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Review: Serum biomarkers in idiopathic pulmonary fibrosis and systemic sclerosis associated interstitial lung disease – frontiers and horizons

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ABSTRACT

Disease behaviour in interstitial lung disease (ILD) is highly variable and accurate clinical tools to predict prognosis and guide management decisions remain unsatisfactorily elusive. Accurate disease stratification would allow clinicians to better distinguish patients at risk of rapid progression requiring urgent treatment, from those indolent disease where potentially toxic drug therapy could be minimised or avoided. Several serum biomarkers have demonstrated potential utility for diagnosis and prognosis of ILD in small retrospective studies, and the hope is future multicentre prospective trials focussed on the markers with most potential will see translation to clinical practice.

Two important and contrasting fibrotic lung diseases with high mortality are idiopathic pulmonary fibrosis (IPF) and systemic sclerosis associated ILD (SSc-ILD). In this era where anti-fibrotics for IPF have proven benefit, there are increasing biologic and non-biologic options for the treatment of connective tissue disease ILD (CTD-ILD), and the incidence of both is increasing, there is an urgent need to improve the diagnostic and prognostic accuracy in these complex patients.

This comprehensive literature review will summarise and discuss the current evidence for the major candidate serum biomarkers in IPF and SSc-ILD. Biomarkers will be categorised by the following major mechanistic pathways (1) alveolar epithelial cell damage; (2) aberrant fibrogenesis, fibroproliferation and matrix remodelling; (3) immune dysregulation; and (4) vascular and endothelial damage. The aim is to review the rationale, potential and limitations of current candidate biomarkers and their utility in IPF and SSc-ILD to help direct future research and translation to clinical practice.

Contents

Abbreviations: ADAM, a disintegrin and metalloproteinase; AEC, alveolar epithelial cell; BLys, B lymphocyte stimulator; Ca15.3, carbohydrate antigen 15.3; CC16, Clara Cell Protein 16; CCL, C-C motif chemokine ligand; CTD-ILD, connective tissue disease-associated interstitial lung disease; CTD, connective tissue disease; CXCL, C-X-C motif chemokine; DLCO, diffusing capacity; ECM, extracellular matrix; ET-1, Endothelin 1; FVC, forced vital capacity; HRCT, high resolution computed tomography; HSP, Heat shock protein; ICAM1, intercellular adhesion molecule 1; IIP, idiopathic interstitial pneumonia; IL, interleukin; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; KL-6, Krebs von den Lungen; MMP, matrix metalloproteinase; OPN, osteopontin; PM/DM, polymyositis/dermatomyositis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; sLOXL2, serum lysyl oxidase-like 2; SP, surfactant protein; sRAGE, soluble receptor for advanced glycosylated end products; SSc, systemic sclerosis; TIMP, tissue inhibitors of metalloproteinase; TLC, total lung capacity TLC; VCAM1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

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1. Introduction and background

Interstitial lung diseases (ILDs) are a diverse group of chronic lung disorders characterised by damage to lung tissue by inflammation and fibrosis. Diagnosis requires meticulous evaluation for an identifiable aetiology, particularly an underlying connective tissue disease (CTD). Distinguishing CTD-associated ILD (CTD-ILD) from "idiopathic interstitial pneumonia" (IIP), where no cause can be identified, has major implications for prognosis and management (Park et al., 2007; Raghu et al., 2011; Solomon, Chartrand, & Fischer, 2014; Vij & Strek, 2013). Disease behaviour is highly unpredictable and variable between, and even within, ILD subtypes, and no current measures can accurately and reproducibly detect early disease, predict future decline in lung function, identify individuals more likely to benefit from and response to therapy (Bonella & Costabel, 2014; Winstone et al., 2014).

The most common IIP is "idiopathic pulmonary fibrosis" (IPF), an invariably progressive and fatal disease with a median survival time from diagnosis of 2 to 5 years (ATS/ERS, 2002). On radiology and histopathology, IPF is characterised by a pattern of usual interstitial pneumonia (UIP). Age, gender, smoking history, body mass index, the presence of pulmonary hypertension or emphysema, pulmonary function and composite staging systems (eg. Gender-Age-Physiology [GAP] and Composite-Physiological-Index [CPI] scores) and have demonstrated utility predicting survival and outcomes in IPF, but do not accurately predict future rate of pulmonary function decline (Ley, Brown, & Collard, 2014; Salisbury et al., 2016). The incidence of IPF is increasing worldwide, and with approval of the first disease-modifying agents in IPF, anti-fibrotics pirfenidone and nintedanib, a robust clinical tool to predict future disease progression, guide treatment and monitor treatment response is urgently required (Navaratnam et al., 2011).

CTD-ILD is most commonly associated with systemic sclerosis (SSc; also known as scleroderma) but can occur in any CTD, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), idiopathic inflammatory myopathy including polymyositis/dermatomyositis (PM/DM), Sjögren's syndrome and mixed connective tissue disease (MCTD) (Fischer & du Bois, 2012). ILD usually develops in the setting of an established CTD, but can be the first and only presentation of the CTD (Castelino & Varga, 2010; Fischer & du Bois, 2012; Mittoo et al., 2009; Tzelepis, Toya, & Moutsopoulos, 2008). Up to 33% of SSc-related deaths may be attributed to pulmonary fibrosis, but there are no validated measures to accurately and safely predict disease progression (Steen & Medsger, 2007).

Autoantibodies (eg. anti-Scl70, anti-centromere and anti-RNA polymerase-III autoantibodies), correlate with the likelihood of developing SSc-ILD but do not predict lung function decline (Bahmer, Romagnoli, Girelli, Claussen, & Rabe, 2016). The ILD-GAP index (ILD subtype, gender, age and pulmonary function) enables risk prediction and survival estimates across a range of ILDs, and includes but is not specific to CTD-ILDs (Ryerson et al., 2014). Disease extent on high-resolution computed tomography (HRCT) at baseline combined with reduced or declining forced vital capacity (FVC) and diffusing capacity (DLCO) is predictive of mortality (Goh et al., 2008; Goh et al., 2017; Moore et al., 2013; Moore et al., 2015). However, radiation exposure limits routine repeated imaging, and neither HRCT nor pulmonary function alone correlate with disease progression (Goh et al., 2008; Moore et al., 2013). Shorter duration of ILD since SSc diagnosis may predict physiologic worsening but is affected by survivorship bias (Winstone et al., 2014). Histopathology and cytology from surgical lung biopsy or bronchoalveolar lavage (BAL) are invasive and do not correlate with disease progression (Winstone et al., 2014).

Thus, there is a current and critical need for reliable, safe and feasible markers that can accurately predict disease progression and therapeutic response, to enable more precise and personalised treatment of patients with ILD.

1.1. Role of biomarkers in ILD

Biomarkers are defined as "characteristics that are objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Biomarkers Definitions Working Group, 2001). In its broadest definition, biomarkers of respiratory disease may include any measure on imaging, lung function, genetic polymorphisms and biochemical molecules identifiable in lung tissue, BAL fluid and/or blood. Advances in next-generation sequencing techniques and our understanding of the complex pathogenic mechanisms underpinning fibrotic ILDs has sparked major interest identifying serum biomarkers for the following uses (Fig. 1): 1) Predisposition – identify the risk of a patient developing ILD or detect early disease; 2) Diagnostic – identify the presence of ILD and aide classification; 3) Prognostic – predict disease progression and clinical events (eg. exacerbations); 4) Therapeutic – predict and quantify response to therapy; and 5) Research/Clinical – act as a surrogate endpoint in research or clinical practice and identify novel treatment targets (Ley et al., 2014; Ray et al., 2010).

In IPF, despite advances in imaging technology and disease classification systems, diagnosis can remain elusive thus there is a need for more accurate, non-invasive diagnostic markers. The diagnosis of SSc-ILD is often more straightforward, nonetheless markers of early ILD remain lacking. And although distinct clinical entities, both IPF and SSc-ILD demonstrate highly variable disease courses and prognostic markers that could predict future disease behaviour to aide more personalised management decisions are urgently needed.

1.2. Pathophysiology and serum biomarkers

The pathogenesis of ILD is complex and not completely understood, but thought to result from aberrant fibro-proliferation, excess extracellular matrix (ECM) deposition, immune system activation and vascular and endothelial damage, triggered by alveolar epithelial cell injury (eg. from viral infection, tobacco smoking, occupational exposures) in genetically susceptible individuals (King Jr., Pardo, & Selman, 2011; Ley et al., 2014; Selman, King, & Pardo, 2001). Persistence of the abnormal wound healing leads to architectural distortion, organ dysfunction and eventual failure (Daccord & Maher, 2016; Ley et al., 2014). Complete discussion of the pathophysiology of pulmonary fibrosis is beyond the scope of this article but some understanding is essential when evaluating the validity of candidate biomarkers. Biomarkers themselves can improve our understanding of underlying biological mechanisms. Although very distinct clinical entities, idiopathic and CTD associated ILD demonstrate overlap in many of these core cellular pathways (Bagnato & Harari, 2015; Ley et al., 2014). Fig. 2 illustrates how current candidate biomarkers of ILD may fit into proposed cellular mechanisms.

Key cells in the pro-fibrotic process include myofibroblasts, alveolar epithelial cells (AECs), fibroblasts, immune cells and endothelial cells.

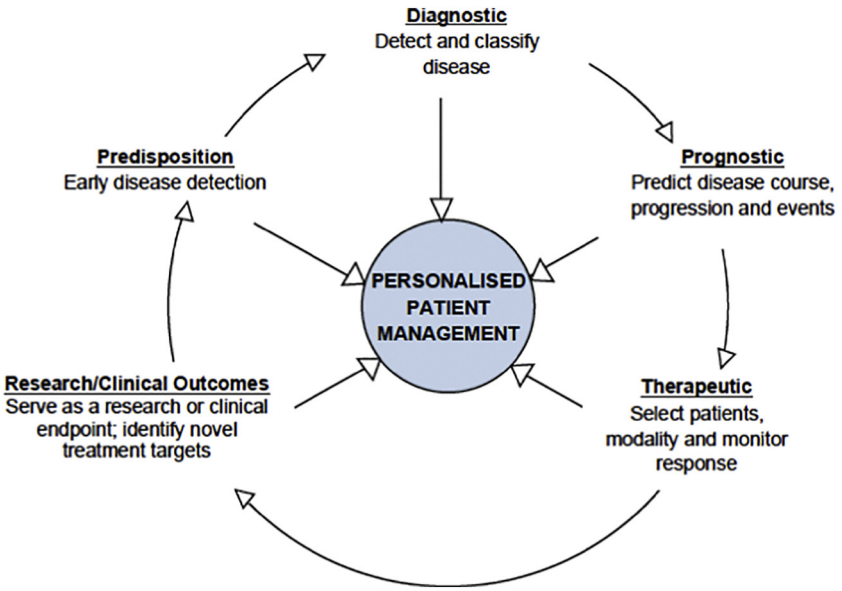

Fig. 1. Potential roles of biomarkers in ILD patient management.

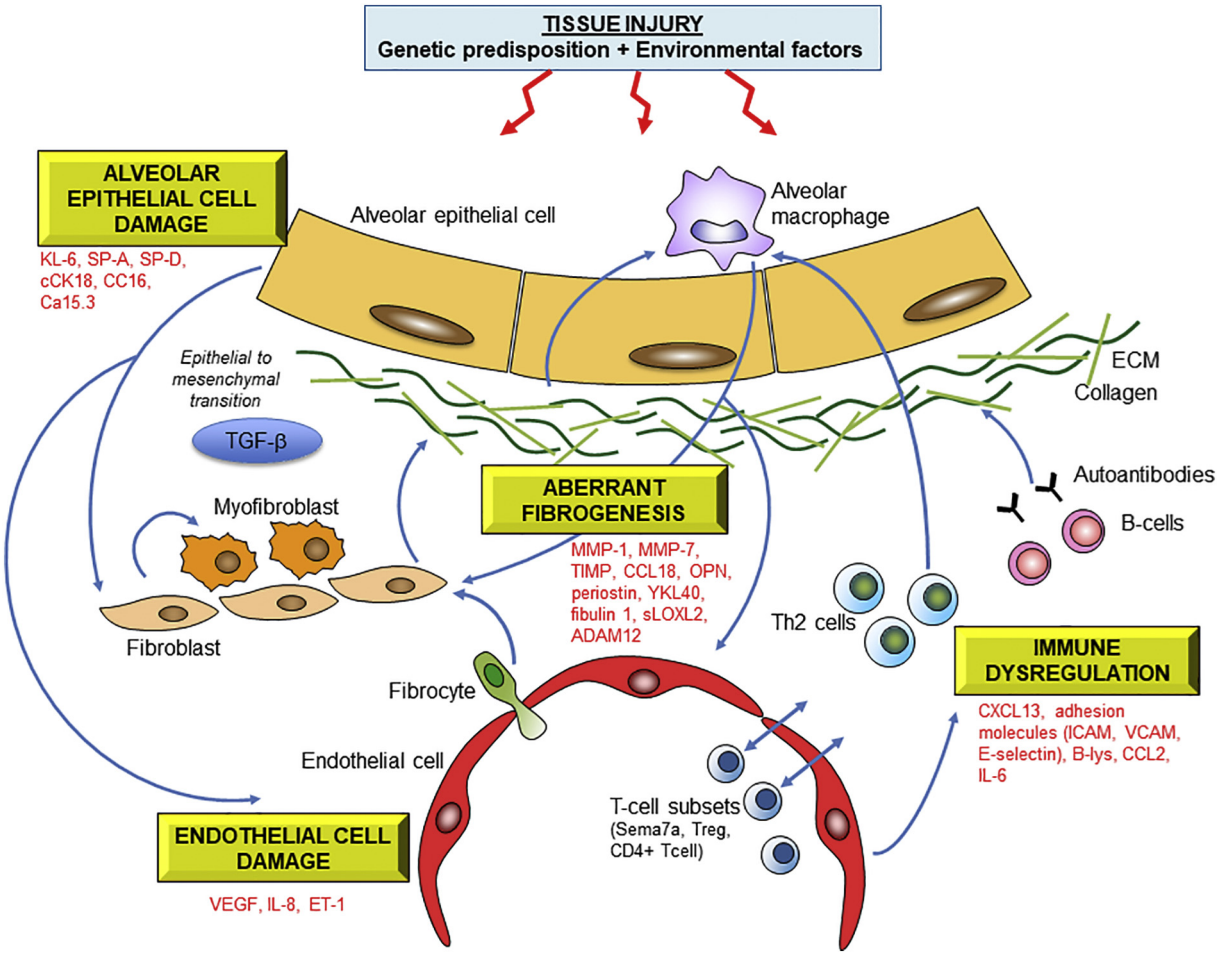

Fig. 2. Core cellular mechanisms of fibrotic lung injury and candidate biomarkers of ILD. Abbreviations: KL-6 Krebs von lungen 6; SP-A/SP-D surfactant protein A/D; cCK18 cleaved cytokeratin 18; CC16 Clara cell protein 16; MMP matrix metalloproteinase; OPN osteopontin, HSP47 heat-shock protein 47; sLOXL2 serum lysyl oxidase-like 2; ADAM a disintegrin and metalloprotease; CCL18 chemokine ligand 18; ICAM intercellular adhesion molecule; VCAM vascular cell adhesion molecule; CXCL13 C-X-C motif chemokine 13; CCL2 CC chemokine 2; IL-6 interleukin-6

Myofibroblasts secrete ECM matrix proteins (eg. collagen) and likely have multiple origins, including differentiation from resident and recruited fibroblasts (Bagnato & Harari, 2015; Wells & Denton, 2014). Abnormally activated AECs are implicated through transformation to fibroblasts via 'epithelial mesenchymal transition' (EMT), and expression of chemokines (eg. CXCL12) that attract circulating fibrocytes and promote collagen production (Bagnato & Harari, 2015; Teoh, Tan, & Tran, 2016; White et al., 2003).

Fibroblasts are key regulators of ECM homeostasis via multiple paths including the expression of cytokines and epigenetic mediators of fibrosis including matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), genetic expression of collagen types I and III, insulin-like growth factors binding proteins (IGFBPs), interferon-gamma receptors, connective tissue growth factor (Bagnato & Harari, 2015). Aberrant accumulation and activation of immune cells including macrophages, T- and B-cell lymphocytes express pro-fibrotic and inflammatory chemokines, cytokines and autoantibodies (eg. platelet-derived growth factors, TGF-β, reactive oxygen species, IL-6 and many others) promoting collagen production, pro-fibrotic cell-cell interactions and Th2 T-cell responses mediating tissue fibrosis (Bagnato & Harari, 2015). Activated endothelial cells up-regulate the expression of adhesion molecules and secrete chemokines, such as vascular endothelial growth factor (VEGF), that lead to endothelial dysfunction, defective repair and vascularisation (Bagnato & Harari, 2015).

There are an increasing number of genomic approaches to diagnosis and prognostication of ILD including the identification of microRNAs, polymorphism in the promoter region of MUC5B, and other rare mutations (eg. SFTPC, SFTPA2, TOLLIP, TERT and TERC). Whilst very important, discussion of genomic markers are beyond the scope of this article (Peljto et al., 2013; Yang & Schwartz, 2015).

1.3. Aims

This review will summarise the current evidence for major candidate serum biomarkers in IPF and SSc-ILD and their utility for diagnosis and prognosis. Biomarkers will be categorised by the following major mechanistic pathways (1) alveolar epithelial cell damage; (2) aberrant fibrogenesis, fibroproliferation and matrix remodelling; (3) immune dysregulation; and (4) vascular and endothelial damage. Tables 1 and 2 summarise the level of evidence supporting the potential clinical role for each candidate biomarker in IPF and SSc-ILD.

Strength of evidence: +, consistent or strong evidence to support role; ± equivocal, low-level evidence, insufficient to recommend clinical use; – no evidence to support role; blank, no data

2. Biomarkers of alveolar epithelial cell damage and dysfunction

2.1. Krebs von den Lungen (KL-6)

Krebs von den Lungen (KL-6) is a high-molecular-weight mucin glycoprotein, encoded by Mucin 1 (MUC1) (Kohno et al., 1989). Predominantly expressed on the surface of type II AECs – but also found on epithelial cells of the pancreas, oesophagus and stomach – its pathogenic role in pulmonary fibrosis is suggested by its pro-fibrotic, anti-apoptotic effects on fibroblasts (Kohno et al., 1989; A. Tzouvelekis et al., 2005). Serum KL-6 is elevated across multiple ILDs limiting its diagnostic utility, but studies report prognostic potential (Ishii et al., 2003; Ohnishi et al., 2002; A. Tzouvelekis et al., 2005; van den Blink et al., 2010).

In small studies, a serum KL-6 level threshold ranging from 500 to 1300U/mL was predictive of mortality and survival in IPF patients,

Table 1
Peripheral blood biomarkers in IPF and level of evidence supporting potential roles.

Biomarker	Diagnosis	Prognosis	Therapeutic	References
Alveolar epithelial cell damage and dysfunction				
KL-6/MUC1	±	±	–	Ishii et al., 2003, Ohnishi et al., 2002, Tzouvelekis, Kouliatsis, Anevlavis, & Bouros, 2005, van den Blink, Wijsenbeek, & Hoogsteden, 2010, Satoh, Kurishima, Ishikawa, & Ohtsuka, 2006, Yokoyama et al., 2006, Kobayashi et al., 2001, Salazar et al., 2018, Ohshimo et al., 2014
SP-A and SP-D	±	±	–	(Barlo et al., 2009; Greene et al., 2002; Ishii et al., 2003; Kinder et al., 2009; Ohnishi et al., 2002; Okuda et al., 2013; Song et al., 2013; Takahashi et al., 2000)
Aberrant fibrogenesis and matrix remodelling				
MMP7	±	+		(Flynn, E, & Kass, 2015; Fujishima et al., 2010; Jenkins et al., 2015; Richards et al., 2012; Rosas et al., 2008; Song et al., 2013; Argyris Tzouvelekis et al., 2017)
MMP3	±	±		(Craig et al., 2014; DePianto et al., 2015)
Periostin		±		(Naik et al., 2012; Okamoto et al., 2011)
Circulating fibrocytes		±		(Fujiwara et al., 2012; Moeller et al., 2009)
Osteopontin	–	±		(Kadota et al., 2005; Pardo et al., 2005)
Fibulin-1		±		(Jaffar et al., 2014)
HSP47	±			(Kakugawa et al., 2005; Kakugawa et al., 2013)
LOXL2		±	–	(Chien et al., 2014; Gibson, 2006; Raghu et al., 2013; Raghu, 2017)
Insulin like growth factor binding proteins IGFBPs	±	–	±	(Ruan & Ying, 2010)
Immune dysregulation and inflammation				
CCL18	–	±		(Cai et al., 2013; Hoffmann-Vold et al., 2016; Kodera et al., 2005; Ohshimo et al., 2014; Prasse et al., 2009; Tiev et al., 2011)
YKL-40		±		(Furuhashi et al., 2010; Korthagen et al., 2011; Korthagen, van Moorsel, Zanen, Ruven, & Grutters, 2014)
ICAM, VCAM, E-selectin	–	±		(Gruschwitz, Hornstein, & von Den Driesch, 1995; Ihn, Sato, Fujimoto, Takehara, & Tamaki, 1998; Sfikakis et al., 1993) (Richards et al., 2012)
T cell subsets		±		(Kotsianidis et al., 2009; Reilkoff et al., 2013)
Anti-HSP70 IgG		±		(Kahloon et al., 2013)
CXCL-13		±		
BLyS/BAFF		±		(Hamada et al., 2015)
IL-6	±	±		(De Lauretis et al., 2013)
Serum RAGE		±		(Manichaikul et al., 2017)
Damaged endothelium				
VEGF, ET-1, IL-8	±	±		(Ando et al., 2010; Simler et al., 2004; Smadja et al., 2014); (Richards et al., 2012; Simler et al., 2004; Tsoutsou et al., 2006; Ziegenhagen, Zabel, Zissel, Schlaak, & Muller-Quernheim, 1998)
Other				
Oxidative stress markers		±		(Daniil et al., 2008)

Table 2
Peripheral blood biomarkers in SSc-ILD and level of evidence supporting potential roles

Candidate biomarker	Diagnosis	Prognosis	Therapeutic	References
Alveolar epithelial cell damage and dysfunction				
KL-6/MUC1		±		
SP-A and SP-D	±	±		(Asano et al., 2001; Bonella et al., 2011; Hant et al., 2009; Takahashi et al., 2000; Yanaba, Hasegawa, Takehara, & Sato, 2004)
CC16	±	±		(Hasegawa et al., 2011; Olewicz-Gawlik et al., 2016)
Ca15.3		±		(Celeste et al., 2013; Marzano, Morabito, Berti, & Caputo, 1998; Ricci et al., 2009)
Aberrant fibrogenesis and matrix remodelling				
MMP7	±	±		(Kikuchi, Kubo, Sato, Fujimoto, & Tamaki, 1995; Manetti et al., 2012; Moinzadeh et al., 2011; Oka et al., 2013)
Osteopontin	–			(Corallo et al., 2014)
ADAM12	±			(Taniguchi et al., 2013)
Immune dysregulation and inflammation				
CCL18		±		(Elhaj et al., 2013; Hoffmann-Vold et al., 2016; Salazar et al., 2018)
YKL-40		±		(La Montagna, D'Angelo, & Valentini, 2003; Nordenbaek et al., 2005)
ICAM, VCAM, E-selectin	±			(Gruschwitz et al., 1995; Ihn et al., 1998; Sfikakis et al., 1993)
Anti-HSP70 IgG		–		(Kahloon et al., 2013)
BLyS/BAFF		±		(Hamada et al., 2015)
CCL2/MCP-1		±		(Assassi et al., 2010; De Lauretis et al., 2013; Wu et al., 2017)
IL-6		±		(De Lauretis et al., 2013)
CXCL4	±	±		(van Bon et al., 2014; Volkmann et al., 2016)
Damaged endothelium				
VEGF, ET-1, IL-8		±		(De Santis et al., 2016; Kuryliszyn-Moskal, Klimiuk, & Sierakowski, 2005; Vancheeswaran et al., 1994)

Strength of evidence: +, consistent or strong evidence to support role; ± equivocal, low-level or small single-study evidence, insufficient to recommend clinical use; –, evidence against potential role; blank, no data.

with 67.2 - 92% sensitivity and 60.2 – 70.6% specificity (Kobayashi et al., 2001; Salazar et al., 2018; Satoh et al., 2006; Yokoyama et al., 2006). Wakamatsu demonstrated higher FVC%predicted decline in a retrospective cohort of 89 IPF patients with rising KL-6 compared with patients with no KL-6 increase. Ohshimo et al. found KL-6 more than 1300U/mL predicted IPF exacerbation with 92% sensitivity, but with low specificity 61% and high false-positive rate 39% (Ohshimo et al., 2014). In small studies KL-6 levels decreased in stable IPF patients on pirfenidone and after corticosteroid therapy for acute exacerbations, but validation in prospective, larger cohorts is required (Kuwano et al., 2002; Okuda et al., 2013; Yokoyama et al., 1998).

In SSc-ILD, several studies demonstrate elevated KL-6 compared with healthy controls and SSc patients without ILD (Bonella et al., 2011; Hant et al., 2009; Yamane et al., 2000; Yanaba et al., 2003; Yanaba et al., 2004). Longitudinal studies demonstrate correlation with baseline and serial changes in pulmonary function (FVC and DLCO), HRCT fibrosis scores, serial measures of skin involvement, and composite disease activity indices (Bonella et al., 2011; Yanaba et al., 2003). Preliminary studies suggest KL-6 may have utility assessing response to treatment with cyclophosphamide or azathioprine, but larger studies are required (Boerner, Wessendorf, Ohshimo, Costabel, & Bonella, 2015; Shirai, Takeuchi, & Kuwana, 2015).

Despite the large body of research, studies in KL-6 are predominantly in Japanese cohorts with limited access to the assay outside of Japan, thus better understanding of pathophysiological mechanisms and a reliable, accessible assay thoroughly tested in diverse populations is required before clinical implementation.

2.2. Surfactant proteins

Surfactant proteins are lipoprotein complexes contained within pulmonary surfactant and produced by type II AECs and Clara cells important for innate immune defence mechanisms and modulating the inflammatory response at the alveolar air-liquid interface (Pastva, Wright, & Williams, 2007). Four surfactant proteins have been identified (SP-A, SP-B, SP-C, SP-D), with SP-A and SP-D of particular interest in IPF and CTD-ILD (Ley et al., 2014).

SP-A and SP-D are elevated in IPF compared with healthy controls, but do not consistently distinguish IPF from other ILDs (Greene et al., 2002; Ishii et al., 2003; Ohnishi et al., 2002). Elevated SP-A has been associated with 3.3 times increased mortality and SP-D with reduced one-year survival (sensitivity 62.5%, specificity 78.3%) (Barlo et al., 2009; Kinder et al., 2009). Kinder et al. demonstrated improvement of one-year mortality prediction models when SP-A and SP-D were added to clinical predictors alone (age, gender, race, smoking status, FVC, DLCO and alveolar-arterial oxygen gradient), but this was not replicated in a later study (Kinder et al., 2009; Song et al., 2013). Lower baseline SP-D has been associated with future declines in FVC and total lung capacity (TLC), but longitudinal changes in SP-D are unknown (Takahashi, Fujishima, et al., 2000). Reduced SP-D levels have also been demonstrated after pirfenidone therapy in IPF, but data is limited (Okuda et al., 2013).

In SSc-ILD, several studies have found serum SP-D to be more sensitive (range 77 – 89.4%) but less specific (range 80 – 83%) than KL-6 for detecting disease (Hant et al., 2009; Takahashi, Kuroki, et al., 2000; Yanaba et al., 2004). Several studies have also demonstrated negative correlation between serum SP-D and lung function, and worsening symptoms and lung function in longitudinal measures (Asano et al., 2001; Hant et al., 2009; Yanaba et al., 2004). Takahashi et al. showed a strong correlation between SP-D and extent of ground-glass change on HRCT, not confirmed on further studies (Bonella et al., 2011; Hant et al., 2009; Takahashi, Kuroki, et al., 2000).

2.3. Clara cell protein (CC16)

Clara cells are multifunctional cells predominantly localised at terminal bronchioles that secrete 16kDa Clara cell protein (CC16), with important protective, immunosuppressive and anti-inflammatory functions (Broeckaert & Bernard, 2000). Serum CC16 is elevated across several pulmonary conditions including SSc-ILD, sarcoidosis and inhalant lung injury, and reduced in asthmatics and smokers (Hasegawa et al., 2011). CC16 is eliminated by glomerular filtration thus cannot be interpreted in severe renal impairment (Broeckaert & Bernard, 2000).

CC16 is elevated in SSc-ILD compared with healthy controls and SSc-without ILD, with one study reporting 46 ng/mL diagnostic for SSc-ILD with 51.8% sensitivity and 88.8% specificity (Hasegawa et al., 2011; Olewicz-Gawlik et al., 2016). In the same study CC16 was inferior to

KL-6 and comparable to SP-D (Hasegawa et al., 2011) Correlation between lung function and serum CC16 is conflicting between studies. A small study of 28 SSc patients observed greater HRCT involvement, lower TLC and shorter 6-minute walk test distance with elevated CC16, but this has not been validated (Olewicz-Gawlik et al., 2016).

2.4. Carbohydrate antigen 15.3 (Ca 15.3) and other epithelial markers

Cancer associated antigen carbohydrate antigen 15.3 (Ca15.3) is produced from the same MUC1 gene that encodes for KL-6 and is expressed on various epithelial cells, including type II AECs (Jaffar et al., 2014). Elevated Ca15.3 has been demonstrated in IPF and SSc-ILD, although association with outcomes has only been investigated for SSc (Celeste et al., 2013; Marzano et al., 1998; Ricci et al., 2009). Among 221 SSc patients, Ca15.3 level correlated strongly with HRCT scores and was associated with progression-free survival by univariate analysis (Celeste et al., 2013). 20% or more lung involvement on HRCT was best at distinguishing high-risk for an unfavourable outcome, validating the staging system previously described by Goh et al. (Celeste et al., 2013; Goh et al., 2008). Furthermore, if HRCT was unavailable, Ca15.3 outperformed FVC in predicting survival (Celeste et al., 2013). However, its performance has not been compared with other biomarkers and it has low sensitivity and specificity and also increases in non-pulmonary malignancy and hepatic disease.

Other epithelial markers investigated as biomarkers of ILD without current evidence for utility include cleaved cytokeratin 18 (cCK18), cytokeratin 19 (CK19), Ca19-9 and sialyl Lewis x (SLX, also known as stage specific embryonic antigen or SSEA-1) (Dobashi et al., 1999; Fujita et al., 1999; Ley et al., 2014; Satoh et al., 1991; Spagnolo, Cordier, & Cottin, 2016; Takayama et al., 1990).

3. Fibrogenesis, fibroproliferation and matrix remodelling

3.1. Matrix metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) are tightly regulated proteases important in mediating ECM degradation, activity of inflammatory mediators and growth factors in the lung (Craig, Zhang, Hagood, & Owen, 2015; Dancer, Wood, & Thickett, 2011). There are 23 known MMPs, variably expressed by activated type II AECs, macrophages and fibrocytes (Pardo at al., 2008). Dysregulation of cellular expression has been implicated in the pathogenesis of pulmonary fibrosis (DePianto et al., 2015; Fujishima et al., 2010; Pardo et al., 2005; Rosas et al., 2008).

Serum MMP7 is elevated in IPF compared with healthy controls but its ability to differentiate IPF from other ILDs is conflicting (Fujishima et al., 2010; Rosas et al., 2008; Tzouvelekis et al., 2017). However, MMP7 is one of the most promising prognostic biomarkers in IPF, with serum levels reproducibly correlating with lung function, clinical predictors of all-cause mortality and transplant free survival in several studies (Flynn et al., 2015; Richards et al., 2012; Song et al., 2013; Tzouvelekis et al., 2017). Its inclusion in prognostic models with clinical parameters (gender, FVC and DLCO) has demonstrated good discriminative performance for survival (Richards et al., 2012; Tzouvelekis et al., 2017). MMP7 alone has only moderate sensitivity and specificity, but combining MMP7 with other candidate biomarkers (eg. SP-A, KL-6) may improve predictive accuracy and warrants validation in larger, prospective cohorts (Jenkins et al., 2015). MMP3 has also demonstrated negative correlation with FVC and survival over 3 years in IPF (Craig et al., 2014; DePianto et al., 2015). Other MMPs are yet to demonstrate correlation with lung function or mortality.

Extracellular collagen fragments generated by MMPs and released into circulation may provide an indirect measure of MMP activity. (Jenkins et al., 2015) The PROFILE (Prospective Observation of Fibrosis in Lung Clinical Endpoints) study is a prospective, multicentre observational cohort study that measured 11 such collagen fragments, or neoepitopes, in 189 IPF patients. 6 neoepitopes were higher in patients with progressive IPF compared with patients with stable disease: biglycan degraded by MMP-2/9 (BGM), collagen 1 degraded by MMP-2/9/13 (C1M), collagen 3 degraded by MMP-9 (C3M), collagen 3 degraded by ADAMTS-1/4/8 (C3A), collagen 6 degraded by MMP-2/9 (C6M), C-reactive protein degraded by MMP-1/8 (CRPM) (Jenkins et al., 2015). Higher baseline C1M and C3A were associated with increased mortality, and increasing rate of change at 3 months of six neoepitopes (BGM, C1M, C3M, C5M, C6M and CRPM) were strongly predictive of worse overall survival (Jenkins et al., 2015). Further study will clarify the prognostic utility of serum matrix degradation products as prognostic biomarkers in IPF.

In SSc-ILD, serum MMP7, MMP12 and TIMP1 are elevated when compared with healthy controls and SSc without ILD, and correlate inversely with pulmonary function (Kikuchi et al., 1995; Manetti et al., 2012; Moinzadeh et al., 2011). Oka et al. analysed 30 biomarkers in patients with acute-onset diffuse ILD (defined as onset and decline of lung function within a month; presence of fever, dry cough or dyspnoea; hypoxia; and ILD on HRCT) (Oka et al., 2013). SSc, RA and polymyositis/dermatomyositis patients were included and MMP1, MMP8, MMP9, TIMP3, and osteopontin were increased and the MMP3 to MMP1 ratio was decreased in patients who died, whilst TIMP2 and MMP3 were increased in surviving patients (Oka et al., 2013). Limited by small numbers and a mixed CTD cohort, the prognostic utility of MMPs/TIMPs in SSc-ILD remains to be validated.

3.2. A distintegrin and metalloprotease (ADAM) and ADAMTS

ADAMs (a disintegrin and metalloproteinase) are a group of multifunctional proteins that share the metalloprotease domain with MMPs (Mochizuki, Ikari, Yano, Sato, & Okazaki, 2018) ADAMs have a unique multifunctional domain with both proteolytic and adhesive functions, and play an important role in cell binding, migration and signalling (Pardo, Cabrera, Maldonado, & Selman, 2016). They are implicated in a variety of diseases including CTD, malignancy, Alzheimer's, Crohn's disease and possibly pulmonary fibrosis (Giebeler & Zigrino, 2016; Pardo et al., 2016; Taniguchi et al., 2013). ADAM15 gene expression is increased in IPF but the cell source and role in IPF is unknown [Pardo]. In the PROFILE study, collagen 3 degraded by secreted-type ADAM with thrombospondin motifs (ADAMTS)-1/4/8 was higher in IPF patients with more progressive disease and associated with higher mortality (Jenkins et al., 2015).

In a study of 56 SSc patients, serum ADAM12 levels were elevated in SSc-ILD compared with SSc-alone, and correlated with FVC (Taniguchi et al., 2013). ADAM12 also correlated positively with serum CRP, extent of ground-glass opacities on HRCT and negatively with HRCT fibrosis score (Taniguchi et al., 2013). Its role as a marker of active inflammation requires further investigation.

3.3. Periostin

Periostin promotes ECM deposition, mesenchymal cell proliferation, and fibrosis in the lung and other organs, with elevated levels demonstrated in IPF compared with controls (Naik et al., 2012). Inverse correlation with pulmonary function and an association with progression at 48 weeks (defined as death, acute exacerbation of IPF, transplant, decline in FVC or DLCO by ≥10 and ≥15% respectively) has also been demonstrated and requires validation (Naik et al., 2012; Okamoto et al., 2011). In SSc, no relationship with lung disease has been demonstrated (Yamaguchi et al., 2013).

3.4. Circulating fibrocytes

Circulating fibrocytes are bone-marrow-derived progenitor cells that circulate in the bloodstream, migrate to sites of tissue injury, differentiate into fibroblasts and are capable of producing ECM components.

(Flynn et al., 2015; Moeller et al., 2009). In IPF, elevated fibrocyte levels have been associated with worse survival and inversely correlated with FVC and DLCO (Fujiwara et al., 2012; Moeller et al., 2009). Higher levels have been demonstrated in SSc, but association with ILD remains undetermined (Mathai et al., 2010). There is also no standardised set of fibrocyte markers or protocol for the identification of fibrocytes by flow cytometry (Moore & Kolb, 2014).

3.5. Osteopontin

Osteopontin (OPN) is a multifunctional protein expressed in many tissues that regulates inflammation, cellular immune response and T cell function, with a pro-fibrotic effect in ILD through mechanisms not completely understood (Mazzali et al., 2002; Pardo et al., 2005). Serum OPN levels are elevated in IPF and SSc compared with controls, but cannot differentiate between ILD subtypes (Corallo et al., 2014; Kadota et al., 2005; Pardo et al., 2005). One study demonstrated correlation with arterial oxygenation in a mixed ILD population (including IPF and CTD-ILD), but no relationship with other measures of pulmonary function have been demonstrated (Kadota et al., 2005).

3.6. Fibulin-1

Fibulin-1 is a glycoprotein identifiable in blood and pulmonary ECM essential for alveolar septal formation and embryonic morphogenesis, and possible pathogenic role in IPF (Jaffar et al., 2014; Kostka et al., 2001). A multicentre international study demonstrated elevated serum fibulin-1 levels among 72 IPF patients compared with healthy controls, and inverse correlation with FVC (Jaffar et al., 2014). Fibulin-1 levels above 1.6 units identified patients with progressive disease (≥10% fall in FVC, ≥15% fall in DLCO or death) with 70% sensitivity and 71% specificity, and each unit increase carried a 1.69 hazard ratio for likelihood of disease progression (Jaffar et al., 2014). As the only known study to date, further prospective longitudinal studies are required to validate fibulin-1 as a prognostic biomarker in IPF and other ILDs.

3.7. Heat shock protein (HSP) 47

Heat shock protein 47 (HSP47) is a collagen-specific molecular chaperone, involved in the biosynthesis and secretion of collagen molecules, increased in a number of fibrotic conditions (Kakugawa et al., 2005). In small studies, serum HSP47 is higher in idiopathic usual interstitial pneumonia (UIP) compared with UIP when associated with connective tissue disease, and able to distinguish acute-exacerbations from stable IPF (threshold 559.4 pg/mL, 100% sensitivity, 93.9% specificity) (Kakugawa et al., 2005; Kakugawa et al., 2013). These studies are limited by very small patient numbers.

3.8. Lysyl oxidase-like (LOXL) 2

Serum lysyl oxidase-like 2 (sLOXL2) is an enzyme secreted by activated fibroblasts that promotes collagen synthesis and directly involved in matrix remodelling and fibrogenesis (Barry-Hamilton et al., 2010). The ability of sLOXL2 to predict disease progression in IPF was evaluated using baseline sera and data collected from two independent clinical studies: *ARTEMIS-IPF,* a randomised, multicentre, double-blind, placebo-controlled trial evaluating the ability of ambrisentan (a selective endothelin receptor antagonist) to prevent IPF disease progression, and the *Genomic and Proteomic Analysis of Disease Progression (GAP) in IPF* study, an ongoing observational study of prognostic markers in IPF (Gibson, 2006; Raghu et al., 2013). Elevated sLOXL2 was associated with increased risk for lung function decline in both cohorts, respiratory hospitalisation in the ARTEMIS cohort, and mortality risk in the GAP cohort (Chien et al., 2014). Limitations include small study numbers, confounding from the study drug, and lack of a standardised assay. sLOXL2 has been proposed as a therapeutic target but an IPF clinical trial investigating the efficacy and safety of simtuzumab, a humanised monoclonal antibody that inhibits LOXL2, was terminated in 2016 due to lack of efficacy (Raghu, 2017).

3.9. Insulin-like growth factor binding proteins

Insulin like growth factors (IGFs) are important in cell growth, differentiation, apoptosis and metabolism (Ruan & Ying, 2010). IGF activity is modulated by IGF binding proteins (IGFBPs) (Ruan & Ying, 2010). In the human lung, IGFBPs have been implicated in the pathogenesis of pulmonary fibrosis via fibroblast activation, differentiation to myofibroblasts (epithelial-to-mesenchymal cell transition) and aberrant ECM deposition/degradation (Ruan & Ying, 2010). A cross-sectional study of 50 IPF patients demonstrated increased serum IGFBP-1 and -2 levels compared with healthy controls (Guiot, Bondue, Henket, Corhay, & Louis, 2016). IGFBP-2 levels were lower in patients treated with anti-fibrotics and levels did not correlate with lung function (Guiot et al., 2016). Further longitudinal studies are required.

4. Immune dysregulation and inflammation

4.1. Chemokine ligand 18 (CCL18)

C-C motif chemokine ligand 18 (CCL18), previously known as pulmonary and activation-regulated chemokine (PARC), is primarily produced by alveolar macrophages with an important role stimulating fibroblasts to synthesise collagen in fibrotic lung diseases (Flynn et al., 2015; Prasse et al., 2007). Although unable to differentiate between ILD subtypes, longitudinal CCL18 levels have demonstration correlation with lung function and mortality in both IPF and SSc-ILD patients (Cai et al., 2013; Hoffmann-Vold et al., 2016; Kodera et al., 2005; Ohshimo et al., 2014; Prasse et al., 2009; Tiev et al., 2011). A prospective study of 298 SSc, including SSc-ILD, patients demonstrated higher rates of annual FVC decline and worse 5- and 10-year cumulative survival, but this was not confirmed on a recent study by Salazar et al. which only included SSc-ILD patients (Elhaj et al., 2013; Hoffmann-Vold et al., 2016; Salazar et al., 2018).

4.2. YKL-40

YKL-40 is a chitinase-like glycoprotein, thought to have a role in regulating connective tissue cell proliferation and angiogenesis (Lee et al., 2011). Serum YKL-40 is unable to distinguish between ILD subtypes, with elevated levels identified across a variety of inflammatory and fibrotic diseases including ILD, liver fibrosis, inflammatory arthropathies, asthma and chronic obstructive pulmonary disease (COPD) (Flynn et al., 2015; Lee et al., 2011; Nordenbaek et al., 2005).

The prognostic potential of YKL-40 in both IPF and SSc-ILD requires further validation. A study of 85 Japanese IPF patients demonstrated worse 4-year survival (48% versus 86%) at a serum threshold 79 ng/mL (Furuhashi et al., 2010; Korthagen et al., 2011; Korthagen et al., 2014). Elevated YKL-40 has also been demonstrated in SSc populations and associated with decreased FVC and DLCO, in addition to features of obstructive lung disease (La Montagna et al., 2003; Nordenbaek et al., 2005). Nordenbaek et al. demonstrated a trend towards shorter survival in 88 SSc patients with high YKL-40 levels, but this did not maintain statistical significance after controlling for age, FVC and DLCO (Nordenbaek et al., 2005).

4.3. Adhesion molecules (ICAM, VCAM and E-selectin)

Adhesion molecules, including intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), and E-selectin, are expressed on leucocytes and vascular endothelial cells and are important mediating adhesion and interaction of these cells

(Agassandian et al., 2015; Kuryliszyn-Moskal et al., 2005; Shijubo et al., 1992). Elevated levels are detectable across a number of inflammatory and fibrovascular conditions including SSc, SLE, RA, vasculitis, radiation-pneumonitis and IPF (Shijubo et al., 1992). Integrins, transmembrane proteins that form a large family of cellular adhesion molecules, interact with adhesion molecules such as VCAM and ICAM, and other growth-factor receptors and ECM components to activate downstream signalling pathways and TGF-B mediated pulmonary fibrosis (Agassandian et al., 2015; Teoh et al., 2016).

Early studies of ICAM1 in IPF did not demonstrate correlation with pulmonary function, but a recent study of 241 IPF patients found ICAM1 was predictive of poor overall, transplant-free and progression-free survival regardless of age, sex or baseline pulmonary function (Richards et al., 2012; Tsoutsou et al., 2004). VCAM1 predicted outcomes in the derivation but not validation cohorts (Richards et al., 2012). ICAM2 and E-selectin have also demonstrated elevated levels in IPF, but there is no data regarding outcomes (Hayashi et al., 2004; Tsoutsou et al., 2004).

Elevated levels of ICAM, VCAM and E-selectin have been demonstrated in SSc and ICAM has been associated with the combined presence of diffuse, rapidly progressive SSc, digital contractures, ILD and joint involvement (Gruschwitz et al., 1995; Ihn et al., 1998; Sfikakis et al., 1993). Longitudinal measures and specific outcomes in SSc-ILD remain unknown.

4.4. T cell subsets ($CD4+CD28^{null}$ T cells and Sema 7a)

CD28 is a co-stimulatory molecule expressed on the surface of nearly all CD4+ T cells in healthy individuals. Repeated cycles of antigen-driven T-cell proliferation down-regulate CD28 expression, thus $CD4+CD28^{null}$ cells are considered a marker of chronic adaptive immune activation, although the pathophysiologic importance of CD28 down-regulation is uncertain (Gilani et al., 2010; van den Blink et al., 2010). An exploratory study found a reduction in the proportion of $CD4+CD28^{null}$ T cells in 89 IPF patients compared with healthy controls, and association with low DLCO and increased likelihood of requiring lung transplantation within one year (Gilani et al., 2010). A small subset also showed correlation with longitudinal changes in FVC but requires confirmation (Gilani et al., 2010).

Regulatory T cells (Tregs) also play an important role in modulation of the adaptive immune response, and whilst the pathophysiologic role of Tregs in IPF remains controversial, lower Treg levels and impaired function have been associated with disease severity in IPF (Kotsianidis et al., 2009). Semaphorin 7a (Sema 7a) is a membrane protein expressed on Tregs that modulates lymphocyte function and induces lung fibrosis in animal models (Reilkoff et al., 2013). A study of 38 IPF patients demonstrated increased Sema 7a expression in subjects with rapidly progressive disease (composite of >10% decline in FVC and acute exacerbation or death), although no correlation with FVC or DLCO alone was found (Reilkoff et al., 2013).

4.5. Adaptive immunity, B-cell pathways

4.5.1. Autoantibodies to heat shock protein (HSP) 70

Heat shock protein 70 (HSP70) induces T-cell proliferation and pro-fibrotic cytokine production. Autoantibodies to HSP70 may have pathogenic potential in ILD by augmenting neutrophil recruitment, complement activation and production of inflammatory mediators in target organs (Kahloon et al., 2013; Ley et al., 2014). Kahloon et al. demonstrated a higher prevalence of anti-HSP70 autoantibodies in IPF compared with controls, and greater subsequent FVC decline and reduced one-year survival (Kahloon et al., 2013). CTD-ILDs have also demonstrated higher prevalence of anti-HSP70 antibodies, but no association with clinical progression (Kahloon et al., 2013). Current anti-HSP70 antibody assays still require refinement and validation prior to clinical use.

4.5.2. C-X-C motif chemokine (CXCL) 13

C-X-C motif chemokine 13 (CXCL13) is overexpressed in the lungs and circulation of IPF patients and appears important in homing B lymphocytes to inflammatory foci (Vuga et al., 2014). Vuga et al. demonstrated elevated CXCL13 levels in IPF patients compared with COPD and healthy controls, and negative correlation with lung function and survival, which was replicated by DePianto et al. (DePianto et al., 2015; Vuga et al., 2014).

4.5.3. B lymphocyte stimulator (BLys) or B-cell activating factor (BAFF)

B lymphocyte stimulator (BLyS), also known as B-cell activating factor (BAFF), is a cytokine belonging to the tumour necrosis factor family, critical to B cell maturation and antibody production with a possible pathogenic role in several autoimmune diseases and IPF (Hamada et al., 2015, pp. 110-117). Xue et al. demonstrated elevated BLyS levels in IPF compared with healthy controls and COPD, and an association with elevated pulmonary arterial pressure, greater subsequent decline in FVC and diminished one year survival (Xue et al., 2013).

Negative correlation with FVC has been reported in mixed CTD cohorts, but not specific association with SSc-ILD (Hamada et al., 2015; Matsushita et al., 2006). Belimumab, a human monoclonal antibody targeting BLyS, is approved for the treatment of SLE but effectiveness for CTD-ILD is unknown (Furie et al., 2011; Navarra et al., 2011).

4.5.4. C-C chemokine 2 (CCL2)

C-C motif chemokine 2 (CCL2), previously known as monocyte chemoattractant protein-1 (MCP-1), plays an important role in innate immunity and inflammation, with a potential pro-fibrotic effect in SSc, IPF, RA, and sarcoidosis (Antonelli et al., 2008; Antoniades et al., 1992; Carulli, Handler, Coghlan, Black, & Denton, 2008; Hasegawa, Sato, & Takehara, 1999; Ling et al., 2010; Suga et al., 1999). Dedicated study in IPF populations is lacking. A study of SSc-ILD patients that included 58 IPF patients found that CCL2 was an independent predictor of progression free survival in IPF (hazard ratio 2.4) (De Lauretis et al., 2013).

The *Genetics versus Environment in Scleroderma Outcome Study* (GENISOS) is a prospective, observational cohort of patients with early SSc, aimed at identifying predictors of disease progression including a panel of 11 cytokines and chemokines (Assassi et al., 2010). In preliminary analysis of 266 participants, CCL2 predicted faster FVC decline and worse survival (hazard ratio 1.93), while IL-10 predicted a slower FVC decline, but requires further study (Wu et al., 2017).

4.6. Pleiotropic interleukins including IL-6

Numerous cytokines involved in the regulation of T-lymphocytes have been detected and implicated in the pathogenesis of IPF and SSc including interleukin (IL)-6, IL-10, IL-18, IL-15, IL-17, IL-23, and TNF-related apoptosis inducing ligand (TRAIL) (Bonella & Costabel, 2014; Gono et al., 2010; Sato et al., 2013; Shen, Xia, & Lu, 2013; Truchetet, Brembilla, Montanari, Allanore, & Chizzolini, 2011; Wuttge et al., 2007). To our knowledge, only IL-6 has a reported association with disease progression.

IL-6 is involved in the differentiation of CD4+ T cells to pro-fibrotic Th2 type cells, and implicated in the activation of fibroblasts (Glimcher & Murphy, 2000; Kondo et al., 2001; Laurence & O'Shea, 2007). An exploratory study of 8 cytokines (IL-6, IL-8, IL-10, CCL2, CXCL10, vascular endothelial growth factors, fibroblast growth factor 2 and CX3CL1) found IL-6 was an independent predictor of progression-free survival (hazard ratio 1.69) and the only biomarker predictive of DLCO decline in both IPF and SSc (De Lauretis et al., 2013). In SSc patients, IL-6 was also associated with shorter survival and time to decline in FVC adjusted for age, smoking, composite physiologic index, pulmonary hypertension and skin disease (De Lauretis et al., 2013). IL-6 is an acute phase reactant and increases in acute exacerbations of IPF, but its ability to predict events is unknown (Collard et al., 2010).

4.7. C-X-C motif chemokine ligand 4 (CXCL4)

CXCL4, also known as platelet factor 4, is a potent anti-angiogenic chemokine whose pathogenic role in ILD is evolving but has generated significant interest in SSc. Levels are also elevated in liver fibrosis and anti-phospholipid syndrome, and the ability to discriminate SSc-without and with ILD is unknown (Distler et al., 1999; Kahaleh, Osborn, & LeRoy, 1981). Van Bon et al. observed serum CXCL4 levels 270-times higher in SSc patients compared with healthy controls and an association with decline in DLCO (van Bon et al., 2014). The Scleroderma Lung Study II (SLS II) did not demonstrate correlation between baseline CXCL4 and physiologic or radiographic measures of severity of SSc-ILD (Volkmann et al., 2016). However, CXCL4 levels declined at 12 and 24 months after immunosuppressive therapy (cyclophosphamide and mycophenolate), and a fall in CXCL4 from baseline-to-12 months was associated with improvement of FVC from 12-to-24 months (Volkmann et al., 2016).

4.8. Other cytokines

Other proposed cytokines that have either no data or a lack of association with disease outcomes in IPF and/or SSc include monocyte inflammatory protein-1a (MIP-1a), CXCL11 (also known as IFN-inducible T-cell alpha chemoattractant, ITAC), CXCL10, CXCL12 (Antonelli et al., 2008; Hasegawa et al., 1999; Strieter, Starko, Enelow, Noth, & Valentine, 2004; Volkmann et al., 2016).

4.9. Soluble receptor for advanced glycosylated end products (sRAGE)

The receptor for advanced glycosylated end products (RAGE) is a cell surface protein thought to mediate inflammation in several disease processes (atherosclerosis, vascular disease, Alzheimer's disease and diabetic nephropathy) [reference]. It is expressed on AECs and it is hypothesised soluble RAGE (sRAGE) may be protective in ILD by binding RAGE ligands and attenuating RAGE-mediated inflammation (Manichaikul et al., 2017). Manichaikul et al. recently demonstrated lower plasma sRAGE levels in patients with IPF and other ILDs (including CTD-ILD) when compared with healthy controls (Manichaikul et al., 2017). Furthermore, lower plasma sRAGE levels were strongly associated with greater disease severity and increased rate of death or lung transplant, confounded by FVC (Manichaikul et al., 2017). Further study will enhance understanding of the pathobiological and potential prognostic and therapeutic roles of RAGE and sRAGE in ILD.

5. Endothelial damage

5.1. Vascular endothelial growth factor (VEGF)

Aberrant angiogenesis is implicated in the pathogenesis of pulmonary fibrosis and fundamental mediators of this process include vascular endothelial growth factor (VEGF), endothelin 1 (ET-1) and interleukin-8 (IL-8) (Ando et al., 2010; Simler et al., 2004).

VEGF is a central cytokine and growth factor for endothelial and type II AECs and its utility as a diagnostic and prognostic biomarker in IPF is conflicting (Voelkel, Vandivier, & Tuder, 2006). A prospective, multicentre study of 64 patients found serum VEGF was 2.8 times higher in IPF compared with healthy controls, in contrast to earlier studies that showed no difference (Meyer, Cardoni, & Xiang, 2000; Smadja et al., 2014). There was also a trend towards negative correlation with DLCO and five-year survival, but was unable to confirm the association between VEGF and disease severity from previous reports (Ando et al., 2010; Simler et al., 2004; Smadja et al., 2014). Nintedanib, a tyrosine kinase inhibitor that targets VEGF signalling slows disease progression in IPF but the utility of measuring serum VEGF as a marker of therapeutic response is not determined (Flynn et al., 2015)

In a recent study of 44 SSc patients elevated VEGF correlated with disease severity measured by DLCO and HRCT involvement and larger, longitudinal cohorts would help confirm these findings (De Santis et al., 2016; Kuryliszyn-Moskal et al., 2005; Vancheeswaran et al., 1994).

5.2. IL-8 and endothelin 1 (ET-1)

IL-8 (also known as CXCL8) is produced by phagocytes when exposed to inflammatory stimuli and attracts neutrophils and promotes angiogenesis (Flynn et al., 2015; Simler et al., 2004). Endothelin-1 (ET-1) is a potent vasoactive peptide with diverse properties including vasoconstriction, bronchoconstriction, cell growth, turnover and fibroblast activation (Bonella & Costabel, 2014; Fagan, McMurtry, & Rodman, 2001; Simler et al., 2004). Both IL-8 and ET-1 are elevated in idiopathic ILD compared with controls and have demonstrated correlation with HRCT fibrosis scores, pulmonary function decline and reduced overall, transplant-free and progression-free survival (Richards et al., 2012; Simler et al., 2004; Tsoutsou et al., 2006; Ziegenhagen et al., 1998). Prospective validation is required. In SSc, studies have demonstrated correlation of ET-1 with vascular markers of disease severity including digital ulcers, renal vascular disease and pulmonary hypertension (Aghaei et al., 2012; Cozzani, Javor, Laborai, Drosera, & Parodi, 2013). Whilst studies have included patients with SSc-ILD in small numbers, to our knowledge, correlation with pulmonary fibrosis = has not been demonstrated.

6. Other biomarkers

6.1. Oxidative stress biomarkers in IPF

Oxidative stress has been implicated in the pathogenesis of pulmonary fibrosis for some time, although the exact mechanism remains unclear (Cheresh, Kim, Tulasiram, & Kamp, 2013). Levels of total hydroperoxide, as a marker of oxidative stress, were elevated in 21 IPF patients compared with healthy controls, and correlated with Medical Research Council dyspnoea score, and inversely with lung volume and DLCO (Daniil et al., 2008). It must be noted that treatment targeting mechanisms of oxidative stress, such as N-acetylcysteine (NAC), have overall not shown benefit in ILD and a greater understanding of the pathogenic role of oxidative stress and larger, prospective studies is required.

7. Discussion: Limitations and future perspectives

Despite great interest and motivation, a serum biomarker that reliably predicts disease progression and outcomes in ILD is yet to be validated for clinical use. An ideal biomarker needs to demonstrate adequate reproducibility, accuracy, sensitivity and specificity, be safe and clinically feasible, have an acceptable cost, add value to currently available tests and finally, be validated in well-designed multicentre studies across heterogeneous populations (Andersen et al., 2011; Flynn et al., 2015; Ray et al., 2010).

Although multicentre prospective studies are emerging, the majority of current data derive from small, retrospective, single-centre studies, with measurements at a single time-point. Many biomarkers are non-specific and unable to predict lung disease independent of confounders such as malignancy and other inflammatory states. Other challenges that preclude pooling of results and accurate comparison of studies include the lack of a gold standard for measuring disease activity, standardised assays for biomarker testing, discrepancy in thresholds defining positive and negative results, and the highly heterogeneous and relatively rare nature of ILD itself. Furthermore, the classification of ILD is in constant evolution as our understanding of disease pathophysiology and behaviour advances, but this may also change how a patients' disease is classified in studies. This is especially relevant for

studies performed before the 2001 ATS/ERS consensus classification for idiopathic interstitial pneumonias.

As discussed in detail by Clarke et al. in a recent issue of *Pharmacology and therapeutics*, the interest in biomarkers also lies in their potential to better stratify the heterogeneity of ILD patients in order to provide more precise and personalised therapy and as a target of novel therapies able to not just slow, but cease disease progression (Clarke, Murray, Crestani, & Sleeman, 2017). Two anti-fibrotic drugs, nintedanib and pirfenidone, are now generally considered the standard of care in IPF to slow disease progression, but there is no consensus who to treat, when to commence, cease or change therapy in order to maximise efficacy whilst minimising side effects.

Despite the methodological weaknesses of current studies, frequently acknowledged by the study authors themselves, selected biomarkers such as SP-A, SP-D, MMP7, periostin, osteopontin, CCL18, and markers of endothelial damage (eg. CCL2, IL-6, CXCL4, VEGF, IL-8, ET-1) show promise. The broad heterogeneity of the ILD population and disease behaviour suggests that to improve sensitivity and specificity the likely way forward is to validate composite biomarker panels that can be integrated with demographic, radiographic, physiologic and possibly genetic features to develop a clinical tool that can predict disease progression to guide management.

Results must always be interpreted within the patient's clinical context, and thorough clinical assessment and multidisciplinary collaboration between respiratory physicians, rheumatologists, immunologists, radiologists and pathologists remains paramount in the management of ILD patients.

The successful translation of molecular biomarkers into clinical practice requires validation in large, multi-centre, prospective studies with long-term, longitudinal follow-up, supported by a better understanding of biological mechanisms driving disease, standardisation of molecular assays, and intervention trials that show changes related to clinical disease state. This is particularly pertinent to IPF and SSc-ILD, which have heterogeneous disease courses, high mortality, and no current means to accurately identify individuals at greater risk of progression.

It is with urgency that we continue to work towards an ultimate goal of developing a clinical tool that can safely and accurately predict individual outcomes, thus maximising the therapeutic benefits for patients at the highest risk, while minimising exposure to harm in others. It seems likely that patients have several distinct mechanisms important to their individual pathobiology, and better characterisation through biomarker profiles combined with thorough clinical assessment may in the future aid our provision of personalised and precision-based therapy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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