with radiological evidence of parenchymal lung fibrosis evidenced by reticulation and traction bronchiectasis, with or without honeycomb change will be recruited. Those with inflammatory radiological changes without evidence of fibrosis will not be deemed suitable regardless of clinical phenotype.

Recruitment will be reviewed on an ongoing basis and should rates fall below the expected levels, additional National Health Service (NHS) sites within the UK will be considered for participation. Eligible patients who meet the inclusion/exclusion criteria will be invited to consent. Most participants will be identified through outpatient clinics, but recruitment will not be restricted to this route. It will be explained to participants that entry into the study is entirely voluntary and that further treatment and care will not be affected by a decision to not partake. It will be clearly stated that participants are free to withdraw from the trial at any time. All participants will provide written informed consent, which will be countersigned by a member of the study team.

Inclusion criteria:

- Male or female aged ≥18 years.
- Able and willing to give written informed consent.
- Recently diagnosed (defined as diagnostic CT scan or surgical lung biopsy (if applicable) within 18 months of study start date).
- An MDT diagnosis of fibrotic ILD defined as the presence of traction change and reticulation with or without honeycombing within the lung parenchyma associated with either:
  - Rheumatoid arthritis (rheumatologist diagnosed with anti-cyclic citrullinated peptide antibodies and/or rheumatoid factor positive).
  - Asbestosis (appropriate occupational history and radiological evidence of asbestos exposure).
  - Chronic HP in accordance with consensus criteria[11] (appropriate exposure history, radiological features ± avian and fungal precipitins).
  - Unclassifiable fibrotic lung disease (fibrotic lung disease otherwise unclassifiable despite extensive clinical and radiological examination).
  - IPF in accordance with consensus criteria (American Thoracic Society (ATS), European Respiratory Society (ERS), Japanese Respiratory Society (JRS, Latin American Thoracic Society (ALAT) guidelines).[18 19]

Exclusion criteria:

- Participating in an interventional clinic trial.
- Asymptomatic ILAs and normal lung function.
- Change in clinical phenotype from initial radiological diagnosis to screening.
- Any connective tissue disease other than rheumatoid arthritis.
- Acute HP.
- Participants who do not possess a smartphone cannot partake in the domiciliary spirometry.

Study regimen

Both cases and IPF controls will undertake the same investigations. Following informed consent, the following test results, previously carried out as part of the participant's usual NHS care, will be used for the purposes of the study:

- HRCT findings.
- Blood results.
- Lung function tests.
- Bronchoscopy samples if already taken.

All participants will have baseline investigations at the first visit having provided informed consent. At the first visit, 40 mL of blood will be obtained, full lung function tests and a 6 min walk test will be performed. Participants will also be asked to complete QoL Questionnaires (Medical Research Council Dyspnoea Scale,[20] Leicester Cough Questionnaire,[21] IPF-abridged Profile for Assessment and Referral to Care,[22] King's Brief ILD Questionnaire[23] and EQ-5D-5L).[24] If consent is given for optional bronchoscopy, this will be subsequently performed and bronchoalveolar lavage carried out. Participants with a smartphone will be given the option of a home handheld spirometer and asked to provide daily FVC readings for the first 3 months of the study period.

Further visits at 3 months, 12 months and 24 months will include further 40 mL blood sampling, QoL and full lung function analysis. At 12 months and 24 months a 6 min walk test will be repeated (figure 1).

The majority of initial sample processing will be performed at the participant's local NHS hospital trust via the Clinical Research Network. Participation in the

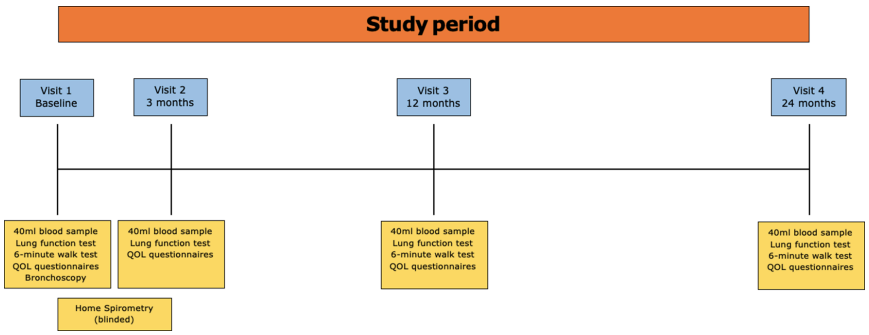

Figure 1 Legend – participant flow through the study.

study will be for 2 years. Other than initial assessment and follow-up visits at 3 months, 12 months and 24 months, there will be no further assessments. Only participants recruited at Nottingham University Hospitals NHS Trust will be offered a study bronchoscopy. It is not anticipated that any data will be obtained before completion of study that would lead to discontinuation of the study. For individual participants, discontinuation will be decided on an individual basis. The end of study will be defined as the last patient to complete the 2 years of follow-up.

Specimen processing and analysis

All human tissue (blood, lavage, biopsy, etc.) samples will be processed and stored in accordance with the Human Tissue Act (2004). All biological samples will be processed within 2 hours of being obtained according to local standard operating procedures and stored in 500 µl aliquots at −80°C until analysis. Sample analysis will take place either at the University of Nottingham or in the laboratories of third party national, or international, academic partners or contract research organisations following appropriate tissue transfer agreements.

The primary analysis will include measurement of epithelial biomarkers [matrix metalloproteinase-7 (MMP-7), cancer antigen 125 (CA-125), carbohydrate antigen 19-9 (CA19-9), surfactant protein D (SP-D)]) and markers of matrix turnover [C-reactive protein degraded by MMP-1/8 (CRPM), collagen 3 degraded by MMP-9 (C3M) and collagen 6 degraded by MMP-2/9 (C6M)) as well as genotyping for mucin 5B (*MUC5B),* desmoplakin (*DSP)* and A-kinase anchoring protein 13 (*AKAP13).* Exploratory analysis will include whole genome sequencing, RNA sequencing, proteomic and metabolomic analysis to identify novel biomarkers that predict fibrotic disease behaviour. Biopsy material will be used to culture cellular components, frozen for extraction of protein, RNA and DNA or formalin-fixed paraffin embedded.

Any additional samples will be archived for future genetic and biomarker studies in the University of Nottingham premises at the Nottingham City Hospital. The Human Tissues Authority license number is 12 265. Where participants do not agree to the future use of the samples they will be destroyed in accordance with the Human Tissue Act, 2004.

Details of spirometry

A secondary objective of this study is to determine whether change in daily home (domiciliary) handheld spirometry values over 3 months can predict disease progression and overall survival.

Participants wishing to take part in home spirometry will be provided with a portable handheld spirometer (MIR Spirobank Smart) linked to an electronic health journal (patientMpower smartphone application) on enrolment. Participants will be trained to undertake daily spirometry readings (one forced expiratory manoeuvre/day; seated) for the first 3 months of the study period. All spirometry readings will be blinded to participants and automatically uploaded to patientMpower via the smartphone application, ensuring full encryption throughout. Participants will therefore need to possess, and be confident in using, a smartphone device and have an email address. The spirometry data will then be transferred to the University of Nottingham for further analysis. Participants will be able to continue to use the spirometer and patientMpower application with open display of FVC readings after the initial 3-month observation period if they wish.

Statistics

The primary end point of disease progression (10% relative FVC decline or death within 12 months) will be used dichotomously across all subgroups collectively analysed together, with the exception of IPF controls. Association of baseline biomarker levels with dichotomous disease progression and overall survival will be analysed using binomial family of generalised linear models, while Gaussian family or otherwise appropriate models will be used to assess associations with continuous secondary end points. The Benjamini-Hochberg procedure will be applied to account for multiplicity as appropriate. Repeated measures mixed models will be used to assess associations over time, which includes time points within one model and circumvents correction for multiple testing. Fixed factors will include baseline demographic information. Where putative biomarkers are identified, end point data will be used to compare biomarker levels by time-to-event through proportional hazard models; comparable ability to predict end points will be assessed through sensitivity and specificity analyses (receiver operator characteristic curves).

To identify transcriptomic biomarkers, RNA-seq libraries will be prepared and entered into a workflow for read count normalisation enabling quantification of transcript expression; normalisation ensures length and abundance of cDNA reads are corrected according to other genes (reads per kilobase per million) and further library scaling can occur.[25 26] Libraries, aligned to an appropriate reference genome, will enable detection of differential gene expression and SNPs/variants according to primary end point and secondary measures of disease severity. Transcript-discovery artefacts, transcripts that remain below detectable levels of change across compared samples, as well as any with zero mapped reads will be excluded. R statistical packages will be used for these bioinformatics workflows.

Further statistical analyses will be performed to determine associations between identified biomarkers and exploratory secondary objectives. Statistical approaches include, but are not limited to, correlation and analyses of variance between biomarker levels and patient-reported QoL outcomes or disease exacerbations; machine learning strategies on home spirometry to detect decline in lung function, and subsequent comparison of sensitivity

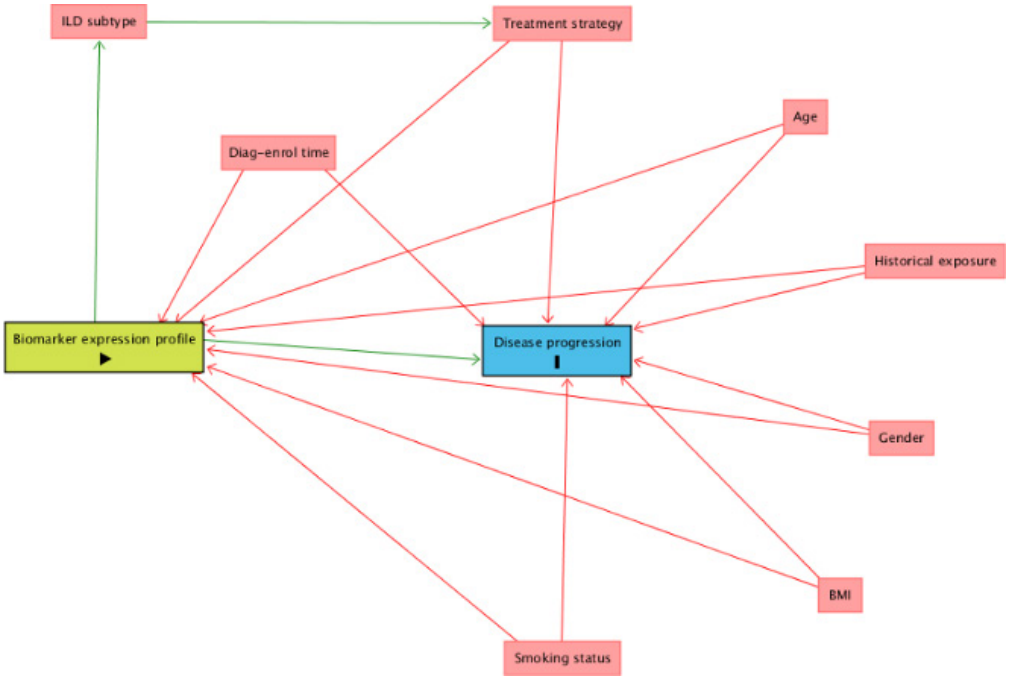

Figure 2 Directed acyclic graph (DAG) illustrates proposed model of causal inference and minimal sufficient adjustment sets (confounders; red) for estimating the direct effect of biomarker expression profiles (exposure; green) on disease progression (outcome; blue). Image generated using DAGitty.net environment. QoL, quality of life. BMI, body mass index; ILD, interstitial lung disease.

with traditional spirometry measures at predicting end points. Factor analysis approaches will be used to reduce dimensions of data sets and assess clustering; generalised linear models will be used to test associations.

Confounding factors are particularly important in studies that hope to offer causal inference of biomarkers on clinical outcomes,[27] and corrections to adjust for these will be applied in the analysis as proposed by the directed acyclic graph (figure 2).[28] Fixed factors in mixed models will include baseline demographic information: age, gender, body mass index and smoking status. Exposure history in patients with chronic HP will be collected as will time from diagnosis to enrolment across all subgroups and adjusted for as a potential confounder. Some participants may be on background therapies such as immunosuppression and corticosteroids. While these therapies are likely to be confounders, it is possible they may decrease or increase progression of fibrosis based on the PANTHER (Prednisone, Azathioprine, and *N*-Acetylcysteine: A Study That Evaluates Response in Idiopathic Pulmonary Fibrosis) Study.[29] Therefore, models will include treatment strategy as part of the minimal set of adjustment for estimating direct effect on end point. The objectives of the study do not extend to assessing treatment effect. Further unknown confounders will be identified from the spirometry smartphone application that will collect various data such as air quality index to inform limitations and future study.

Sample size justification

A power calculation has been conducted using prior data obtained at 3 months in the PROFILE (Prospective Observation of Fibrosis in the Lung Clinical Endpoints) study of patients with IPF.[8] The study demonstrated that 90 stable and 90 progressive individuals were sufficient to detect a twofold change in MMP7 with >90% power and 5% type I error rate. MMP7 was selected for power calculation as the most conservative of the biomarkers identified in the PROFILE Study, with the lowest threshold for biomarker change and least statistical precision, thus powering on MMP7 ensures that other biomarkers will be analysed with adequate power. The calculation demonstrates that a sample of 100 participants with progressive fibrosis and 100 with stable fibrosis will be large enough to detect dynamic differences in biomarkers over 3 months with 80% power and a type I error rate of 5% (α=0.05). Therefore 200 participants with ILD will be recruited assuming 50% disease

progression. Fifty positive IPF controls will be recruited to take part in the study to benchmark progressive fibrotic lung disease but will not be included in the final analysis.

The study is also appropriately powered for genetic risk scores. An individual SNP with 25% minor allele frequency would have >70% power to detect an OR of 1.8 for stable versus progressive disease. The power for a risk score comprising multiple variants is expected to be greater. This assumes an additive genetic model, $p<0.05$, in 100 stable versus 100 progressive patients whereby with each additional marker added to the model the power is actually increased rather than reduced. The markers used will all be defined a priori based on data obtained from the PROFILE Study[7 8] but are likely to include MUC5B, DSP, AKAP13, SP-D, CA-125, CA19-9, MMP-7, CRPM and C6M.

Following the recruitment of 100 participants who complete 1 year in the study, an interim sample size re-estimation by an independent data monitoring committee will be conducted to determine the progression status between blinded subgroups. If the blinded progression status is approximately 50%, recruitment will continue as described. If however, any subgroups show a relatively stable phenotype they will be excluded from subsequent recruitment, after being unblinded to the data monitoring committee. To attain an adequately powered sample size with 50% progressive fibrosis, further recruitment will be enriched with participants from the progressive phenotypical subgroups relative to rate of progression in interim analysis. Those recruited from subsequently excluded subgroups will be removed from the primary analysis, as they will be sources of inconsistency. However, if all subgroups progress at a substantially lower rate than expected and conditional power calculations at interim analysis suggest futility then all subgroups will be included in an analysis that demonstrates the null hypothesis (fibrotic lung disease progress at a rate specific to aetiology) could not be rejected. The study is not statistically powered to detect differences between ILD subgroups, although exploratory analyses will be carried out to inform future studies and support replication studies.

Patient and public involvement

The Action for Pulmonary Fibrosis (APF)[30] charity have been consulted during the design of the study and will sit on the steering committee as patient representatives, which will inform study conduct and recruitment. All patient information material has been reviewed by patient representatives. Study findings will be communicated to participants, and the APF will also support the dissemination of the study's finding to patients with pulmonary fibrosis and their families.

ETHICS AND DISSEMINATION

Monitoring

Monitoring of study data will include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, backup and disaster recovery of any local databases and validation of data manipulation. Entries on case report forms (CRFs) will be verified by inspection against the source data. A sample of CRFs (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the study database will be checked. Where corrections are required, these will carry a full audit trail and justification.

The study coordinator, or where required, a nominated designee of the sponsor, shall carry out monitoring of study data as an ongoing activity Trial data and evidence of monitoring and systems audit will be made available for inspection by the research ethics committee as required.

Safety reporting

No significant safety concerns are anticipated in relation to any measurements carried out as part of this trial. For patients undertaking bronchoscopy, the possible risks are the same as described in the hospital information sheet given prior to the procedure. All adverse events will be recorded and closely monitored until resolution, stabilisation or until it has been that the study intervention is not the cause. The chief investigator shall be informed immediately of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners.

Trial monitoring and oversight

The trial will be overseen by the INJUSTIS steering group consisting of the chief investigator, centre manager, research officer, research fellow, statistician, patient representatives (APF) and an independent chairperson. This committee will meet every 4 months.

Interim analysis will be undertaken by an independent data monitoring committee that will comprise two clinicians with expertise in clinical trials in ILD and a statistician not directly involved in this study.

Dissemination

All data will be anonymised and grouped for presentation and publication. The results from this study will be publicised at regional and national conferences as well as being submitted for publication in open access peer-reviewed journals in accordance with UK Research Council policies. No participants will be identified in any publications that arise from this research.

CONCLUSION

The INJUSTIS is a prospective longitudinal study of non-idiopathic fibrotic ILD, that will identify biomarkers of progression of fibrotic lung disease regardless of aetiology should such biomarkers exist. However, this study is not powered to detect differences between fibrotic lung

diseases of specific aetiologies, although it may provide insights into specific fibrotic lung diseases for further investigation.

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Contributors GJ, GS, FK, LH, IS were involved in designing the study. FK wrote the manuscript and all authors reviewed it prior to submission. IS provided the sample size and statistical evaluation of the study design and is the trial statistician. CE was involved in writing the areas relevant to handheld spirometry and agrees with the content. SJ is the patient representative for the study.

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Competing interests CE is an employee and shareholder of patientMpower. RGJ reports grants from GlaxoSmithKline, UK Medical Research Council, Biogen, Galecto, MedImmune; as well as personal fees from Boehringer Ingelheim, Galapagos, GlaxoSmithKline, Heptares, MedImmune, Roche and Pulmatrix outside the submitted work; served as consultant for NuMedii and Pliant; a trustee for charities Action for Pulmonary Fibrosis and the British Thoracic Society.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

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10.10.4 Clinical Utility of Home versus Hospital Spirometry in Fibrotic ID: Evaluation Following INUSTIS Interim analysis – Khan et al (ATS 2022 In press)

Clinical Utility of Home versus Hospital Spirometry in Fibrotic ILD: Evaluation Following INJUSTIS Interim Analysis

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Domiciliary monitoring of physiological variables has become routine in many chronic conditions owing to technological advances(1). Restricted clinical capacity and patient safety during the coronavirus-disease 19 (COVID-19) pandemic have identified an urgent need to consider remote lung function monitoring of chronic respiratory disease(2). Home handheld spirometry enables repeated measurements, offering opportunities for real-time disease evaluation, without the risk of nosocomial infection.

Interstitial lung disease (ILD) encompasses a heterogeneous range of immuno-inflammatory and fibrotic diseases. Forced vital capacity (FVC) correlates with outcome in ILD and remains the most commonly used biomarker of disease progression(3), with clinical trials consistently adopting hospital FVC measurements as the primary endpoint(4-6). We assessed interim data from the multi-centre It's Not Just Idiopathic Pulmonary Fibrosis Study (INJUSTIS, NCT03670576)(7) to evaluate the clinical utility of home spirometry as an alternative to hospital spirometry in participants with fibrotic ILD.

Methods

The INJUSTIS study is an ongoing multi-centre prospective, observational cohort study aiming to identify blood and physiological biomarkers that may predict disease progression in a mixed cohort of participants with multi-disciplinary confirmed fibrotic ILD (unclassifiable, hypersensitivity pneumonitis, asbestosis, rheumatoid-associated ILD and IPF)(7). A subgroup of participants possessing a smartphone were offered a portable handheld spirometer (MIR Spirobank Smart) linked via Bluetooth to a smartphone

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application and asked to perform a single, blinded forced expiratory manoeuvre daily for at least three months. Hospital spirometry was collected according to international guidelines(8) at baseline and three months.

Home spirometry readings falling within the upper and lower percentile (1%/99%) of aggregated group data were excluded to limit effects of substandard blows. Baseline measurements were calculated as the mean of daily readings obtained during the first seven days. Three-month measurements were calculated as the mean of readings obtained between days 90 and 96.

Correlation and inter-observer reliability between home and hospital spirometry for corresponding timepoints were assessed using Pearson correlation and intra-class correlation coefficients in a two-way random effects model. Bland-Altman plots were generated to assess the number of measurements that were outside the 95% limits of agreement. Adherence was calculated as the number of days where a participant provided at least one reading divided by 105 days. Change in King's Brief Interstitial Lung Disease Health status questionnaire (K-BILD) over three-months was calculated according to adherence categories (<60%, 60%-80%, >80%). We assessed consistency of measures across each week of study, by calculating a weekly coefficient of variation where three or more daily values were provided. This was assessed in a generalised estimating equation population-averaged model with exchangeable correlation matrix and robust sandwich variance estimators. Association of diagnostic subgroup (IPF vs non-IPF), week and interaction of week and subgroup were estimated. All analyses were performed using Stata v.16 (StataCorp, College Station, TX, USA).

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Results

Eighty-two participants were included in analysis, of whom 23 had IPF (28%) and 59 had non-IPF ILD (72%). Forty-three participants had three-month data for both home and hospital spirometry, with 19 participants excluded due to missing hospital spirometry attributable to the Covid pandemic (Table 1). Median adherence to daily home spirometry for all participants was 81% (IQR 61-94%), increasing to 91% (IQR 79-97%) in those who completed three-months. Individuals with adherence lower than 80% reported increased symptoms between baseline and three-months as measured by decreases in K-BILD-scores for total, activities, and chest domains.

Of the total 6202 daily FVC measurements, values in the upper and lower percentiles (below 0.9L or above 5.4L) were excluded. High correlation was observed between home and hospital spirometry at baseline (r=0.89) and three-months (r=0.82) (Table 1). The intra-class correlation coefficients between hospital and home spirometry at baseline and three-months were 0.92 (95%CI 0.79-0.96) and 0.91(95%CI 0.82-0.95) respectively. Bland-Altman plots demonstrated more-than 90% of home spirometry values were within agreement limits of hospital values at both timepoints (Figure 1), although home values more frequently underestimated hospital values. There was no significant difference between baseline and three-month spirometry measurement whether measured by hospital or home spirometry.

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The median coefficient of variation (CoV) for all participants was 8.2% (IQR 5.6-12.1%). A slightly higher CoV was observed in the phenotypically more diverse and larger non-IPF ILD subgroup, although no significant association with CoV was observed in longitudinal analysis (coefficient 2.11, 95%CI -1.60;5.83, p=0.144) (Figure 1C). Overall, weekly CoV did not significantly change (-0.22, 95%CI -0.52;0.08, p=0.144), indicating that weekly averages reliably reflect daily values for comparison to a single time point of hospital spirometry. No interaction with ILD subgroup was observed at any week.

Discussion

Our findings in the largest prospective study of mixed fibrotic ILD support the clinical utility of home spirometry in the remote monitoring of patients. Although participants were blinded, adherence to daily spirometry remained high, and was similar to adherence rates in non-blinded studies(9). We stipulated the performance of daily measures rather than a minimum number of weekly blows,(9, 10) with reliable adherence in the three-month design. Home and hospital measurements were highly correlated at complementary time points, though home spirometry tended to underestimate measurements when compared with hospital spirometry(11). The mean difference at baseline was 0.25L lower with over 90% of measurements within agreement limits. Furthermore, although variability was observed, daily measures indicated minimal influence of time or disease and at 8.2% was comparable to the CoV in prior non-blinded studies (range 3.9-8.2%)(9, 11-14). A suggestive, non-significant reduction in variability over time may be attributable to learning and improved technique. Whilst we demonstrate comparability of measurements, we

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emphasise the importance of longitudinal modelling of daily spirometry for clinical endpoint precision.

Recent studies using home spirometry support feasibility in idiopathic pulmonary fibrosis (IPF) (9-12, 15), but fewer data exist regarding the acceptance of home spirometry in non-IPF ILD and its comparability to hospital spirometry(13, 14). In a single centre study of mixed fibrotic ILD, including 27 non-IPF patients, there was strong correlation between hospital and home spirometry at baseline, three-months, and six-months(14). Our results generated in a multi-centre study comprising a majority of subjects with non-IPF diagnoses demonstrate good agreement and inter-observer reliability between home and hospital measures of FVC in fibrotic ILD. We addressed potential bias associated with participant drop out due to falling spirometry values by asking participants to perform blinded readings in a prospective study design. Additionally, this is the first study in non-IPF ILD to explore adherence according to patient reported outcomes, describing worsening in symptoms where adherence was less than 80%.

Our study was limited by modest interim sample sizes and a restricted follow up due to interim censoring attributable to the COVID-19 pandemic. Participants were asked to perform a single reading without replication to minimise potential intrusiveness of multiple daily expiratory manoeuvres. Exclusion of participants without a smartphone may have enriched the cohort to be more competent in the use of home technology. Baseline hospital spirometry was obtained pragmatically as a standard of care and the acceptable timeframe from recruitment may have contributed to larger discrepancies with home spirometry at this time point compared with three-month research visits. We were unable to validate the

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quality of participant attempts as the handheld device did not record flow-volume loops. It is likely these factors would be compensated in longitudinal modelling of daily spirometry, whilst the intention here was to assess comparability to hospital spirometry when evaluated as a single value.

In summary, we demonstrate that blinded, daily home spirometry in fibrotic non-IPF ILD is feasible, reliable and within acceptable levels of agreement to hospital spirometry for clinical measurement. This is likely to be particularly relevant where clinical access or trial participation is limited due to geographical factors, patient choice, service pressures and future pandemics.

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Demographics	All	IPF	Non-IPF	Completed 3 months
Baseline, N	82	23	59	43
Male, n (%)	59 (72%)	19 (83%)	40 (68%)	34 (79%)
Mean age (sd)	69.8 (8.1)	70.7 (7.0)	69.4 (8.5)	70.1 (7.7)
FVC, litres (sd)	2.96 (0.88)	3.38 (0.90)	2.80 (0.82)	3.01 (0.90)
FVC, % predicted (sd)	80.6 (18.0)	85.0 (15.5)	78.9 (18.7)	80.7 (20.6)
DL_{CO}, % predicted (sd)	55.1 (16.2)	54.3 (14.6)	55.4 (16.9)	52.6 (16.4)
6MWD, m (sd)	332 (101)	354 (103)	324 (100)	330 (101)
Three months, n (%)	43 (52%)	12 (52%)	31 (53%)	43 (100%)
Median Adherence, % (IQR)	81% (61-94)	79% (53-93)	85% (61-95)	91% (79-97)
Mean change in KBILD scores	**All**	**Adherence <60%**	**Adherence 60-80%**	**Adherence >80-100%**
Total (sd)	0.08 (6.75)	-1.66 (7.31)	-0.98 (6.62)	1.25 (6.56)
Chest domain (sd)	1.61 (18.13)	0.39 (15.93)	-3.56 (15.93)	4.44 (19.71)
Activities domain (sd)	-1.92 (12.92)	-5.38 (13.78)	-1.63 (12.03)	-0.68 (13.11)
Psychological domain (sd)	1.12 (10.65)	-0.72 (11.33)	1.5 (12.08)	1.67 (9.95)

Table 1: Baseline demographics and mean change in Kings Brief Interstitial Lung Disease health related quality of life scores between baseline and 3 months visit in total and in individual domains. Adherence calculated as number of daily readings out of 105 days. Mean values presented with standard deviation (sd); median values presented with interquartile range (IQR).

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		Comparison			Agreement		Pearson correlation			Intra-class coefficient
FVC sample	N	*Mean Hosp. (SD)*	*Mean Home (SD)*	*Mean diff (SD)*	*n Outside limits*	*% Within limits*	*r*	*R2*	*P*	*Coefficient (95%CI)*
All										
Baseline	*82*	*2.96 (0.88)*	*2.71 (0.86)*	*-0.26 (0.41)*	*7*	*91.5*	*0.89*	*0.79*	*<0.0001*	*0.92 (0.75;0.96)*
3 months	*43*	*2.91 (0.93)*	*2.74 (0.90)*	*-0.17 (0.52)*	*1*	*97.7*	*0.84*	*0.70*	*<0.0001*	*0.91 (0.82;0.95)*
Δ 3 months	43	-0.103 (0.27)	-0.088 (0.44)	0.014 (0.49)	3	93.0	0.11	0.01	0.50	0.18 (-0.55;0.56)
Non-IPF ILD only										
Baseline	*59*	*2.80 (0.82)*	*2.57 (0.84)*	*-0.23 (0.39)*	*4*	*93.2*	*0.89*	*0.79*	*<0.0001*	*0.92 (0.80;0.96)*
3 months	*31*	*2.83 (0.99)*	*2.63 (0.91)*	*-0.20 (0.53)*	*0*	*100*	*0.85*	*0.72*	*<0.0001*	*0.91 (0.80;0.96)*
Δ 3 months	*31*	*-0.071 (0.23)*	*-0.082 (0.35)*	*0.012 (0.40)*	*2*	*93.5*	*0.07*	*0.01*	*0.70*	*0.13 (-0.86;0.59)*

Table 2: Comparison of FVC shown in litres after FVC <1st and >99th percentile excluded. Values shown for all patients, and for non-IPF ILD separately. Agreement after values plotted on Bland-Altman plot, with n the total number of participants with values outside limits. Correlation presented between hospital (hosp.) and home spirometry.

Figure 1:

A. Correlation of home and hospital FVC (litres) measurements at baseline and 3 months, coloured differently for IPF (n=23 at baseline; n=12at 3 months) and non-IPF (n=59 at baseline; n=31 at 3 months). Black reference line represents y=x.

B. Bland Altman plot for baseline and 3 months. Mean difference of hospital relative to home spirometry was 0.26L (SD 0.41) at baseline and 0.17L (SD 0.52) at 3 months. The red lines represent the 95% limits of agreement. Baseline measurements were calculated as the mean of daily readings

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obtained during the first seven days. Three-month measurements were calculated as the mean of readings obtained between days 90 and 96.

C. Weekly coefficient of variation (CoV) (%) in home spirometry across study time for ILD subtype. Blue and red lines represent estimated CoV (and 95% confidence intervals) in IPF and non-IPF group, respectively. Scatter points for observed individual participant weekly CoV. Number of participants included at each week (p-value for ILD subtype interaction): week 1, 76 (0.987); week 2, 72 (0.946); week 3, 73 (0.695); week 4, 69 (0.790); week 5, 70 (0.756); week 6, 69 (0.574); week 7, 68 (0.617); week 8, 65 (0.791); week 9, 63 (0.619); week 10, 59 (0.903); week 11, 58 (0.734); week 12, 58 (0.742); week 13, 55 (0.842); week 14, 52 (0.490); week 15, 46 (0.391). P values from generalised estimating equation shown for change in coefficient of variation per week, and ILD subtype (IPF and non-IPF).

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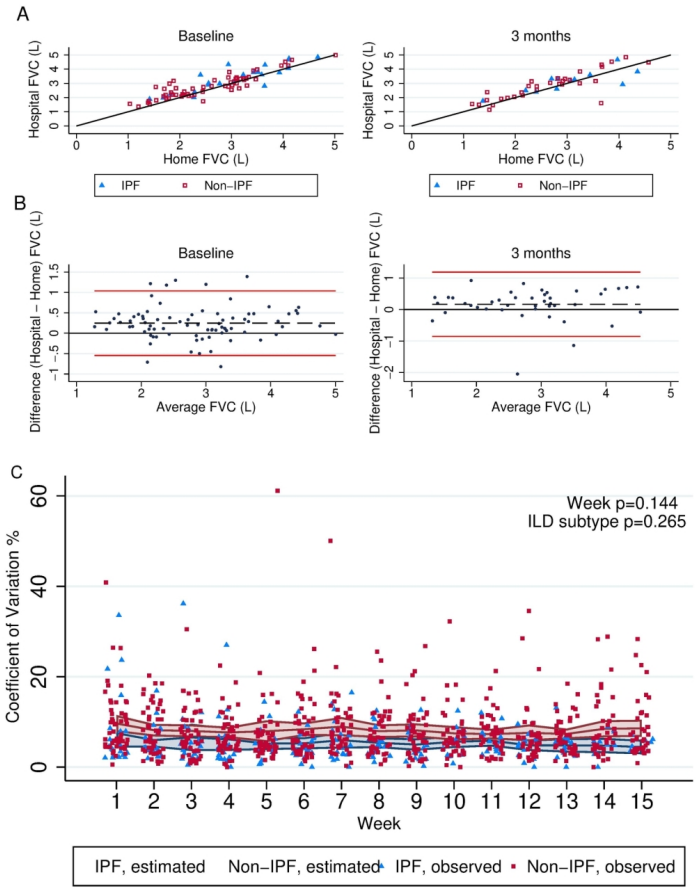

A. Correlation of home and hospital FVC (litres) measurements at baseline and 3 months, coloured differently for IPF (n=23 at baseline; n=12at 3 months) and non-IPF (n=59 at baseline; n=31 at 3 months). Black reference line represents y=x.

B. Bland Altman plot for baseline and 3 months. Mean difference of hospital relative to home spirometry was 0.26L (SD 0.41) at baseline and 0.17L (SD 0.52) at 3 months. The red lines represent the 95% limits of agreement. Baseline measurements were calculated as the mean of daily readings obtained during the first seven days. Three-month measurements were calculated as the mean of readings obtained between days 90 and 96.

C. Weekly coefficient of variation (CoV) (%) in home spirometry across study time for ILD subtype. Blue and red lines represent estimated CoV (and 95% confidence intervals) in IPF and non-IPF group, respectively. Scatter points for observed individual participant weekly CoV. Number of participants included at each week (p-value for ILD subtype interaction): week 1, 76 (0.987); week 2, 72 (0.946); week 3, 73 (0.695); week 4, 69 (0.790); week 5, 70 (0.756); week 6, 69 (0.574); week 7, 68 (0.617); week 8, 65 (0.791); week 9, 63 (0.619); week 10, 59 (0.903); week 11, 58 (0.734); week 12, 58 (0.742); week 13, 55 (0.842); week 14,

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139x177mm (300 x 300 DPI)

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