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REVIEW

Recent advances in the pathogenesis and clinical evaluation of pulmonary fibrosis

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ABSTRACT: Interstitial lung diseases (ILDs) are a group of heterogeneous disorders, either idiopathic or associated with injurious or inflammatory causes, in which the major site of damage is the lung interstitium. For a long time, knowledge regarding pathogenesis was trivial and there were difficulties in diagnosing and subsequently treating these diseases.

During the past decade, however, there has been an impressive development in the field of ILDs. Idiopathic pulmonary fibrosis, the most common and fatal form of ILD, was initially believed to be due to an inflammatory response to unknown lung injury, whereas nowadays it is believed to be the result of multiple injuries at different sites of the lung followed by aberrant repair.

The integration of clinical, radiological and histological data, namely a multidisciplinary team (MDT) approach, has provided grounds for a more accurate diagnosis of ILDs, and helped the identification of different entities and development of different therapeutic approaches. However, because of the complexity of ILDs, even this approach may fail to establish a confident diagnosis.

How should the clinician behave in this case and what are the pitfalls of the MDT approach? In addition, since diagnosis is the major predictor of prognosis, are there any other tools available to predict prognosis?

KEYWORDS: Idiopathic pulmonary fibrosis, interstitial lung disease, multidisciplinary team meeting, pathogenesis

Interstitial lung diseases (ILDs) are a group of heterogeneous lung disorders which include idiopathic interstitial pneumonias (IIPs), ILDs of known cause, granulomatous disorders and orphan lung diseases. The main challenge in the field of ILDs is the early and accurate diagnosis of the most common and devastating form, namely idiopathic pulmonary fibrosis (IPF), which frequently has a worse prognosis than that of many cancers [1, 2]. It has become clear that in ILDs, diagnostic precision is paramount, because diagnosis determines prognosis as the rest of the ILDs have, on average, a more benign course than IPF. Moreover, early diagnosis allows lung transplantation enrolment and access of more patients to clinical trials, which is very important as there is not yet an approved pharmacological treatment for IPF.

IPF is a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults and limited to the lungs [1]. Despite recent progression, the pathogenesis of the disease is not fully understood. However, it is well recognised that IPF is caused by an unknown insult to the lung that leads to alveolar epithelial cell injury and subsequent dysregulated repair, characterised by excessive deposition of extracellular matrix (ECM) and loss of normal parenchymal architecture and lung function. Fibroblasts, which produce ECM proteins in fibrosing diseases such as IPF, exhibit unregulated proliferation and differentiate into myofibroblasts. The latter is considered the hallmark cell in the development and establishment of lung fibrosis. Multiple pathways, such as coagulation, apoptosis and oxidative stress, are also implicated in the pathogenesis of the disease [3–6]. In this review we aim to highlight recent advances in the pathogenesis of IPF and diagnosis of ILDs.

Following the European Respiratory Society research seminar which took place in Athens, Greece (November 11–12, 2010), pirfenidone has received marketing authorisation in European Union.

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ADVANCES IN THE PATHOGENESIS OF IPF

Origin of myofibroblasts

In response to tissue injury, myofibroblasts arise from a resident pool of tissue fibroblasts. These cells organise into small areas of active fibrosis, namely fibroblastic foci, and produce excessive amounts of ECM proteins. It is believed that injured alveolar epithelial cells (AECs) produce mediators, such as platelet-derived growth factor, transforming growth factor (TGF)-β and tumour necrosis factor (TNF)-α, which can lead to the migration of local fibroblasts to the injured site and to their subsequent differentiation into myofibroblasts [7].

Recently, it has been demonstrated that myofibroblasts may also derive from resident epithelial cells through a process called epithelial mesenchymal transition (EMT). This process is characterised by a series of events such as loss of epithelial cell polarity, loss of adhesion molecules such as E-cadherin and zona occludens-1, reorganisation of cytoskeleton, acquisition of mesenchymal markers such as fibronectin and α-smooth muscle actin, and possible acquisition of a migratory phenotype [8, 9]. TGF-β is a major inducer of EMT in the lungs. It has been shown that both primary rat AECs and an AT2 cell line (RLE-6TN) can undergo EMT *in vitro* and *in vivo* following extended exposure to TGF-β1 [8]. Epithelial markers such as Nkx2.1 (also known as thyroid transcription factor-1), pro-surfactant protein B and C, as well as mesenchymal markers such as α-smooth muscle actin and N-cadherin, have been co-localised in hyperplastic epithelial cells overlying fibroblastic foci in lung biopsies of IPF patients [8, 10]. TGF-β1-induced EMT in type II alveolar cells (ATII) is driven by the expression and nuclear translocation of mediators such as SNAI transcription factors. Increased SNAI1 and SNAI2 expression in experimental and human IPF *in vivo* suggests that SNAI-mediated EMT may contribute to the fibroblast pool in IPF [11].

MicroRNAs, post-transcriptional gene regulators, play a critical role in various physiological processes, such as tissue development and differentiation, cellular proliferation and tissue repair. Let-7d, a microRNA expressed in epithelial cells of normal lung, is downregulated in IPF, while its target molecule, high-mobility group AT-hook 2, a known regulator of EMT, is overexpressed [12]. Inhibition of let-7d *in vitro* induces EMT whereas inhibition *in vivo* causes alveolar septal fibrosis.

In light of these observations, inhibition of EMT could be a plausible therapeutic target. Recently, it has been demonstrated that *N*-acetyl-cysteine (NAC), a drug with antioxidant properties, can slow the decline of lung function, mainly in less severe cases of IPF [13, 14]. The exact mechanism of action is not known but it has been hypothesised that NAC prevents EMT in AECs *in vitro*, at least in part through replenishment of intracellular glutathione stores and limitation of TGF-β1-induced intracellular reactive oxygen species (ROS) generation [15]. Hepatocyte growth factor has also been found to have an inhibitory effect on EMT *in vitro via* induction of Smad7, an inhibitor of TGF-β signalling, in a mitogen-activated protein kinase dependent manner [16].

Circulating fibrocytes have been identified as another source of myofibroblasts [17]. These cells derive from the bone marrow and express markers of either mesenchymal cells, such as collagen I, collagen III and fibronectin, or haematological cells, such as CD45 and CD34 [18]. The CXCR4–CXCL12 axis plays a cardinal role in mobilising fibrocytes to the site of injury; it was observed that the chemokine receptor CXCR4 is expressed on the surface of fibrocytes, whereas CXCL12 is expressed by AECs [17, 19, 20].

Alveolar epithelial cells

Injury and apoptosis within the alveolar epithelium, with subsequent ATII cell hyperplasia, are consistent findings in experimental and human lung fibrosis. Deregulation of AEC developmental programmes might contribute to the pathogenesis of IPF. The Wnt family includes highly conserved secreted growth factors that participate in organ development and which are defective in organ failure. The best characterised signalling pathway, the Wnt/β-catenin pathway, has been found to be overexpressed and operative in adult lung epithelium of patients with IPF [21, 22]. Furthermore, WNT1-inducible signalling protein-1 (WISP1), a member of the CCN family of secreted cysteine-rich matricellular proteins which is encoded by a WNT target gene, is highly expressed in hyperplastic ATII cells, mediates enhanced ATII cell proliferation and induces the release of pro-fibrotic markers such as matrix metalloproteinase (MMP)-7, plasminogen-activator inhibitor 1 and SPP1 by ATII cells. Moreover, it induces EMT of ATII cells as well as ECM production by fibroblasts whereas it has no effect on fibroblasts proliferation. Interestingly, neutralisation of WISP1 leads to marked attenuation of bleomycin-induced lung fibrosis, suggesting that it could represent a potential therapeutic target [23].

Mediators of fibrosis

TGF-β1 is a central mediator in the development of lung fibrosis, playing a crucial role in each of the individual processes that leads to fibrosis. TGF-β1 inhibits the proliferation of epithelial cells, promotes epithelial migration, stimulates EMT and enhances epithelial apoptosis. In addition, it stimulates fibroblast proliferation, collagen production and inhibits fibroblast apoptosis. It has been found that TGF-β1 can be activated through interaction with integrins, which are heterodimeric transmembrane proteins consisting of α and β subunits. Cytoskeletal changes activate the αvβ6 integrin, which subsequently activates TGF-β1. Thrombin and lysophosphatidic acid released by platelets following injury bind to epithelial cell surface receptors, such as protease activator receptor (PAR-1) and lysophosphatidic acid receptor-2, induce cytoskeletal changes and activate αvβ6 integrin which subsequently activates TGF-β1 [24, 25]. Mice that do not express the β6 subunit can develop mild inflammation but are protected from lipopolysaccharide, ventilator-associated lung injury and bleomycin-induced lung fibrosis [24, 26]. Anti-αvβ6 monoclonal antibody can prevent lung fibrosis and acute lung injury *in vivo* [27, 28].

It has recently been observed that serotonin induces fibroblast proliferation *via* the 5-HTR_{2A} and 5-HT_{2B} receptors [29]. The expression of serotonin's receptors has been investigated in IPF and experimental models of lung fibrosis [30]. The expression of 5-$HTR_{1A/B}$ and 5-HTR_{2B} was upregulated in the lungs of patients with IPF and nonspecific interstitial pneumonia (NSIP), whereas the expression of 5-HTR_{2A} was increased specifically in IPF. Moreover, the authors observed that 5-HTR_{2A} protein largely localised to fibroblasts whereas 5-HTR_{2B}

localised to the epithelium. Subsequently, they investigated the efficiency of the 5-$HTR_{2A/B}$ antagonist terguride in a mouse model of bleomycin-induced lung fibrosis and, interestingly, they observed that there was a significant improvement in either lung function or histology together with decreased collagen content compared with vehicle-treated mice. Treatment of human lung fibroblasts also significantly reduced collagen production in response to TGF-β and WNT3a. It should be stressed that serotonin is a potent vasoconstrictor and may have a role in the pathogenesis of pulmonary hypertension which is commonly observed in patients with IPF [31].

Oxidative stress

It is well known that in IPF there is an excess of oxidant and a deficiency of antioxidant factors which contributes to the pathogenesis of the disease [32]. The level of the oxidative stress correlates negatively with the lung function and can be used as a marker of disease severity [33]. On the one hand, inflammatory cells from bronchoalveolar lavage (BAL) produce ROS which can have an effect on pulmonary cells and cytokines, such as TGF-β1, and promote fibrogenesis [32, 34]. On the other hand, glutathione, an antioxidant agent, has been found to be decreased in IPF [35] and this could be partially due to activation of TGF-β1, which inhibits glutathione synthesis, as has been shown in human epithelial cell lines [36]. It has been demonstrated that NAC is capable of stimulating glutathione synthesis, increasing the intra- and extracellular levels and thereby partially restoring glutathione levels [37, 38]. NAC also has an effect on apoptosis, inhibiting H_2O_2-mediated induction of ceramide, a membrane sphingolipid implicated in the process of apoptosis [39]. Other beneficial effects of NAC include the inhibition of pro-fibrotic fibroblast response to TGF-β1 and the dose-dependent inhibitory effect on interleukin (IL)-8 and MMP-9 release, and intracellular adhesion molecule expression by BAL macrophages and lymphocytes from patients with IPF and sarcoidosis [40, 41]. In agreement with these observations, NAC has been found to have favourable effects on the lung function of patients with IPF, and mainly in those with less progressed disease [13, 14].

Apoptosis

In the process of normal wound healing, myofibroblasts should be eliminated through apoptosis. In the case of IPF though, myofibroblasts present a decreased sensitivity to apoptosis whereas epithelial cells are more sensitive to apoptosis, a phenomenon called apoptosis paradox [4]. A plausible explanation of this phenomenon has been proposed recently. Patients with IPF have a reduced capacity to produce prostaglandin E2, resulting in increased AEC and reduced fibroblast sensitivity to Fas ligand-induced apoptosis [42]. TGF-β1 produced by AECs is likely to contribute to myofibroblast resistance to apoptosis *via* signalling pathways involving PI3K/Akt [43]. Phosphatase and tensin homologue, which is implicated in the control of processes such as apoptosis, is downregulated in myofibroblasts within fibroblastic foci and may also contribute to the decreased resistance to apoptosis [44].

Is IPF an autoimmune disease?

Recent studies have supported the issue of autoimmunity as a possible pathogenetic pathway for IPF. It has been observed that neolymphoidogenesis can occur in the lungs of IPF patients. B-cell aggregates, activated T-cells and mature dentritic cells have been demonstrated in IPF lung, increasing the likelihood of antigen presentation activity [45, 46]. Moreover, activated CD4+ T-cells produce either cytokines, which induce the production of auto-antibodies from B-cells, or mediators, such as IL-10, TGF-β1 and TNF-α, which promote fibrogenesis [47]. In addition, CD4+ T-cells purified from lymph nodes from patients with IPF proliferate when cultured with autologous lung tissue protein extracts [47]. It has been suggested that periplakin, a small protein from the plakin family and localised to desmosomes may represent a possible target of auto-antibodies [48]. Desmosomes have a crucial role in the maintenance of integrity of the alveolar epithelium. The presence of auto-antibodies against periplakin in the serum and BAL of IPF patients was associated with severe disease. It is thought that they may alter the reparative ability of the epithelium.

Coagulation cascade

It is well recognised that the activation of the coagulation cascade has pro-fibrotic effects in IPF [7, 49]. Thrombin might influence the deposition of connective tissue proteins during normal wound healing and the development of fibrosis by stimulating fibroblast pro-collagen production [50]. Thrombin may exert this effect by inducing the proteolytic activation of PAR-1, which is found to be overexpressed in the activated epithelium of fibrotic areas in IPF [51]. Both thrombin and PAR-1 may promote the development of fibrosis by upregulating fibroblast connective tissue growth factor expression [52]. Factor X has also been observed to play an important role in the pathogenesis of IPF. Tissue factor, together with factor VIIa and factor X, form a ternary complex which co-localise in the epithelium overlying fibroblastic foci leading to the activation of factor X. Subsequently, factor Xa activates fibrogenetic pathways by either activating TGF-β1, the main pro-fibrotic cytokine, or inducing fibroblast to myofibroblast differentiation through the PAR-1 signalling pathway [3]. Targeting factor Xa could be an intriguing therapeutic approach for IPF.

ADVANCES IN THE DIAGNOSIS OF ILDS

Is the diagnosis always straightforward? The multidisciplinary team approach

It has long been considered that surgical lung biopsy was the ''gold standard'' procedure for the diagnosis of ILDs, but currently it seems that this is not the case because of major limitations. Disease severity, comorbidities, interobserver variation [53] and ''sampling error'', *i.e.* a sample taken from an area not representative of the predominant process [54, 55], are some of the constraints that made surgical lung biopsy lose its golden glow. Over recent years, there has been a progressive shift to a combined silver standards approach where more or less equal weight is given to clinical radiological and histopathological information [1]. The importance of a multidisciplinary team (MDT) approach has been highlighted by the fact that it improves interobserver variation and diagnostic confidence [56]. However the level of experience may influence the efficiency of the MDT approach. It was shown that more interobserver variation exists in community-based clinicians, radiologists and pathologists than in their academic counterparts [57].

Clinicians should have a high index of suspicion in the right clinical context and not overlook symptoms such as subtle cough and/or shortness of breath as these symptoms may represent early manifestations of ILDs, and mainly IPF. Frequently, these symptoms may be attributed to smoking or asthma which delays the need for further investigations and early diagnosis. In case of collagen tissue disorder (CTD) associated-ILD the presence of dyspnoea may be masked by inactivity due to underlying arthritis [58]. It should be noted that as lung involvement may be the presenting manifestation of CTDs, physical examination, laboratory findings and close follow-up are of significant importance as interstitial pneumonias have, on average, better prognosis when associated with CTDs than in their idiopathic forms, and in this case the need for further invasive diagnostic procedures is obviated [58]. Longitudinal behaviour, such as duration of dyspnoea and cough, pattern of symptomatic progression and previous responsiveness (or non-responsiveness) to corticosteroid therapy, may provide valuable diagnostic clues. Previous full lung function test or spirometry may provide useful information regarding the progression of the disease.

Both radiologists and pathologists have to deal with the problem of interobserver variation, which may reflect the presence of overlap patterns in ILDs [59]. Trying to tease out the overlap issue, one may argue that sameness on either histology or imaging, coexistence of various patterns or transformation of one pattern of disease to another may be potential contributors. Smoking-related ILD could be an example of overlap. Respiratory bronchiolitis (RB), RB-ILD and desquamative interstitial pneumonia (DIP) are all characterised, in terms of pathology, by accumulation of pigmented macrophages in the alveoli and the profusion of inflammation could differentiate some of these entities. From the imaging point of view, it has been shown that RB is predominantly characterised by centrilobular nodules and DIP is characterised predominantly by ground-glass opacities, whereas RB-ILD shares features of both entities. That makes RB-ILD difficult to distinguish from RB and DIP based only on radiological findings. These three disorders may represent different stages of small airway and parenchymal reaction to smoke [60]. Other examples of similarities are subacute hypersensitivity pneumonitis (HP) and lymphoid interstitial pneumonia, in both of which patchy ground-glass and randomly distributed thin-wall cysts may be observed [61].

It is well established that usual interstitial pneumonia (UIP) and NSIP can coexist in different segments or lobes, and that most of these cases behave as IPF [54]. Integration of radiological data is paramount in terms of prognosis as biopsy-proven cases of UIP with typical findings on high-resolution computed tomography tend to have a worse outcome than the ones with atypical presentation in HRCT [54]. NSIP, in the context of polymyositis, can coexist with peribronchovascular consolidations suggestive of organising pneumonia [62–65]. More interestingly, patients with different clinical presentation had surgical lung biopsy features of both pulmonary alveolar proteinosis and HP, whereas most of them had HRCT features suggestive of neither of the two entities [66].

Another pitfall that may potentially undermine histopathological and imaging diagnoses is the longitudinal behaviour of ILDs, as patterns may change over time. For instance, diffuse alveolar damage, the histological counterpart of acute interstitial pneumonia, may transform in NSIP. It was observed that, in the acute phase of adult respiratory distress syndrome, ground-glass opacities and airway dilatation were the predominant findings, while on follow-up scans airway dilatation was still present and, moreover, it was admixed with a reticular and linear pattern suggestive of supervening pulmonary fibrosis [67, 68].

Is BAL useful in the diagnosis and management of ILDs?

The utility of BAL in the diagnosis of ILDs merits some consideration. In diseases such as sarcoidosis, HP and IIPs, diagnosis cannot be made without integration of HRCT data [69, 70]. It should be stressed though, that when HRCT findings are atypical for either sarcoidosis or HP, an absence of BAL lymphocytosis may exclude these disorders. Recently, it was suggested that a cut-off value of 30% of lymphocytes in the BAL of patients with clinical and HRCT findings typical for IPF may change the diagnostic perception [71]. In six out of 74 patients diagnosis was changed and further confirmed by surgical lung biopsy in two cases and by the non-IPF outcome in the rest of the cases. Of course, this finding needs to be further explored in larger cohorts. BAL can be diagnostic in the field of rare ILDs, such as alveolar proteinosis [72], lipoid pneumonia, acute eosinophilic pneumonia, pulmonary lymphoma [73], diffuse alveolar haemorrhage and drug-induced lung diseases. In cases of acute deterioration of ILDs while on immunosuppressive treatment, BAL is extremely important in order to diagnose an opportunistic infection and decide whether, if this is the case, to reduce the level of immunosuppression and treat the infection or, in contrast, to increase the level of immunosuppression if there is no infection.

How should clinicians deal with diagnostic and management uncertainties?

As mentioned previously, a firm diagnosis is needed in order to estimate prognosis and make decisions about best treatment options. According to the recently published American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines, the diagnosis of IPF should be based on the exclusion of other known causes of ILDs (environmental exposures, drugs and collagen tissue disorders) and presence of UIP on HRCT, *i.e.* reticular abnormalities with subpleural and basal distribution, honeycombing with or without traction bronchiectasis and absence of features inconsistent with UIP such as extensive ground-glass opacities, diffuse mosaic attenuation, profuse micronodules and consolidations [1]. In the most experienced hands, a firm diagnosis of IPF could be reached with moderately high confidence from HRCT images [74, 75] even without lung biopsy. A large subgroup of cases with atypical images, such as reticular abnormalities with subpleural and basal predominance without honeycombing or traction bronchiectasis, can be classified by experienced radiologists as possible IPF [1]. Clearly, this uncertainty is greater in the hands of less experienced radiologists and, overall, it becomes obvious that problems for radiologists are translated as problems for clinicians who need a guide on how they should treat the patients. The next reasonable step as suggested by ATS/ERS guidelines is surgical lung biopsy. A typical UIP pattern is characterised by the combination of

TABLE 1 Diagnosis of idiopathic pulmonary fibrosis based on a combination of high-resolution computed tomography (HRCT) and histopathology patterns

HRCT pattern	Histopathology pattern				
	UIP	Probable UIP	Possible UIP	Non-classifiable fibrosis	Not UIP
UIP	Yes	Yes	Yes	Yes	No
Possible UIP	Yes	Yes	Probable	Probable	No
Not UIP	Possible	No	No	No	No

UIP: usual interstitial pneumonia. Modified from [1, 76] with permission from the publisher.

marked fibrosis with architectural distortion with or without honeycombing that presents a predominantly subpleural and paraseptal distribution. Patchy involvement of lung parenchyma by fibrosis, presence of fibroblastic foci and absence of features suggestive of an alternative diagnosis are also cardinal criteria for the diagnosis of UIP in biopsy samples. As mentioned previously, the final diagnosis requires an MDT approach combining imaging and pathological data (table 1) [1, 76]. The main challenge is to differentiate, with confidence, IPF from fibrotic NSIP. But what if the patient declines to undergo surgical lung biopsy or, in the worst case scenario, is not fit for the procedure because of advanced age, stage of disease and presence of comorbidities? Unfortunately this issue is not covered by the recent ATS/ERS guidelines [1]. However, this issue has been argued in the recent British Thoracic Society guidelines [77] where recommendation for treatment of ''definite IPF with fibrotic NSIP not a realistic possibility'', ''IPF and fibrotic NSIP both plausible diagnoses'' and ''definite (biopsy-proven) NSIP'' have been made. Recently it has been shown that in patients without honeycombing in HRCT, older age and modest amount of fibrosis, such as reticular changes are highly predictive of IPF. However, it should be stressed that these findings need further confirmation in multicentre prospective studies [78].

The scenario of diagnostic uncertainty may also be applied outside the field of IPF/fibrotic NSIP. What if, as mentioned previously, the integration of clinical, radiological and, when available, histological information are compatible with more than one realistic diagnosis? It is suggested that ILDs, based on longitudinal behaviour, can be subdivided in five patterns of disease behaviour and if a patient fits into one of these patterns with confidence then treatment decisions could be taken (table 2) [79]. When HRCT images and longitudinal behaviour are suggestive of either self-limited inflammation or stable fibrosis and an underlying causative agent can be identified, removal of this agent is more likely to be the key management option, obviating the need for more invasive procedures. Long-term follow-up to assess stability is warranted. It is in the field of idiopathic ILDs that input from pathologists is usually needed. The main challenges are to differentiate between stable disease, in which the treatment goal is to assess stability, and progressive fibrosis, in which the treatment goal is to prevent progression; and between major inflammation that is likely to progress, in which the realistic goal is to treat for an initial response and subsequently rationalise therapy in order to preserve the gains, and inexorably progressive fibrosis, in which the aim is to slow disease progression and assess the need for transplant or palliative care.

In ILD associated with systemic sclerosis (SSc), in which the pattern of major inflammation with progressive fibrosis is usually observed, a staging system has been proposed in order to give clinicians a ''helping hand'' to avoid unnecessary treatment in stable and limited disease and to treat the disease

TABLE 2 Patterns of disease behaviour

Self-limited inflammation	Drug-induced lung disease (acute onset)
	Hypersensitivity pneumonitis (short-term exposure)
	Sarcoidosis (distinct subset with usually acute onset)
	COP, DIP and LIP
Stable fibrotic disease	Drug-induced lung disease (residual fibrosis after cessation)
	Hypersensitivity pneumonitis (after prolonged exposure)
	Sarcoidosis (residuum of burnt-out fibrotic disease)
	Non-progressive pneumoconiosis after cessation of exposure (*e.g.* silicosis)
	NSIP
Major inflammation, risk of fibrotic progression	Drug-induced lung disease (unusually florid reactions)
	Hypersensitivity pneumonitis (usually continuing exposure)
	Sarcoidosis (prolonged severe inflammation)
	NSIP, COP, LIP and DIP
Inexorably progressive fibrosis	Drug-induced lung disease (continuing exposure)
	Hypersensitivity pneumonitis (antigen usually unknown)
	Sarcoidosis (small subset of patients)
	Progressive pneumoconiosis after exposure (*e.g.* asbestosis)
	UIP
Explosive acute diffuse lung disease	Drug-induced lung disease
	AIP

COP: cryptogenic organising pneumonia; DIP: desquamative interstitial pneumonia; LIP: lymphocytic interstitial pneumonia; NSIP: nonspecific interstitial pneumonia; UIP: usual interstitial pneumonia; AIP: acute interstitial pneumonia.

aggressively when it is progressive [80]. It is a user-friendly system which allows, even in moderately experienced hands, the distinction between limited and extensive disease which has prognostic significance, simply by integrating information obtained by HRCT and lung function tests. A threshold of 20% of disease extent in HRCT is used to distinguish patients in two prognostically different groups. In borderline cases, a threshold of 70% of forced vital capacity can differentiate limited from extensive disease.

Beware of comorbidities

Patients with ILDs and apparently stable disease may deteriorate because comorbidities remain undiagnosed and untreated. It is now well recognised that smoking is associated with worse outcome in IPF [81]. The presence of emphysema, *i.e.* a well-known smoking-related disease, is associated with a more severe course of IPF and with a higher prevalence of pulmonary hypertension [82–85]. The entity of combined pulmonary fibrosis and emphysema can also be observed in the context of CTD-ILDs [86]. Gastro-oesophageal reflux (GER) has been associated with the development of pulmonary fibrosis and mainly IPF [87, 88]. Recently, it was shown that IPF patients with asymmetric disease on HRCT had a higher rate of GER and acute exacerbations than patients with symmetric disease [89, 90]. Interestingly, there was an association with sleeping position as the most involved lung was the dependent one in 94% of the cases. Pulmonary hypertension is not uncommon in ILD and in IPF, sarcoidosis and SSc-ILD it is mainly associated with reduced diffusing capacity of the lung for carbon monoxide and desaturation in exercise, and is a predictor of poor survival [91–98]. Recently, it was proposed that pulmonary vascular resistance measured during right heart catheterisation may predict mortality in ILD [99]. Brain natriuretic peptide levels, when combined with echocardiogram markers of right heart dysfunction, can also predict mortality [100, 101].

The role of biomarkers

In the field of ILD and IPF in particular, for which there is ongoing research for effective treatment, a vast number of biomarkers have been proposed as predictors of disease severity and progression, and have been used as end-points in clinical trials. Unfortunately, most of them still remain experimental and are not used in everyday clinical practice. Serum levels of KL-6, a high-molecular weight mucin-like glycoprotein, have been found to be associated with decreased survival in IPF [102]. Surfactant protein A and D are secreted by type II alveolar epithelial cells. Serum levels are increased in IPF as well as in other ILDs, and can predict survival [103–107]. MMPs are believed to participate in the pathogenesis of pulmonary fibrosis and, in fact, MMP-3, -7, -8 and -9 are elevated in the BAL of IPF patients who had worse outcome [108]. Serum levels of CCL-18, a chemokine produced by alveolar macrophages, is independently predictive of mortality in IPF [109]. Circulating fibrocytes are mesenchymal cell progenitors and involved in the pathogenesis of IPF. They are found to be increased in IPF and during acute exacerbations, and can independently predict disease mortality. There is some evidence that markers of oxidative stress are increased in exhaled breath condensate in IPF and that H_2O_2 can be associated, although weakly, with disease severity [110]. Serum levels of oxidative stress are also increased in IPF and associated with disease severity [33]. Data regarding the prognostic value of BAL neutrophilia are conflicting. It has been shown in IPF that there is an association, although weak, with increased early mortality after adjustment for disease severity, in contrast to what was observed in previous studies [111, 112]. In pulmonary fibrosis associated with SSc, BAL neutrophilia is a marker of disease severity as it is linked to disease extension in HRCT and greater lung function impairment [113, 114] but it showed no prognostic significance after adjustment for disease severity [113]. Periostin, a 90-kDa ECM protein found to be involved in the process of fibrosis, was observed to be strongly expressed in the lungs of patients with IPF and fibrotic NSIP, whereas it was only weakly expressed in patients with cellular NSIP and cryptogenic organising pneumonia and normal lungs. Thus, it may be used as a biomarker in order to distinguish fibrotic from non-fibrotic IIPs [115].

CONCLUSION

It has become clear that the diagnosis of ILDs is not always straightforward. MDT meetings are now considered the cornerstone for accurate diagnosis; however, in less straightforward cases the simple knowledge of disease behaviour may also provide the guide for the right treatment. Patients with suspected ILDs should always be referred to tertiary care centres in order to achieve a more confident diagnosis by experts in this field. Clearly, delays in referral may mean delays in diagnosis and management, with significant impact on survival, and possible enrolment in transplant lists and clinical trials. The main challenge is the diagnosis of IPF, the most frequent and devastating form of ILD, for which there is not yet an approved pharmacological treatment in Europe. However, following the ERS research seminar which took place in Athens, Greece (November 11-12, 2010), pirfenidone has received marketing authorisation in the European Union. To date, it has been shown that the combination of low-dose steroids, azathioprine and NAC may provide some benefit, mainly in patients with mild-to-moderate disease defined by a composite physiological index <50 [13, 14]. Pirfenidone, a drug with antifibrotic and anti-inflammatory properties has been proven to significantly slow the decline of forced vital capacity and to increase progression-free survival with an acceptable safety profile [116–119]. Recently, it has been suggested that multiple pathways could be simultaneously involved in the pathogenesis of IPF [120]. Moreover, this hypothesis has been strengthened by the observation that there are certain similarities between IPF and lung cancer biology [2]. In the latter, the hypothesis of multiple pathogenetic pathways led to the development of therapeutic approaches which simultaneously target these pathways, and this hypothesis should be taken into account for the design of future therapeutic trials in IPF.

STATEMENT OF INTEREST

None declared.

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